Inhibition and Potentiation of Platelet Function by Lysolecithin

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The effects of lysolecithin (LPC) on aggregation, serotonin release, shape, and lysis of rabbit, pig, or human platelets in platelet-rich plasma (PRP) or Tyrode albumin solution were examined during prolonged incubation. LPC added to citrated or heparinized PRP from humans or rabbits at a final concentration above 100 μM caused instantaneous inhibition of platelet aggregation induced by adenosine diphosphate (ADP), epinephrine (human PRP only), collagen, or thrombin. The inhibitory effect of LPC was found to be partially reversible over a period of 60–90 min. LPC at final concentrations above 30 μM also caused inhibition of ADP-, collagen-, and thrombin-induced aggregation and collagen- and thrombin-induced release of serotonin in suspensions of rabbit, pig, or human platelets. With washed platelets, the inhibitory effect not only rapidly disappeared but was followed by transient potentiation of aggregation and serotonin release. This potentiating effect of LPC was most pronounced when thrombin was used as stimulus.

Both inhibition and potentiation were observed at concentrations of LPC that did not cause a significant change in platelet shape or loss from platelets of lactic dehydrogenase. Inhibition and potentiation were also observed when platelets were added to suspending medium containing LPC, although considerably higher concentrations of LPC were required under these conditions. Potentiation was not observed when LPC was added to citrated or heparinized rabbit or human PRP or to washed rabbit platelets suspended in a medium containing 4% bovine serum albumin. It seemed likely that some or all of the observed effects of LPC on platelet function were due to structural modification of the platelet membrane insufficient to result in gross membrane damage or platelet lysis. In addition, the results of experiments using 14C-LPC seemed to indicate that the observed potentiating effect of LPC on platelet function may be related to its rapid uptake and metabolism by the platelets.

There is evidence that changes in vitro in plasma phospholipid composition may affect platelet behavior. Among the major phospholipids that have been tested in vitro, lysolecithin (LPC) in concentrations which may be found in plasma has been reported to induce platelet aggregation, to increase the sensitivity of human platelets to ADP as determined by electrophoretic mobility, and to inhibit adenosine diphosphate (ADP)-, epinephrine-, or collagen-induced platelet aggregation in human platelet-rich plasma (PRP). We have recently observed that when washed platelets from one rabbit are resuspended in platelet-free plasma from another rabbit, in which all of the major phospholipids have been labeled in vivo with 32P-orthophosphate, labeled...
plasma LPC is rapidly taken up and incorporated by the platelets while little or no uptake of the other plasma phospholipids occurs. In view of the possibility that LPC might be an important link in the interaction between platelets and plasma lipoproteins and the apparent contradictions in the data reported previously, the effect on platelet function of the addition of LPC to platelets in vitro has been examined in plasma and in suspensions of washed platelets.

**MATERIALS AND METHODS**

**Materials**

Lysophosphatidylcholine (LPC, egg), phosphatidylcholine (PC, egg), and glycerophosphorylcholine (GPC) were purchased from Supelco, Bellefonte, Pa. The purity of the compounds was verified by thin-layer chromatography (LPC, PC) according to the method of Lloyd et al. or by paper chromatography (GPC), which revealed single spots. LPC and GPC were dissolved in 0.85% saline to give a concentration of 2 mM and were stored in 0.5-ml aliquots at -20°C. Dilutions were made from these stock solutions each day using saline. PC was suspended in saline at a concentration of 1 mM by ultrasonication. Chromatographically pure lysophosphatidyl-1,2-14C-choline (117 mCi/mM) was obtained from International Chemical Nuclear Corporation, Irvine, Calif.

ADP (Sigma Chemical Co., St. Louis, Mo.) and bovine thrombin (Parke, Davis & Co., Ann Arbor, Mich.) were dissolved in and diluted with modified Tyrode's solution. Purified bovine thrombin was a gift of Mr. E. T. Yin, Department of Medicine, The Jewish Hospital, St. Louis, Mo.

Acid-soluble collagen was prepared as previously described, stored at 5°C, and diluted with 0.85% saline.

Human fibrinogen (Grade L Kabi, Stockholm, Sweden) was dissolved in distilled H2O to give a 4% solution. The solution was adsorbed twice with aluminum hydroxide. Then 0.5 ml of 0.1 M diisopropylfluorophosphate (DFP) in propylene glycol was added to 20 ml of fibrinogen solution and the mixture was incubated for 60 min at room temperature and dialyzed against two changes of saline for 12 hr at room temperature to eliminate unbound DFP. The solution was stored in small aliquots at -20°C until use.

Blood was obtained from anesthetized (pentobarbital 30 mg/kg) rabbits through a carotid artery cannula into plastic syringes. Pig blood was collected in polyethylene bottles at a slaughterhouse (Canada Packers, Toronto, Ont., Canada). Human blood was collected from healthy volunteers by venipuncture of an antecubital vein.

PRP was prepared from blood collected into 0.1 volume of 3.8% sodium citrate solution by centrifugation at 120 g for 15 min at room temperature. The platelet count was adjusted to 300,000/cu mm. Platelet counts were done in a Model 1 12 TH Electrone/Celloscope (Particle Data, Elmhurst, Ill.) or by phase-contrast microscopy according to the method of Brecher and Cronkite.

Suspended platelets in Tyrode solution containing 0.35% or 4% bovine albumin (fraction V, Pentex, Kankakee, Ill.) were prepared from blood collected into acid citrate dextrose according to the method of Ardlie et al. Suspensions of washed platelets from humans were prepared according to the method of Mustard et al. The platelet count was adjusted to 300,000/cu mm. The suspensions were stored at 37°C with the addition of potato apyrase.

**Platelet aggregation.** This was studied by a turbidimetric method using 1.0-ml aliquots of either PRP (stored at room temperature and prewarmed at 37°C for 4 min) or suspensions, in response to the addition of 0.1 ml of ADP, collagen, or thrombin. Since washed pig or human platelets do not normally aggregate upon the addition of ADP without the prior addition of fibrinogen to the suspending fluid (probably owing to a loss of membrane-bound fibrinogen during the washing process), 0.02 ml of a 4% DFP-treated, human fibrinogen solution was added to all samples of the suspensions in the aggregometer cuvette 30 sec before the addition of either ADP or collagen. The extent of aggregation was determined by measuring the maximal height of the aggregation curve in units of chart paper 3 min after the addition of each stimulus. The values
obtained using the platelet preparations to which LPC had been added were expressed as a percentage of the values obtained at corresponding times using the control preparations to which saline had been added.

**Release of platelet serotonin.** This was studied with 5-hydroxytryptamine-\(^{3}H\) (G) creatinine sulfate (2 mCi/μM 0.01 μCi/ml suspension, Amersham/Searle Corp., Des Plaines, Ill.) using washed rabbit, pig, or human platelets according to the method described previously,\(^{15}\) in conjunction with the tests of platelet aggregation.

**Platelet shape.** Platelet shape was assessed by measuring the height in millimeters of the oscillations recorded during stirring of either PRP or platelet suspension in the aggregometer at 37°C. Addition of saline to these platelet preparations did not result in a measurable change in the height of the oscillation over a period of 5 min. Platelet shape 1 min after the addition of different concentrations of LPC was expressed as a percentage of that determined in the PRP or suspension 3 min after the addition of saline solution.

**Platelet lysis.** This was studied by determining the amount of lactic dehydrogenase (LDH) in the PRP or suspending medium 3 min after the addition of either LPC or saline to the platelet preparation and subsequent centrifugation at 12,000 g for 1 min. LDH was measured according to the method of Bergmeyer et al.\(^{16}\) The values were expressed as a percentage of those obtained using a sonicated sample of the suspension.

**Incubation Procedure**

In this procedure 50-60 ml of PRP or 50 60 ml of platelet suspension were equally divided between two tubes and incubated at 37°C. Then 0.1 volume of LPC was added to tube A and, after rapid mixing 1.0 ml of suspension, was transferred to an aggregometer cuvette for testing of platelet aggregation and/or platelet serotonin release upon the addition of ADP. Then 0.1 volume of saline was added to tube B and the same measurement was repeated as for tube A. This procedure was followed by measurements of platelet aggregation (and/or serotonin release) induced by collagen in tubes A and B, followed by the same tests with thrombin in tubes A and B, followed by tests with ADP, and so on. Similar experiments were carried out on the same suspension or on different suspensions by reversing either the sequence of tubes A and B or the order of the stimuli.

In two experiments washed rabbit platelets in Tyrode’s albumin solution (10⁶/cu mm) were incubated at 37°C with \(^{14}\)C-LPC (0.04 μCi/ml suspension) at a concentration of 50 μM. Aggregation in response to thrombin was tested as described above. At the same time intervals after the addition of the \(^{14}\)C-LPC, two 1.6-ml aliquots of suspension were removed from the incubation mixture. One aliquot was sonicated briefly and subjected to lipid extraction. The second aliquot was centrifuged at 12,000 g for 1 min and the supernatant was removed. The platelet sediment was washed twice with Tyrode’s solution containing 0.05 M EDTA and extracted. Radioactivity was determined in the aqueous and lipid phase of the partitioned extracts and in the individual phospholipid fractions after two-dimensional thin-layer chromatography. Material concentrated from the aqueous phases of the extracts was subjected to paper chromatography\(^{17}\) for identification of labeled compounds.

**Phospholipid analysis.** Lipids were extracted according to the method of Bligh and Dyer\(^{17}\) as modified by Lloyd et al.\(^{6}\) The phospholipids were separated by two-dimensional thin-layer chromatography\(^{18}\) and stained by exposure of the plates to iodine vapor. The spots were identified by comparison of the mobility on thin-layer chromatography in the same system of known pure phospholipid reference compounds. The spots were scraped into separate test tubes and the phospholipids were eluted from the silica gel with CHCl₃:CH₃OH(1:2).\(^{18}\)

**Liquid scintillation counting.** Next 0.1 ml of the total platelet suspension incubated with \(^{14}\)C-LPC, aqueous and lipid phase of the partitioned lipid extracts or silica gel eluates were added to vials containing 2.0 ml 95% ethanol and 10.0 ml scintillator solution (5 g of 2,5-diphenyloxazole and 0.3 g of 2,2-1-phenylenbis (5-phenyloxazole) in 1 liter of toluene). All samples were counted for 4 min and the results were expressed as cpm (background subtracted).

**Identification of labeled compounds in aqueous phase.** The aqueous phases of the partitioned platelet lipid extracts (derived from samples of suspensions taken at various time intervals during the incubation experiments described above) were lyophilized and the material recovered was dissolved in 0.5 ml of distilled H₂O. Next 0.1-ml aliquots were applied to Whatman
No. 1 filter paper together with a pure GPC reference compound and descending chromatography was carried out using N-propanol:ammonia:H₂O (6:3:1) as solvent. After completion of the run, the paper was dried in a stream of cold air and stained for phosphorus. Radioactivity was located by autoradiography on Kodak No-Screen medical X-ray film (NS54T).

RESULTS

LPC inhibited platelet aggregation in citrated human or rabbit PRP induced by low concentrations (3–5 μM) of ADP (Fig. 1). Inhibition was evident with final concentrations of added LPC as low as 100 μM. The addition of high concentrations of LPC (300 μM or greater) produced changes in the amplitude of oscillation of light transmission, probably indicating a change in platelet shape. At concentrations between 100 and 250 μM, at which a change in platelet shape was not detected (Table 1, Fig. 1), inhibition was characterized mainly by a reduction of the maximal extent of aggregation and by the early onset of deaggregation; at higher concentrations, at which changes in platelet shape were frequently observed, there was also a slight effect on the initial response of the platelets to ADP (Fig. 1). With citrated human PRP, inhibition by LPC of aggregation induced by epinephrine (3–5 μM) was similar to LPC inhibition of aggregation induced by ADP in that LPC concentrations between 100 and 250 μM mainly inhibited the second phase of the aggregation curve, while higher

![Diagram]

Fig. 1. Effect of LPC on aggregation of platelets in citrated rabbit or human PRP induced by ADP: 0.1 ml of LPC (final concentration in μM indicated in the boxes) was added (first arrow) to 1-ml aliquots of PRP (300,000/cu mm) followed by 0.1 ml of ADP (second arrow) to give a final concentration of 5 μM. Change in platelet shape is indicated by a decrease in the amplitude of the oscillations and a decrease in light transmission, while aggregation is indicated by an increase in light transmission.
Table 1. LPC-induced Change in Platelet Shape and Platelet Lysis of Rabbit Platelets in Citrated PRP in Comparison to Effect of LPC on Thrombin-induced Aggregation

<table>
<thead>
<tr>
<th>LPC (µM)</th>
<th>Platelet Shape (Per Cent of Control)</th>
<th>LDH Loss (Per Cent of Total)</th>
<th>Thrombin-induced Aggregation (Per Cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0.6</td>
<td>100</td>
</tr>
<tr>
<td>75</td>
<td>100</td>
<td>0.8</td>
<td>100</td>
</tr>
<tr>
<td>150</td>
<td>100</td>
<td>0.8</td>
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<tr>
<td>300</td>
<td>68</td>
<td>0.9</td>
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</tr>
<tr>
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<td>0.9</td>
<td>11</td>
</tr>
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<td>825</td>
<td>14</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>4</td>
<td>2.6</td>
<td>0</td>
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PRP was stored at room temperature and prewarmed to 37°C for 4 min before addition of LPC. The experimental procedures and methods of analysis were as described for washed platelets in Fig. 5. LPC-induced platelet shape change (as amplitude of oscillations of light transmission) was determined 1 min and LDH loss was determined 3 min after the addition of LPC. The background value for LDH in platelet-free plasma was subtracted from the values measured in PRP before calculation of the per cent values given. Thrombin (0.25 U/ml) was added 1 min after LPC.

concentrations also affected the slope and extent of the primary wave of aggregation. LPC also inhibited ADP-induced aggregation in citrated rabbit PRP in which a secondary wave of aggregation and release did not occur.

LPC at concentrations above 100 µM also inhibited aggregation in citrated human or rabbit PRP induced by low concentrations of thrombin (0.1–0.3 U/ml) (Table 1) or collagen. At concentrations of LPC below 200 µM, the inhibitory effect on ADP-, collagen-, or thrombin-induced aggregation in citrated PRP was shown to be partially reversible over a period of 60–90 min. Figure 2 shows the results of such an experiment in which collagen was used as stimulus. Similar results were obtained using heparinized rabbit or human PRP. Addition of LPC to platelet-poor plasma 10 min before the platelets were added to the plasma also caused reversible inhibition of aggregation induced by ADP and collagen. However, the concentration of LPC required to induce the same degree of inhibition was three times greater than that required when it was added to PRP. The platelets did not change shape when added to LPC-containing plasma.

With suspensions of washed rabbit platelets, lower concentrations of LPC (final concentrations above 30 µM) also caused an immediate inhibition of the aggregating response to either ADP, collagen, or thrombin (Fig. 3). In the case of thrombin at a final concentration of 0.03 U/ml, the inhibitory effect not only

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**Fig. 2.** Effect of LPC on collagen-induced aggregation of rabbit platelets in citrated PRP incubated at 37°C. LPC to give a final concentration of 150 µM was added to one portion of PRP and saline was added to the other portion of the PRP. Aggregation induced by a suboptimal concentration of collagen was tested in successive samples of both suspensions. Maximal aggregation was measured in chart paper units at 3 min after the addition of collagen. The values obtained with PRP to which LPC had been added were expressed as percentages of those obtained with PRP to which saline had been added.
disappeared within the first 15 min but was followed by potentiation of aggregation. The period of potentiation lasted 20-30 min, after which time the response returned to the control value. The results obtained with crude bovine thrombin were similar to those obtained using purified bovine thrombin. With the concentrations of ADP or collagen used, the reversal of inhibition was usually slower and the potentiation of aggregation was less pronounced than with thrombin. In a series of experiments carried out as described for Fig. 3, the percentage maximal inhibition and potentiation by LPC (50 μM) of platelet aggregation during a 60-90 min incubation time were as follows: for ADP, 66 ± 22 (1 SD), 18 ± 12 (8 experiments); for collagen, 94 ± 8, 44 ± 16 (7 experiments); and for thrombin, 71 ± 13, 88 ± 26 (16 experiments).

When washed rabbit, pig, or human platelets were labeled with tritiated serotonin and then exposed to LPC, inhibition and subsequent potentiation of thrombin-induced release of radioactivity from the platelets were observed which paralleled inhibition and potentiation of thrombin-induced aggregation. Figure 4 shows such an experiment with washed human platelets. Similar results were obtained using collagen as a stimulus. Figure 5 shows an experiment with washed pig platelets and thrombin. In Fig. 5A, LPC was added directly to the platelet suspension. At a low concentration (15 μM) LPC had little effect on aggregation. At a higher concentration (50 μM) there was initial inhibition followed by potentiation of aggregation. At an even higher concentra-

Fig. 3. Effect of LPC on aggregation of washed rabbit platelets. LPC was added to one portion of a platelet suspension (platelet count 300,000/cu mm), incubated at 37°C, to give a final concentration of 50 μM, and saline was added to the other portion of the suspension. After brief mixing successive samples of the incubation mixtures were tested for platelet aggregation induced by suboptimal concentrations of ADP (3–5 μM), collagen, or thrombin (0.01–0.03 U/ml) as described in the text. Values for maximal aggregation obtained with the LPC suspension expressed as percentages of those values obtained at corresponding intervals with the saline control suspension. One of seven experiments with similar results is shown.

Fig. 4. Effect of LPC on thrombin-induced aggregation of and serotonin release from washed human platelets. The experimental procedure, concentration of thrombin, and method of analysis of aggregation were as described for Fig. 3, except that only one stimulus was used. The release values obtained with the LPC suspension were expressed as a percentage of those obtained with the saline control suspension. (In the saline control, 32% of the total 3H was released.) One of two experiments with similar results is shown.
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S OF CONTROL

Fig. 5. Effect of different concentrations of LPC on aggregation of washed pig platelets induced by thrombin (0.03 U/ml). (A) LPC was added at 4-min intervals to three portions of a suspension of washed pig platelets to give the micromolar concentrations indicated in the boxes beside each curve, and saline was added to a fourth portion of the same suspension. (B) LPC was added to the suspending fluid 10 min before addition of the platelets. Thrombin-induced platelet aggregation was tested in each incubation mixture immediately after the addition of either LPC or saline (A) or resuspension of the platelets (B) and thereafter at approximately 7-15-min intervals. The values for aggregation obtained with the suspensions to which LPC had been added were expressed as a percentage of those obtained at the corresponding time intervals using the saline control suspensions. One of four experiments with similar results is shown.

Fig. 6. Effect of LPC on thrombin-induced aggregation of washed rabbit platelets in relation to its effect on platelet shape and loss of LDH. LPC (to give the final concentrations indicated on the abscissa) or saline was added to aliquots of suspension stirred in the aggregometer at 37°C; 1 min after the addition of LPC (at which time any reduction in the height of the oscillations recorded had occurred) thrombin (0.03 U/ml) was added to the samples and aggregation was recorded. Platelet lysis was determined separately by adding LPC or saline to aliquots of suspensions stirred in the aggregometer at 37°C for 3 min and measuring the activity of LDH in the supernatant medium after centrifugation of the samples at 12,000 g for 1 min. Results expressed as a percentage of the value obtained using a sample of sonicated suspension.
rabbit platelets. At a concentration of LPC which almost completely inhibited aggregation, there was virtually no LPC-induced change in platelet shape. As the concentration of LPC was further increased, there was a progressive loss of platelet shape. At a concentration of 120 \( \mu M \), loss of LDH was observed, indicating platelet lysis. This level was more than twice the concentration of LPC required to induce complete inhibition of platelet aggregation. The effect of increasing concentrations of LPC on rabbit platelets in PRP is shown in Table I.

In plasma, concentrations of LPC almost sixfold higher were required to inhibit thrombin-induced aggregation, to affect platelet shape, and to induce loss of LDH, as compared to those concentrations exerting a similar effect on washed rabbit platelets resuspended in Tyrode’s solution containing 0.35\(^\circ\) bovine serum albumin (BSA). However, in PRP, too, inhibition of aggregation occurred at LPC concentrations which had no effect on platelet shape or loss of LDH.

Since the Tyrode medium used in the preparation of platelet suspensions contained appreciably less protein than plasma, the effect of increasing the concentrations of BSA in the suspending fluid on inhibition or potentiation by LPC of thrombin-induced aggregation of washed rabbit platelets was examined. Significantly higher concentrations of LPC were required to cause 50\(^\circ\) inhibition of thrombin-induced platelet aggregation in suspensions containing 2\(^\circ\) (170 \( \mu M \)) or 4\(^\circ\) BSA (180 \( \mu M \)) as opposed to a suspension containing 0.35\(^\circ\) BSA (35 \( \mu M \)). Furthermore when LPC was added to suspensions of washed rabbit platelets containing 2\(^\circ\) or 4\(^\circ\) BSA, reversal of inhibition of thrombin-induced aggregation was slower and potentiation was not observed.

When a suspension of washed platelets was incubated at 37\(^\circ\)C with \(^{14}\)C-LPC (final concentration 50 \( \mu M \)) and aliquots of the total incubation mixture were removed at various time intervals for lipid analysis, evidence for rapid metabolism of LPC by the platelets was obtained (Fig. 7). As shown in Fig. 7A, radioactivity disappeared from the lipid phase and appeared in the aqueous phase of the extracts. Paper chromatography of material concentrated from the aqueous phases revealed only a single spot when the paper was stained for phosphorus. This spot, which contained practically all of the radioactivity upon autoradiography (a small amount remained at the origin), was found at a distance from the point of application identical to that obtained using a pure GPC-reference compound. Figure 7A also shows that in the same experiment thrombin-induced platelet aggregation was initially almost completely inhibited and that this inhibition rapidly disappeared, followed by transient marked potentiation of aggregation. When the platelets were isolated from aliquots of the suspension taken at the same time intervals, washed twice, and then extracted, subsequent phospholipid analysis showed that labeling of LPC rapidly decreased, while that of PC proportionately increased (Fig. 7B). Less than 10\(^\circ\) of the total phospholipid radioactivity extracted from the platelets was found in sphingomyelin and (to a lesser extent) in phosphatidyl serine at all time intervals.

The effect of GPC or PC (two of the products of LPC metabolism by platelets) added to suspensions of washed rabbit platelets was examined. Neither GPC (up to a concentration of 500 \( \mu M \)) nor PC (up to a concentration of 200
Fig. 7. Effect of LPC on thrombin-induced aggregation of washed rabbit platelets in relation to its metabolism by platelets. A suspension of washed rabbit platelets (10⁶/cu mm) was incubated at 37°C with either saline (control) or ¹⁴C-LPC as described in detail in Materials and Methods. Thrombin-induced platelet aggregation was tested at various time intervals. At the same time intervals two 1.6-ml aliquots of suspension were removed from the incubation mixtures and phospholipid analysis was carried out on the total suspension and on platelets after repeated washings. (A) Percentage distribution of total radioactivity (RA) (217,129 cpm/ml suspension) in the aqueous (—o—) or lipid (—e—) phase. The open bars indicate the response of the platelets incubated with ¹⁴C-LPC to thrombin (0.03 U/ml) as a percentage of that observed in the control suspension to which saline has been added. (B) Percentage distribution of total platelet phospholipid radioactivity in LPC (—A—) and PC (—B—). Platelet-associated phospholipid radioactivity decreased from 988 cpm/platelet/ml suspension at 6 min to 660 cpm/platelet/ml suspension at 60 min with a corresponding increase in the platelet phospholipid radioactivity (expressed as a percentage of remaining phospholipid radioactivity in the total suspension) from 7.4% to 12.0%, respectively.

μM) inhibited or enhanced platelet aggregation induced by thrombin (0.03–0.05 U/ml) in this system.

DISCUSSION

The data obtained in this study confirm the observations previously reported by Besterman and Gillett⁴ that purified LPC added to human citrated PRP (to yield concentrations which may be found in human plasma) inhibited platelet aggregation induced by ADP, epinephrine, collagen, or thrombin. LPC also inhibited ADP-, collagen-, or thrombin-induced aggregation in rabbit PRP. Furthermore, LPC, at appreciably lower concentrations than those required for inhibition of platelet aggregation in PRP, inhibited ADP-, collagen-, or thrombin-induced aggregation of washed rabbit, pig, or human platelets suspended in an artificial medium. ADP-induced aggregation in rabbit PRP and in the suspensions of washed rabbit or human platelets used in this study was not associated with secondary wave aggregation or release from platelets of granule contents.²⁵ Our data therefore do not support the concept⁴ that the inhibition by LPC of platelet aggregation is mediated through the common mechanism of inhibition of the platelet release reaction. Thus LPC inhibited both the release reaction and the primary phase of ADP-induced aggregation that was not associated with the release reaction.

Inhibition by LPC of aggregation in PRP was partially reversible depending on the concentration of LPC used. In contrast, inhibition by LPC of ADP-, collagen-, or thrombin-induced aggregation in suspensions of washed platelets in Tyrode solution containing 0.35% albumin was not only completely reversible, but was followed by a transient period of potentiation, the most pronounced potentiation being observed when thrombin was used as stimulus. LPC also caused inhibition and subsequent potentiation of thrombin-induced re-
lease of serotonin from washed rabbit, pig, or human platelets in a suspending medium containing 0.35% albumin.

The inhibitory effect of LPC was also observed when LPC was added to the plasma or suspending medium containing albumin before the addition of the platelets. The finding that higher concentrations of LPC were required under these conditions probably reflected the known affinity of LPC for albumin and may indicate that LPC bound to albumin (or, perhaps, other plasma binding sites) does not affect platelets.

Since LPC is a detergent, and thus can damage cell membranes, inhibition (and possibly also potentiation) of platelet aggregation or release could at least in part be due to direct platelet damage. In none of the studies previously reported was this possibility examined. In this study the effects of LPC on platelet function were observed at concentrations which were below those at which LPC-induced platelet shape change or platelet lysis could be demonstrated. However, there is experimental evidence indicating that the incorporation of LPC into a membrane bilayer may produce a transition from a bimolecular leaflet to a localized micellar organization of the membrane lipids or lipoproteins. Thus LPC might exert all of the observed effects or at least its initial inhibitory effect on platelet function by membrane perturbation (structural alteration of the platelet membrane) not sufficient to induce gross leakage of cytoplasmic constituents. There is evidence that this inhibitory effect on platelet function may be dependent on the presence of a high proportion of saturated fatty acids in LPC.

Preliminary evidence presented here seems to indicate that a more specific mechanism may be involved in the effect of LPC on platelet function. Elsbach and associates have demonstrated that human platelets are capable of metabolic conversion of LPC to PC and GPC via two pathways: (1) acylation through the Lands pathway as previously described by Cohen et al., to yield PC; and (2) deacylation to yield GPC and free fatty acids. Our findings with washed rabbit platelets indicate that LPC is rapidly metabolized by platelets in concentrations which, in our experiments, inhibited and subsequently potentiated ADP-, collagen-, or thrombin-induced aggregation of these platelets. Thus it seemed reasonable to suspect that the metabolic products of LPC conversion might be implicated in the transient potentiation of platelet function. In our experiments neither PC nor GPC have had an appreciable effect on aggregation of washed rabbit platelets induced by thrombin. However, both induction of platelet aggregation and the platelet release reaction and potentiation of platelet aggregation induced by ADP, collagen, or thrombin by long-chain, saturated fatty acids have been reported by a number of investigators. Our findings and those of Elsbach et al. indicate that a substantial part of LPC is metabolized by platelets through hydrolysis to GPC and fatty acid. The LPC used in our experiments contained, according to the analysis of the supplier, 74% palmitic acid, 22.6% stearic acid, and 1.8% oleic acid. In the study reported by Haslam the lowest concentration of either palmitic acid or stearic acid necessary to induce aggregation of washed platelets was 20–30 μM. Much lower concentrations seem to be sufficient to potentiate platelet aggregation induced by ADP, collagen, or thrombin. If only half of the added LPC, effective in
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inhibiting and potentiating platelet aggregation in the platelet suspensions in our experiments, were rapidly converted to GPC and free fatty acid, the platelets would be exposed to a concentration of long-chain, saturated fatty acids sufficient to cause potentiation of platelet function, particularly in a medium containing a low concentration of albumin. Thus, one could speculate that fatty acids liberated during LPC hydrolysis by platelets might be responsible for the potentiation of platelet function observed in our experiments. However, we have no direct evidence to support this theory. Uptake and metabolic conversion of LPC to PC and GPC plus fatty acids by platelets appears to be an important mechanism in the interaction between plasma lipoproteins and platelets. The findings presented in this paper seem to indicate that this mechanism may also be important in the modification of platelet function in response to changes in the plasma phospholipid composition and/or alterations in the plasma fatty acid-binding capacity.

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