Platelet-Activating Factor and the Release of a Platelet-Derived Coronary Artery Vasodilator Substance in the Canine

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SUMMARY. Platelet-activating factor (acetyl-glyceryl-ether-phosphorylcholine; 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphorylcholine), which is released by stimulated neutrophils and platelets, possesses the ability to alter vascular tone and permeability and to activate various formed blood elements. We have characterized the hemodynamic effects of intracoronary injections of platelet-activating factor and the influences of pharmacological blockade and platelet depletion on its activity. Intracoronary injections of platelet-activating factor produced maximum increases in left circumflex coronary artery blood flow of 55 ± 8, 52 ± 8, and 52 ± 7 ml/min at 0.5, 1.0, and 2.0 nM, respectively. Only modest changes in systemic arterial blood pressure and regional developed isometric contractile force were associated with the intracoronary artery administration of platelet-activating factor over the range of doses studied. The increase in left circumflex coronary artery blood flow in response to platelet-activating factor was attenuated (44%), but not prevented, by pretreatment with diphenhydramine, (4 mg/kg, iv), and was not affected by pretreatment with aspirin (20 mg/kg, iv) or the systemic administration of the serotonin receptor antagonist, methysergide. The coronary vasodilator response to platelet-activating factor was reduced significantly by the induction of thrombocytopenia (95 ± 3% platelet depletion) through the administration of sheep-derived canine platelet antiserum. The intracoronary artery injection of platelet-rich plasma activated with platelet-activating factor into thrombocytopenic dogs produced a significantly greater increase in coronary artery blood flow than the injection of either non-activated platelet-rich plasma or platelet-depleted plasma to which platelet-activating factor was added. Similar changes in coronary artery blood flow could be obtained with the intracoronary artery injection of cell-free supernates from washed platelets activated with platelet-activating factor. The observed results suggest that circulating platelets, when exposed to platelet-activating factor, can release a coronary dilator substance, and that the coronary artery dilation is not prevented by pharmacological receptor antagonists for histamine, serotonin, or inhibitors of cyclooxygenase. (Circ Res 58: 218-229, 1986)
tabolism, we examined the coronary vasomotor responses to AGEPC after the administration of selective pharmacological inhibitors for histamine, serotonin (5-HT), and cyclooxygenase. Studies employing washed platelets have aided in the further characterization of the AGEPC-induced coronary vasodilator response.

The results of these studies demonstrate that AGEPC is capable of eliciting a coronary artery vasodilator response through the release of a platelet-derived factor which, at present, remains unidentified.

Methods

Surgical Preparation and Instrumentation

Male, mongrel dogs (16-22 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv) and ventilated with positive pressure (Harvard respirator) using room air. Tidal volume and respiratory rate were adjusted to maintain blood PO2, Pco2, and pH within normal limits (Instrumentation Laboratories Blood Gas Analyzer, Micro 13).

A left thoracotomy was performed at the 5th intercostal space, and the heart was suspended in a pericardial cradle. A segment (1-2 cm) of the left circumflex (LCX) coronary artery was isolated proximal to its first descending branch. A precalibrated electromagnetic flow probe (Carolina Medical Electronics) was affixed to the LCX artery for continuous measurement of circumflex coronary artery blood flow (CBF). The baseline for zero blood flow was determined periodically during the experimental procedure by transient (5-second) occlusions of the LCX coronary artery with a Silastic ligature placed distal to the flow probe.

An intracoronary catheter was formed from a 27-gauge needle, tip bent into a “U” configuration and attached to Tygon tubing (0.03 inch, i.d.). The needle was inserted through the vessel wall of the LCX at a distance 2-2.5 mm distal to the electromagnetic flow probe. Patency of the intracoronary catheter was maintained during the course of the study by infusing 0.9% sodium chloride solution at a rate of 3.0 ml/hr (Fig. 1).

Polyethylene catheters (PE-160) were placed in the carotid artery and jugular veins for the measurement of arterial blood pressure and the intravenous administration of drugs, respectively. In some of the experimental protocols, calibrated isometric strain gauge arches (Walton-Brodie) were sewn to the left ventricle in the distribution of the LCX coronary artery with sutures that penetrated to the subendocardial (depth of 7-9 mm) region. The gauges were placed mid-way between the apex and the base of the heart at a 45° angle just lateral to the first diagonal branch of the LCX coronary artery. The gauges were stretched so that the underlying segment was contracting isometrically and the developed tension was at the peak of the length-tension curve. Continuous recordings of regional isometric force development, mean and pulsatile LCX coronary artery blood flow, mean arterial blood pressure, heart rate, and the Lead II electrocardiogram were made on a Grass model 7 polygraph. Upon completion of the surgical preparation and instrumentation of the animals, 30 minutes were allowed for equilibration of all hemodynamic parameters before initiation of the study.

Preparation of Platelet-rich and Platelet-poor Plasma

Venous blood (9.0 ml) was drawn into 3.8% sodium citrate solution (1.0 ml) and was centrifuged at 550 g for 5 minutes to obtain platelet-rich plasma, and again at 1325 g for 10 minutes to yield platelet-poor plasma (PPP). In all the studies to be described, the initial circulating platelet counts ranged from 200,000-400,000/ml of whole blood. The administration of sheep-derived canine platelet antisera resulted in a 95 ± 3% reduction in the circulating platelet count. Whole blood platelet counts were made before and 30 minutes after the administration of antisera.

Preparation of Platelet-rich Plasma

Antiserum to canine platelets was prepared by isolating platelets from platelet-rich plasma (PRP) by gel filtration chromatography utilizing sepharose 2B equilibrated with a calcium-free, 100 mM phosphate buffer (pH 7.0). Sheep were inoculated by intradermal injection of 5 X 10⁸ canine platelets in incomplete Freund’s adjuvant, and after 25 days, the animals were bled and the prepared sera were pooled and heat inactivated. Nonimmune serum was prepared by bleeding nonimmunized sheep, and the serum was processed as described above. In the studies to be described, the initial circulating platelet counts ranged from 200,000-400,000/ml of whole blood. The administration of sheep-derived canine platelet antisera resulted in a 95 ± 3% reduction in the circulating platelet count. Whole blood platelet counts were made before and 30 minutes after the administration of antisera.

Preparation of Solutions of Platelet-activating Factor (AGEPC)

Platelet-activating factor, 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphorylcholine (AGEPC; Bachem) was dissolved in chloroform and dried under a stream of nitrogen gas. AGEPC was then dissolved in 0.25% bovine albumin-saline solution. The AGEPC solutions for intracoronary injection were prepared just before use. The concentration of AGEPC was adjusted so that the injected volume was always 0.2 ml. 1-O-hexadecyl-2-O-lyso-sn-glycero-3-phosphorylcholine (lyso-GEPC, Bachem), the
Preparation of Canine Washed Platelets

PRP was obtained as described above, the preparation was centrifuged (550 g for 7 minutes), and the supernate was removed. The platelets were resuspended in calcium-free Tyrode's solution containing ethylenediaminetetra-acetic acid (0.3 mm) and bovine serum albumin (0.35%) at a pH of 6.5. The cells were agitated gently for 2 minutes, and the centrifugation and wash procedures were repeated twice more. The final washed-platelet pellet was resuspended in calcium-free Tyrode's solution, pH 7.35. Tyrode's solution served as a blank for the control experiments. The washed-platelet preparations were diluted with Tyrode's buffer so as to adjust the platelet count to 250,000-350,000 cells/μl. Calcium chloride was added to the washed-platelet suspensions to a final concentration of 2 mM, followed by the addition of lyso-GEPC (2 nm), AGEPC (2 nm) and/or PPP, and the suspensions were allowed to incubate for 10 minutes, after which the preparations were centrifuged at 1325 g for 10 minutes to remove all cellular constituents.

Aliquots (0.2 ml) of the supernate from the activated, washed-platelet suspensions were administered by intracoronary injection to dogs that had been made thrombocytopenic (platelet antibody), and that had been pretreated with ASA (20 mg/kg) and diphenhydramine (4 mg/kg) before withdrawal of blood for the preparation of PRP. Tyrode's solution with and without the addition of the phospholipids and PPP served as controls.

Statistical Analysis

Differences between groups were determined by paired Student's t-test. Multiple group comparisons were made by a one-way or two-way analysis of variance followed by Student-Neuman-Keuls multiple range test. A value of P < 0.05 was considered as significant. All values in the text are given as mean ± SEM.

Results

Coronary Blood Flow Responses to Intracoronary Administration of AGEPC

A representative example of the LCX coronary artery blood flow response to AGEPC is presented in Figure 2. The observed increase in coronary blood flow occurred within 2-4 seconds after the intracoronary administration of AGEPC, and attained its peak value within 8-12 seconds. The mean control coronary blood flow was 39 ± 8 ml/min and increased to 94 ± 9 ml/min in response to 0.5 nm AGEPC. Similar increases in coronary blood flow were observed in response to 1.0 and 2.0 nm AGEPC (Table 1). Over the dose range of AGEPC studied, a delayed decrease in mean arterial blood pressure (12-22 mm Hg) and an insignificant change in regional isometric contractile force were observed (Table 1). Neither of these events was related temporally to the observed coronary artery blood flow responses (Fig. 2).

Coronary Artery Blood Flow Responses to AGEPC in the Presence of Thrombocytopenia

The administration of sheep-derived canine platelet antiserum (6.0 ml) resulted in a mean decrease

Physiologically inactive phospholipid, was prepared in an identical manner and was used to control for nonspecific phospholipid effects on the coronary circulation. All solutions were prepared in polystyrene vessels because of the tendency of the alkyl phosphoglycerides to adsorb to glass. The prepared stock solutions of AGEPC and lyso-GEPC were quantified by phosphorus analysis (Bartlett, 1959; Ames, 1966) and stored at ~20°C in phase chloroform. The prepared stock solutions contained 10.22 and 7.27 nmol/μl of AGEPC and lyso-GEPC, respectively. All subsequent solutions were prepared by appropriate dilutions of the assayed stock solutions each day immediately before use.

The phospholipid solutions were administered by intracoronary injection (0.2 ml) via the indwelling coronary catheter or were added directly to preparations of PRP (diluted to 200,000 platelets/μl), and to PPP, as well as to washed-platelet preparations. After the addition of AGEPC or lyso-GEPC to the PRP, PPP, or to the washed-platelet preparations, the samples were allowed to incubate for 10 minutes and then were centrifuged at 1325 g for 10 minutes to obtain a cell-free supernatant extract (supernate). In every case, control samples were prepared in a similar manner, but without the addition of the phospholipids. The former plasma supernates are referred to as "activated" and the latter as "nonactivated" plasma supernates. Preliminary studies in this laboratory have shown that PRP prepared from canine blood maintains its responsiveness to AGEPC for periods in excess of 2 hours (unpublished observations) when left at room temperature (25°C).

In some experiments, the dogs received acetylsalicylic acid (aspirin, ASA), 20 mg/kg, before the removal of blood for the preparation of PRP and PPP or the administration of platelet antiserum and the withdrawal of blood for the preparation of PPP.

In another group of experiments, preparations of PRP and PPP were activated with AGEPC or lyso-GEPC, as described above, centrifuged, and the cell-free supernates were treated with apyrase (12 U/ml of plasma; Sigma), an adenosine-5'-triphosphatase, and an adenosine-5'-di-phosphatase, for 30 minutes. Intracoronary injections of the cell-free supernates then were performed. Control injections were made with cell-free supernates from non-activated PRP and PPP which had been treated with apyrase. A second set of plasma samples were prepared in which the adenosine-5'-phosphates, ATP (10 μg) or ADP (10 μg) were added to 1 ml of PPP. Intracoronary injections of supernates from these samples were made to assess the vasodilatory potential of ATP and ADP. The supernates of another set of PPP samples containing ATP or ADP were treated with apyrase and incubated for 30 minutes; intracoronary injections of these samples were then performed.

Freeze-Fracture of Platelets

PRP and PPP fractions prepared from the blood of ASA-treated dogs (activated and nonactivated) were frozen rapidly in a liquid nitrogen and then were thawed abruptly in water at 40°C. The platelets subjected to this procedure underwent morphological disruption with lysis. The supernates from the freeze-thawed plasmas were prepared as described earlier and were administered by intracoronary injection in a volume of 0.2 ml to ASA pretreated, thrombocytopenic dogs.
in the circulating platelet count of 95 ± 3% (circulating platelet counts were between 200,000 and 400,000 platelets/mm$^3$ whole blood) without producing any immediate observable effects upon the recorded basal hemodynamic parameters. The control LCX coronary artery blood flow before the administration of platelet antiserum averaged 31 ± 2 ml/min and was 29 ± 3 ml/min ($n = 7$ dogs) 30 minutes after the induction of thrombocytopenia. The coronary artery vasomotor responses to the direct administration of AGEPC (0.5–2.0 nM) were reduced significantly in the presence of thrombocytopenia. Figure 3 shows the coronary blood flow response to the intracoronary administration of AGEPC before and after the induction of thrombocytopenia by platelet antiserum. The LCX coronary artery flow response to AGEPC (0.5–2.0 nM) was reduced significantly in the presence of thrombocytopenia. Figure 3 shows the coronary blood flow response to the intracoronary administration of AGEPC before and after the induction of thrombocytopenia by platelet antiserum. The LCX coronary artery flow response to AGEPC was reduced significantly (77%) in response to each of the doses of AGEPC (Fig. 3). Methacholine (0.2 μg)-induced increases in LCX coronary artery blood flow (27 ± 2 ml/min) were not altered by the induction of thrombocytopenia (26 ± 3 ml/min). Furthermore, the administration of non-immune sheep serum (6 ml) to each of four dogs did not change the circulating platelet count and did not alter the vasodilator response to the intracoronary administration of AGEPC. These data suggest that a vasodilator substance is derived from platelets upon activation by AGEPC.

Effects of Selected Pharmacological Antagonists upon the Platelet-dependent Coronary Vasodilator Response to AGEPC

In an attempt to elucidate the nature of the vasodilator substance derived from AGEPC-stimulated platelets in our study, we used selective pharmacological receptor antagonists in an effort to prevent the coronary vasodilator response produced by the intracoronary injection of AGEPC. In all instances, methacholine and/or nitroglycerin were included in the experimental protocols to test for the maintenance of vascular reactivity throughout the course of each experiment.

Reduction in the AGEPC-induced Coronary Vasodilator Response by Histamine $H_1$-Receptor Blockade

The histamine H$_1$-receptor antagonist, diphenhydramine (4.0 mg/kg), reduced, but did not prevent, the coronary vasodilator response to 2.0 nM AGEPC administered into the LCX coronary artery. Diphenhydramine reduced (44%) the maximum increase in flow in response to AGEPC from 36 ± 6 ml/min ($n = 10$) to 20 ± 3 ml/min (Fig. 4). Intracoronary administration of histamine (0.2 μg) produced the same increase in coronary blood flow (33 ± 5 ml/min, $n = 10$) as did AGEPC; however, H$_1$-receptor antagonism produced a greater reduction (76%) of the histamine-induced response (8 ± 2 ml/min in

<table>
<thead>
<tr>
<th>AGEPC (nM)</th>
<th>CBF (ml/min)</th>
<th>MAP (mm Hg)</th>
<th>Isometric force (g)</th>
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<tbody>
<tr>
<td>Basal</td>
<td>Peak</td>
<td>Basal</td>
<td>Peak</td>
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<tr>
<td>0.5</td>
<td>41 ± 8</td>
<td>94 ± 9†</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>1.0</td>
<td>39 ± 8</td>
<td>90 ± 8†</td>
<td>105 ± 7</td>
</tr>
<tr>
<td>2.0</td>
<td>36 ± 8</td>
<td>91 ± 11†</td>
<td>100 ± 4</td>
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* Each value represents the mean ± SEM of five experiments. CBF = coronary blood flow; MAP = mean arterial pressure. Statistical significance is denoted by † $P < 0.05$ and ‡ $P < 0.01$ when comparing the peak effect with the basal values.
the presence of diphenhydramine). The addition of the H₂-receptor antagonist, cimetidine (8 mg/kg, iv), did not modify the AGEPC-induced coronary vasodilator response beyond that already obtained in the presence of the H₁-antagonist alone. Figure 4 also illustrates that 4 mg/kg diphenhydramine significantly inhibited the vasodilator response to exogenously administered serotonin (5-HT, 1.0 μg). The intracoronary injection of 5-HT elicited a peak increase in coronary blood flow of 28 ± 7 ml/min (n = 3); which was inhibited by 75% (7 ± 3 ml/min) in the presence of diphenhydramine. These data suggest that a major portion of the AGEPC-induced vasodilator response is related to a vasoactive substance other than histamine on 5-HT.

**Ineffectiveness of the Serotonin (5-HT₁) Antagonist, Methysergide, to Alter the AGEPC-induced Coronary Vasodilator Response**

Platelets are known to release 5-HT upon activation; therefore, the contribution of this autacoid to the AGEPC-induced coronary vasodilator response was assessed in a group of five dogs. Baseline coronary blood flow in these experiments was 33 ± 6 ml/min. The intracoronary injection of 5-HT (2.0 μg) and AGEPC (2.0 nm) produced equivalent increases in coronary blood flow (37 ± 10 and 34 ± 5 ml/min, respectively). In the presence of the 5-HT₁/5-HT₂ antagonist, methysergide (1.0 mg/kg, iv), 2 μg of 5-HT produced only an 8 ± 2 ml/min increase in blood flow, whereas 2 nm of AGEPC produced a 22 ± 5 ml/min increase in blood flow. These results tend to rule out 5-HT as the mediator of the AGEPC-induced coronary vasodilation.

**Effect of Acetylsalicylic Acid (ASA) upon the AGEPC-induced Coronary Vasodilator Response**

To determine whether the coronary vasodilator response to AGEPC was mediated by way of cyclooxygenase-derived products of arachidonic acid, we studied dogs before and after the systemic administration of ASA (20 mg/kg, iv). The coronary vasodilator response to the intracoronary administration of AGEPC (2.0 nm) was not affected by pretreatment with ASA. The maximum increase in coronary flow in response to AGEPC before and after ASA is shown in Figure 5.

Assessment of ex vivo platelet responsiveness to arachidonic acid before and after the administration of ASA confirmed the presence of cyclooxygenase inhibition. Table 2 demonstrates that both arachidonic acid and collagen failed to aggregate platelets when added to PRP prepared after the administration of ASA to the dog. AGEPC (0.30 μM), however, was still able to aggregate platelets after administration of ASA.

**The Coronary Vascular Responses to Intracoronary Injections of Plasma Supernates Given to the Thrombocytopenic Dog**

The previous data suggest that a platelet-derived vasodilator substance is released in response to the intracoronary administration of the acetylated phosphoglyceride. In the subsequent series of experiments, dogs were pretreated with ASA, 20 mg/kg,
given intravenously 30 minutes before initiation of the studies to be described. The purpose of ASA in this series of experiments was to eliminate the potential confounding problems that could arise during the course of platelet isolation and exposure to the phosphoglycerides.

Either lyso-GEPC (1.0 nM) or AGEPC (1.0 nM) was added to PRP and PPP prepared from blood withdrawn from animals pretreated with ASA. The respective plasma samples were allowed to incubate at 37°C for 10 minutes and then were centrifuged to remove all cellular elements. Before the intracoronary administration of the plasma supernates, the dogs were depleted of their circulating platelets by the systemic administration of 6.0 ml of sheep-derived canine platelet antiserum. The plasma supernates were injected (0.2 ml) directly into the coronary circulation of the ASA-pretreated, thrombocytopenic dog. Figure 6 shows a representative recording from one of the animals in this series. The plasma supernate obtained from a PRP fraction exposed to 1.0 nM lyso-GPEC did not elicit a coronary vasodilator response when injected into the LCX. This was in contrast to the response obtained from the injection of supernate prepared from PRP which had been exposed to 1.0 nM AGEPC. Injection of supernates obtained from PPP exposed to 1.0 nM AGEPC and to PRP not exposed to AGEPC failed to produce any significant increase in coronary blood flow (Fig. 6). However, PRP exposed to 1.0 nM AGEPC resulted in the formation of a coronary vasoactive substance that produced an increase in LCX coronary blood flow of 37 ± 4 ml/min. The control LCX coronary artery blood flow in this series of experiments averaged 25 ± 2 ml/min.

To determine whether or not the adenosine-5'-nucleotides, ATP and ADP, were involved in the AGEPC-induced vasodilator response, we conducted a series of experiments to compare the effects of apyrase on supernate from AGEPC-activated PRP and PPP containing exogenous ADP or ATP. The data are summarized in Table 3. The addition of apyrase to the supernate from AGEPC-activated PRP did not significantly reduce the vasodilator response to the intracoronary injection of the AGEPC-activated supernate; however, the addition of apyrase to the samples of PPP containing ATP or ADP significantly reduced the vasodilator responses to the nucleotides. These data would indicate that neither ATP nor ADP is a major component in the AGEPC-induced vasodilator response.

The release of the platelet-derived coronary vasodilator substance also could be achieved by the addition of collagen (1:10 vol/vol dilution) to PRP, as evidenced by the resulting vasodilator response when the supernate was given into the LCX of the ASA-pretreated, thrombocytopenic dog (Fig. 7). The release of the platelet-derived coronary dilator substance in response to 50 μl of 1:10 collagen was equal to that produced by 1.0 nM AGEPC added to PRP, suggesting an all-or-none platelet release response to each of the platelet-activating agents.

Figure 8 shows that the plasma supernate obtained after freezing and thawing of the PRP was...
able to elicit a coronary vasodilator response which was not increased significantly upon the further addition of AGEPC (1.0 nM) to the supernate.

The Effect of AGEPC on Washed Platelets from ASA/Diphenhydramine-pretreated Dogs

Dogs were pretreated with ASA (20 mg/kg) and diphenhydramine (4 mg/kg) before withdrawal of venous blood for the preparation of washed-platelet suspensions. The animals then were rendered thrombocytopenic by the administration of sheep-derived canine platelet antiserum. Washed-platelet suspensions were incubated with AGEPC (2.0 nM) for 10 minutes and then were centrifuged to remove all cellular components. A 0.2-ml aliquot of the aqueous supernate from the washed platelets was administered into the LCX coronary artery of the thrombocytopenic dog pretreated with ASA and diphenhydramine. The AGEPC-activated washed-platelet supernate resulted in a 46 ± 5 ml/min increase in coronary artery blood flow, compared to a 12 ± 3 ml/min increase obtained with the unactivated washed-platelet supernate. The addition of PPP (0.5 ml) to the washed platelet suspension resulted in the production of a coronary vasodilator substance, the activity of which could not be enhanced further by the addition of AGEPC (2.0 nM). The activation of the vasodilator material by PPP could be prevented by the previous addition of the protease inhibitor, aprotinin (2.5 mg/ml), to the washed-platelet preparation. The intracoronary ad-

![Table 3](http://circres.ahajournals.org/)

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<tr>
<th>CFS from</th>
<th>Increase in coronary blood flow* (ml/min)</th>
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<tbody>
<tr>
<td>PRP + AGEPC (1.0 nM/ml)</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>PRP + AGEPC + apyrase (10 U/ml)</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>PPP + AGEPC + apyrase</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>PPP + ATP (2 μg)</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>PPP + ATP + apyrase</td>
<td>10 ± 3‡</td>
</tr>
<tr>
<td>PPP + ADP (2 μg)</td>
<td>48 ± 5‡</td>
</tr>
<tr>
<td>PPP + ADP + apyrase</td>
<td>9 ± 3‡</td>
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</tbody>
</table>

*All values represent the mean ± SEM of four experiments for the PRP + AGEPC and PPP + AGEPC + apyrase values and six experiments for the PPP + ATP or ADP values.

† Denotes a statistical difference at *P* < 0.001 when the apyrase-treated plasma samples are compared with those samples deficient in apyrase (Student’s paired t-test).

![Figure 6](http://circres.ahajournals.org/)

**Figure 6.** Represents the coronary vasodilator responses to the intracoronary injection of supernates prepared from AGEPC and lyso-GEPC-activated platelet-rich plasma (PRP) and platelet-poor plasma (PPP). Each histogram represents the mean ± SEM. The typical recording (taken from one dog in this series of experiments) illustrates the inability of the biologically inactive phosphoglyceride, lyso-GEPC, to activate platelets and result in a vasodilator response.

![Figure 7](http://circres.ahajournals.org/)

**Figure 7.** Represents the effects of the intracoronary injection of supernates prepared from platelet-rich plasma (PRP) and platelet-poor plasma (PPP) activated with collagen or collagen plus AGEPC. Each histogram represents the mean ± SEM. *Denotes a significant (*P* < 0.01) increase in coronary blood flow, compared to activated PPP or nonactivated PRP.
ministration to the thrombocytopenic dog of a 0.2-ml aliquot consisting of Tyrode's solution (used for the washing of platelets) to which had been added AGEPC and PPP in the same proportions as above, failed to elicit a significant coronary vasodilator response. The results are summarized in Table 4.

**Discussion**

The predominant effect associated with the intracoronary administration of AGEPC as observed in these studies was one of coronary artery vasodilation. The coronary vascular response was not related temporally to the relatively small negative inotropic response observed in the region of distribution of the LCX coronary artery. A positive inotropic response to AGEPC has been noted in rat atrial preparations (Kamitani et al., 1984), whereas Levi et al. (1984) have reported that AGEPC administered to the guinea pig-perfused heart resulted in a marked and sustained negative inotropic response. The latter results obtained in the guinea pig have led to the suggestion that AGEPC may be a mediator of anaphylactic shock (Stimler and O'Flaherty, 1983; Levi et al., 1984), whereas, in the dog, the intravenous injection of AGEPC in doses of 5-20 μg/kg causes a shock-like reaction accompanied by a decrease in plasma volume and a depression in cardiac function (Bessin et al., 1983). The studies mentioned have employed systemic doses of AGEPC that were at least 10-fold greater than those used in the present investigation.

Our studies have shown that the intracoronary injection of AGEPC up to a dose of 2 nm is accompanied by a coronary vasodilator response. We did note, however, that intracoronary doses above 2 nm of AGEPC were, on occasion, associated with a secondary decrease in LCX coronary blood flow (data not shown), suggesting a biphasic response to AGEPC similar to that noted by Feuerstein et al. (1984), who suggested that the phosphoglyceride-induced release reaction from aggregating platelets might play a role in modulating coronary blood flow, especially with respect to the potential coronary artery vasoconstrictor effects of platelet-derived thromboxane B₂ (TXA₂).

Feuerstein et al. (1984) noted that, in the pig, a decrease in coronary artery blood flow in response to AGEPC was prevented by the inhibition of cyclooxygenase. In the present studies, the dose of ASA (20 mg/kg) used was sufficient to prevent the ex vivo-induced platelet aggregation in response to arachidonic acid and collagen. On the other hand, AGEPC still was able to aggregate platelets in the presence of cyclooxygenase inhibition. Pretreatment of the dogs with ASA did not affect either the ex vivo platelet reactivity or the in vivo coronary vaso-

**Table 4**

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<tr>
<th>Intra-coronary Injections of Cell-free Supernates (CFS) from AGEPC-Activated Washed Platelets</th>
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<tbody>
<tr>
<td>CFS from</td>
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<tr>
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</tr>
<tr>
<td>Washed platelets (WP)</td>
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<tr>
<td>WP + AGEPC (2.0 nm)</td>
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<td>WP + AGEPC + aprotinin</td>
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<td>WP + PPP</td>
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<td>WP + PPP + aprotinin</td>
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<tr>
<td>Tyrode + AGEPC + PPP</td>
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\( ^{\dagger} \) All values represent the mean ± sem of four experiments.
\( ^{\ddagger} \) Denotes a statistical difference at \( P < 0.01 \), compared to washed platelets (control).
\( ^{\ddagger} \) Denotes a statistical difference at \( P < 0.01 \), compared to WP + PPP.
dilator response to AGEPC. This dose of ASA, however, has been shown to be ineffective in the inhibition of blood vessel production of prostacyclin (PGI$_2$) (Shaikh et al., 1982). Therefore, the possibility that endothelial cell-derived prostaglandins contribute to the platelet-mediated AGEPC-induced coronary vasodilation cannot be excluded on the basis of the present results. A possible counterpoint is that the AGEPC-induced hypotension was not affected by platelet depletion or ASA (present study) or indomethacin (Feuerstein et al., 1982, 1984). Feuerstein et al. (1982) observed that AGEPC administration in the rat did not cause elevations in 6-keto-PGF$_{1alpha}$ (i.e., PGI$_2$) but did elevate TXA$_2$, which was inhibited by indomethacin. These results suggest that a product of arachidonic acid resulting via the cyclooxygenase pathway is not responsible for the vasodilator component of the AGEPC-induced coronary vascular response. It is possible that decrements in coronary artery blood flow, as observed by Feuerstein et al. (1984), represent interactions between the blood platelets and the vascular endothelium which can account for the cyclical reductions in flow observed in stenotic canine coronary arteries (Folts et al., 1982; Schumacher et al., 1984). These responses can be exacerbated by AGEPC (Appril et al., 1984), even in the presence of thromboxane synthetase inhibition.

Many of the coronary vascular effects of AGEPC have been attributed to the release of secondary mediators such as serotonin (5-HT) and TXA$_2$ (Vargaftig et al., 1982). The production of non-deleterious autacoids, such as an augmented PGI$_2$ synthesis by endothelial cells, may also occur upon exposure to the phosphoglyceride (already addressed). In the present experiments, the inhibition (44%) of the AGEPC-induced vasodilator response observed with the H$_2$-antagonist, diphenhydramine, when compared to the inhibition of histamine and 5-HT, would indicate that the release of histamine and 5-HT from platelets, and histamine from tissue stores and/or mast cells, might contribute to the AGEPC-induced relaxation of the coronary vasculature. The major portion, however, is due to some other platelet-derived vasodilator substance.

The AGEPC-induced release of platelet stores of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) could be implicated as possible mediators of the observed increase in coronary artery blood flow, since platelets are rich in ATP and ADP. In the present study, however, ATP and ADP do not appear to be contributing to the AGEPC-induced coronary vasodilation. Apyrase, an ATP-ADP phosphatase, did not inhibit the coronary dilator response when added to AGEPC-activated PRP (Table 3); however, apyrase (when added to PPP) did inhibit the coronary dilatory response to both ATP and ADP by 80%. Recently, Letts et al. (1985a, 1985b) have observed that leukotrienes C$_4$ and F$_4$ were able to elicit a platelet-dependent vasodilator effect in the femoral vascular bed of the pig. These investigators were unable to show a strong absorption maximum at 256 nm in activated-platelet supernates, suggesting that ADP was not present in quantities sufficient to produce the observed degree of vascular relaxation.

Taken together with the present results and those cited, it is unlikely that the coronary vasodilator response to the intracoronary administration of AGEPC or the response to injected plasma supernates from AGEPC-treated PRP could be attributed to platelet-derived ATP, ADP, histamine, or 5-HT. It is interesting to note that aggregating platelets have been reported to relax canine coronary arteries in vitro by an unidentified endothelial-dependent mediator (Cohen et al., 1983). Rat platelets are insensitive to AGEPC (Crespo et al., 1981), yet, the intravenous administration of the phosphoglyceride induces a hypotensive effect. The present investigation demonstrates that, despite the ability of induced thrombocytopenia to reduce or prevent the coronary vasodilation to the intracoronary administration of AGEPC, we continued to observe a peripheral vasodilator response. These results would indicate that the mechanism for smooth muscle relaxation in response to the phosphoglyceride may differ in the respective vascular beds.

A recent study by Sybertz et al. (1985) has shown that AGEPC given intraarterially into the femoral artery of the anesthetized dog resulted in a dose-related vasodilator response that was not altered by denervation or by pretreatment with theophylline, indomethacin, or BW-755C, implying that the vascular response was independent of purinergic P$_1$-receptors, cyclooxygenase products, or arachidonic acid metabolites derived from the lipoygenase pathway. On the other hand, these same investigators failed to observe a change in coronary vascular resistance when AGEPC was given directly into the coronary vascular bed via a cannulated branch of the left anterior descending coronary artery. Unlike our own experiments, the intracoronary injection of AGEPC was associated with a marked and rapid reduction of systemic arterial pressure, a negative inotropic response, a decrease in heart rate and coronary blood flow (Sybertz et al., 1985), all of which could have obscured the direct effects of the phosphoglyceride upon coronary artery vascular hemodynamics.

Some possible explanations for the differences in results obtained by the two groups of investigators might relate to the source of the alkyl-phosphoglyceride employed by each of the laboratories, the manner in which the dose was calculated, and the fact that we used bovine serum albumin as a carrier for the alkyl-phosphoglycerides. Of potential importance may be the fact that we employed the 1-O-hexadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine as supplied by Bachem, whereas other studies have used a mixture of the hexadecyl and octa-
decyl derivatives. The glyceryl backbone of naturally occurring AGEPC is substituted with C-18 (90%) and C-16 (10%) straight chain alkyl ethers at position 1, acetate at position 2, and phosphocholine at position 3 (Hanahan et al., 1980; Polonsky et al., 1980). The C-12 and C-18 alkyl ethers are respectively 10-fold and 4-fold less active than the C-16 alkyl ether (O’Flaherty and Wykle, 1983). In the present study, we calculated our doses of the alkyl-phosphoglycerides (AGEPC and lyso-GPEPC) on the basis of their phosphate content. The relatively small intracoronary doses of AGEPC employed by us, coupled with the rapid inactivation of the phosphoglyceride in the plasma by the action of acetyl hydrolase (Pincock et al., 1979), would limit our chances of observing a marked or sustained hemodynamic response as reported in other studies (Kenzora et al., 1984, Sybertz, 1985). In addition, we were able to demonstrate the specificity of the acetylated alkyl phosphoglyceride in producing the observed coronary vasodilation, in contrast to lyso-GEPC which was devoid of activity on the coronary vascular bed, as well as on PRP in vitro.

The present study has focused upon AGEPC-induced increases in coronary blood flow, and demonstrates that coronary artery vasodilation is secondary to the release of a preformed constituent contained in the blood platelet that can be released in response to AGEPC, as well as in response to collagen and physical disruption of the platelet by freeze-thawing. The induction of thrombocytopenia eliminated the coronary vasodilator response to the direct coronary administration of AGEPC. The observed coronary vascular response was not dependent upon the formation of distal platelet aggregates causing the transient obstruction of regional coronary flow and a subsequent hyperemic response. This was illustrated by the fact that cell-free supernates taken from PRP activated with AGEPC and given to the thrombocytopenic dog retained the ability to induce an identical coronary vasodilator response. Similar treatment of either PRP with lyso-GEPC or PPP treated with AGEPC failed to provide plasma supernates that possessed coronary vasodilator properties. The data illustrate the dependency upon the platelet for the release of a coronary vasodilator substance. It is interesting to note that the activated PRP supernates retained their coronary vasodilator activity even after standing at room temperature (25°C) for periods up to 2 hours, or after being heated at 50°C for 5 minutes. Vargaftig et al. (1980) reported that bronchoconstriction in response to AGEPC was platelet dependent, and could be suppressed by immune platelet depletion. On the other hand, the hypotensive effect of AGEPC was not inhibited by platelet depletion, suggesting a differential mechanism by which the phosphoglyceride affects different smooth muscles.

An important observation in this study that requires further examination is the finding that washed platelets release a factor into the aqueous wash medium that is converted to a coronary artery dilator substance upon the addition of PPP. Addition of PPP to the aqueous supernate of the AGEPC-activated washed platelets did not result in further augmentation of the coronary vasodilator response. Furthermore, the vasodilator substance appears to be contained within the platelet in an inactive form, and requires the presence of plasma for the full expression of its vasodilator activity, or can be activated and released by the direct action of AGEPC. The activation of the vasodilator material by plasma and the inhibition of its activation by aprotinin make it less likely that the vasodilator material is ATP, ADP, histamine, or 5-HT. These findings also call attention to the observations reported by Henson et al. (1976) who provided evidence which suggested the activation of a serine protease in association with the AGEPC-induced platelet secretion. It is suggested that the stimulus (AGEPC) induces a temporary decrease in intracellular cyclic adenosine monophosphate which permits the secretion to occur. An examination of the data by Henson et al. (1976) suggests that AGEPC activates a precursor serine protease which then initiates the secretory process within the platelet. The latter process is a programmed function of the cell, and can be initiated by other stimuli, including collagen and thrombin. AGEPC differs with respect to the uniqueness of its interaction with a specific cell surface receptor and its activation of an apparently specific serine protease (Henson et al., 1974, 1976). The data as presented must be interpreted with caution, since it is possible that the dilator factor released by the platelet in response to AGEPC may be different from the factor released by the addition of PPP to the washed-platelet suspension.

Although AGEPC is a known activator of polymorphonuclear leukocytes, it is unlikely that this cell is involved in the induced coronary artery dilator response, since the thrombocytopenic animals did not show an alteration in the circulating leukocyte count, and the PPP fractions were essentially free of contaminating cells that could account for the release of a vasodilator material. Furthermore, the administration of AGEPC to the thrombocytopenic dog was without an effect on coronary blood flow, despite the presence of circulating polymorphonuclear leukocytes.

The fact that platelets can release vasorelaxant substances in response to the phosphoglycerides should cause us to reevaluate the potential interaction of the blood platelet with the vascular wall and/or the vascular endothelium. At present, we do not know whether the platelet-mediated coronary vasodilator response to AGEPC is dependent upon the presence of a normal endothelium. If so, this could account for observed differences between the coronary artery vascular response to AGEPC, compared to the vasodilator response seen in the systemic...
vascular bed. It is of interest to note that a recent publication has described a protective role of the endothelium in curtailing the in vitro vasoconstrictor responses elicited by substances released from aggregating platelets (Cohen et al., 1983). The observation that platelets can participate in bringing about a relaxation of coronary arteries in the presence of a normal endothelium may have significance with respect to the pathophysiological mechanism(s) by which coronary vasospasm might be facilitated under conditions in which endothelial damage has occurred. The development of platelet aggregation at a site devoid of endothelium might favor local vasoconstriction due to the release of thromboxane A_2, or, if endothelial cells are in close proximity to the releasing platelets, it is possible that a platelet-mediated, endothelial-dependent vasodilator response would favor the removal of an obstructing thrombotic lesion by bringing about local vasodilation (Cohen et al., 1983). The intimate association of leukocytes with aggregating platelets makes it highly possible that an important physiological mechanism in the control of local coronary blood flow during times of vessel injury may reside in the ability of the leukocyte to release AGEPC which, in turn, not only modulates platelet function (aggregation), but also results in the release of a platelet-derived factor that participates in the regulation of coronary blood flow. The absence of AGEPC-induced coronary vasodilation in the thrombocytopenic animal, and the ability to replicate the response by the administration of supernatants from AGEPC-activated PRP and aqueous supernatants from AGEPC activated washed-platelets, demonstrate the obligatory role of the platelet in modulating coronary vascular dynamics in response to the phosphoglyceride. The release of the vasoactive substance from the platelet can be achieved by other activators of platelet function, and should call attention to the dynamic role of interactions between the formed elements of the blood and the vessel wall as potential physiological mechanisms in the control of regional myocardial blood flow.

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