

Human Platelet Stimulation by Acetyl Glycerol Ether Phosphorylcholine

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ABSTRACT Acetyl glycerol ether phosphorylcholine (AGEPC) induced dose-dependent platelet aggregation and release of [³H]serotonin and platelet factor 4 in citrated human platelet-rich plasma. ADP scavengers or indomethacin prevented irreversible platelet aggregation responses induced by 0.2 μM AGEPC but had no effect upon platelet secretion; prostacyclin inhibited AGEPC-induced aggregation and secretion. EDTA or EGTA inhibited AGEPC-induced aggregation but had no effect on platelet secretion.

INTRODUCTION

Platelet activating factor (PAF)¹ was identified nearly 10 yr ago (1), and since then, the role of PAF in mediating various acute allergic and inflammatory reactions has been receiving an ever increasing amount of experimental support (2, 3). Recently, direct structure-proof analyses of PAF released from antigen-stimulated immunoglobulin (Ig)E-sensitized rabbit basophils have demonstrated that PAF is an acetyl glycerol ether phosphorylcholine (AGEPC, 1-*O*-hexadecyl/octadecyl-2-acetyl-*sn*-glycerol-3-phosphorylcholine) (4). The demonstration of PAF synthesis by a variety of inflammatory cells (5–14) has prompted studies to elucidate its role as an inflammatory mediator in man with particular interest focusing on the stimulation of human platelets. However, widely diverse results have been reported

¹*Abbreviations used in this paper:* AGEPC, acetyl glycerol ether phosphorylcholine, 1-*O*-hexadecyl/octadecyl-2-acetyl-*sn*-glycerol-3-phosphorylcholine; CPCK, creatine phosphate/creatine kinase; lyso-GEPC, 1-*O*-hexadecyl/octadecyl-*sn*-glycerol-3-phosphorylcholine; PAF, platelet activating factor; PGI₂, prostacyclin; PRP, platelet-rich plasma.

for washed human platelets ranging from the inability of PAF to initiate human platelet aggregation and/or secretion to the ability of PAF to induce significant platelet secretion (10, 12, 15–18). In view of the preceding observations, we have undertaken studies to answer the question of whether or not chemically pure AGEPC, a synthetic PAF, can initiate human platelet aggregation and secretion.

METHODS

Animals. Randomly bred, male and female California rabbits were obtained from the Penn Acres Ranch, Wimberly, Tex.

AGEPC preparation. Synthesis and chemical characterization of AGEPC and lyso-GEPC (1-*O*-hexadecyl/octadecyl-*sn*-glycerol-3-phosphorylcholine) have been described (19). AGEPC or lyso-GEPC was dissolved in an albumin-saline solution (either bovine serum albumin, Miles Laboratories, Inc., Elkhart, Ill., bovine serum albumin-saline, or human serum albumin, Cutter Laboratories, Inc., Berkeley, Calif., human serum albumin-saline; 2.5 mg albumin/ml of pyrogen-free, 0.15 M sodium chloride).

Platelet-rich plasma (PRP) preparation. 9 vol of human blood, obtained by antecubital venipuncture from volunteers who had taken no drugs for at least 14 d, was mixed with 1 vol of 3.8% trisodium citrate. In some experiments, 9 vol of blood was mixed with 1 vol of either 0.1 M EDTA or 0.1 M EGTA, pH 7.2. Rabbit blood, obtained from the central ear artery using a 19-gauge needle, was processed as described above for human blood.

Anticoagulated blood was immediately centrifuged at room temperature to prepare human or rabbit PRP, 500 g for 10 min or 750 g for 15 min, respectively. The upper one-half of the PRP was removed (350,000–500,000 platelets/mm³) and to each 1 ml of PRP, 1 μCi of [³H]serotonin (New England Nuclear, Boston, Mass., 28.2 Ci/mmol) was added. This mixture was incubated at 37°C for 15 min and then used immediately.

Platelet aggregation. Aggregation studies were conducted in a single channel, platelet aggregometer (Chrono-Log Corp.,

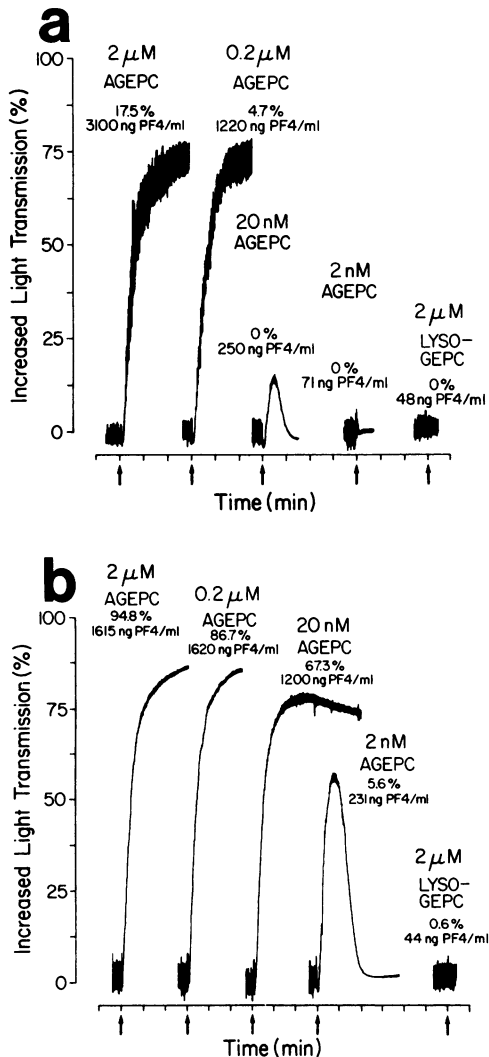


FIGURE 1 AGEPC-induced stimulation of citrated human (A) and rabbit (B) PRP. The numbers above the aggregation curves indicate the final molar concentration of AGEPC or lyso-GEPC added at the time indicated by the arrow, the percent [³H]serotonin released (corrected for nonspecific release after the addition of human serum albumin-saline or bovine serum albumin-saline to human or rabbit PRP, respectively), and the amount of PF4 secreted (ng/ml).

Haverton, Pa.) at 37°C. With constant stirring (900 rpm), aggregation profiles of PRP (500 μl) were monitored after the addition of various test reagents (10 μl). In some studies, prostacyclin (PGI₂) (supplied by Dr. John Pike, The UpJohn Co., Kalamazoo, Mich.) was added to PRP 30 s before stimulation (final concentration, 27 nM PGI₂). In separate experiments, indomethacin (Sigma Chemical Co., St. Louis, Mo.) was added to PRP 90 s before stimulation (final concentration, 10 μM indomethacin). In other studies, creatine phosphate (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and creatine kinase (type III, bovine heart, Sigma Chemical Co.) were added to PRP 30 s before stimulation (final concentration, 2 mM creatine phosphate and 5 U creatine kinase/ml; CPCK).

Platelet secretion. Each test reagent (8 μl) was added to PRP (400 μl) in polystyrene tubes at 37°C (PGI₂, indomethacin, or CPCK addition to PRP was as described above). 60 s later, 40 μl of cold, 3.8% trisodium citrate was added, the reaction tube chilled in an ice bath, and the platelets sedimented (2,500 g, 10 min, 4°C). The supernates were assessed both for platelet factor 4 (PF4) release [by radioimmunoassay (20, 21)] and the percentage of [³H]serotonin release relative to Triton X-100 controls. Monospecific rabbit anti-human PF4 and human PF4 standards were generously provided by Dr. S. P. Levine.

RESULTS

AGEPC reproducibly induced dose-dependent platelet aggregation and secretion in human PRP (Fig. 1A). Irreversible aggregation occurred at 0.2 μM AGEPC with reversible aggregation at lower concentrations. [³H]serotonin and PF4 secretion were maximal within 60 s after AGEPC stimulation. Lyso-GEPC (up to 20 μM) was inactive. AGEPC also induced platelet aggregation and secretion in rabbit PRP with irreversible aggregation at 20 nM AGEPC (Fig. 1B).

The AGEPC-induced dose-response aggregation patterns in human PRP from individual blood donors varied slightly. However, the initial aggregation pattern of PRP at a given AGEPC concentration always decreased with time after PRP preparation (Fig. 2). This transition was not due to a pH change since the PRP in sealed tubes maintained a relatively constant pH (7.45–7.55), nor was it retarded by the inclusion of indomethacin or ADP scavengers in the PRP. Biphasic aggregation patterns were always observed during the PRP transition but were not exclusively due to a progressive decrease in platelet responsiveness since

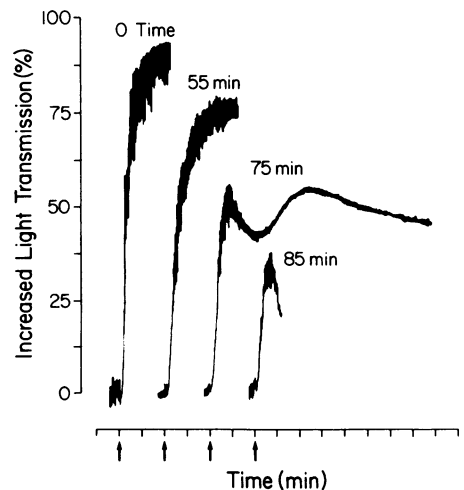


FIGURE 2 Human platelet responsiveness to AGEPC at various times after PRP preparation. The numbers above the aggregation curves indicate the time after citrated PRP preparation at which the aggregation assays were performed. The final molar concentration of AGEPC was 0.2 μM.

stimulation of freshly prepared PRP with appropriate concentrations of AGEPC (usually 100–10 nM) induced similar biphasic aggregation responses. In contrast to human PRP, rabbit PRP remained fully responsive to AGEPC stimulation up to 4 h after preparation and biphasic aggregation patterns were never observed.

The second phase of AGEPC-induced biphasic platelet aggregation was eliminated by the presence of indomethacin or CPCK. Additionally, in freshly prepared human PRP, indomethacin or CPCK prevented the irreversible aggregation response at 0.2 μ M AGEPC without affecting platelet secretion (Fig. 3); at higher AGEPC concentrations (2 μ M), indomethacin or CPCK had no effect on irreversible aggregation responses or on platelet secretion. PGI₂ inhibited both aggregation and secretion at all AGEPC concentrations. EDTA or EGTA prevented AGEPC-induced platelet aggregation in human and rabbit PRP with no effect on the platelet release reaction in human PRP but with reduction of secretion in rabbit PRP.

DISCUSSION

Several possible explanations could account for the previous discrepancies and often conflicting observations with respect to PAF-induced stimulation of human platelets (10, 12, 15–18). First, either crude or partially purified PAF with possible contaminants was used in contrast to the highly purified synthetic AGEPC employed here. Second, other investigators have employed washed human platelets prepared by different procedures and/or different conditions for effecting platelet stimulation. In contrast, we have used freshly prepared human PRP. The observation that human platelet responsiveness to AGEPC decreases with time after preparation (Fig. 2) may explain, in part, the variable findings previously reported with washed platelets prepared by time consuming washing procedures. Alternatively, the preparation of washed human platelets might have removed a possible contaminating cell, e.g., a leukocyte, required for AGEPC-induced human platelet stimulation. In any event, we have observed significant and reproducible dose-dependent AGEPC-induced human platelet aggregation and [³H]-serotonin and PF4 secretion in PRP. Secretion but not aggregation was independent of extracellular calcium, intrinsic ADP release or products derived from the cyclooxygenase pathway of arachidonate metabolism.

The characteristics of human PRP stimulation by AGEPC were similar to those observed in rabbit PRP, an important comparison since the platelet activating characteristics of AGEPC have been derived using washed rabbit platelets (19). Despite these similarities however, several differences were evident. First, with respect to aggregation, human platelets lost their responsiveness to AGEPC with time after preparation,

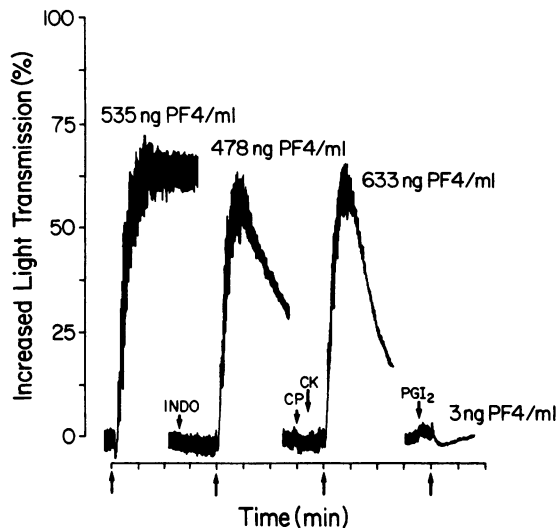


FIGURE 3 The effect of indomethacin (indo), CPCK, and PGI₂ on AGEPC-induced stimulation of citrated human PRP. The final molar concentration of AGEPC was 0.2 μ M.

whereas rabbit platelets were stable. Second, in PRP, human platelets were less sensitive to AGEPC stimulation than rabbit platelets. These differences could be explained either by the relative instability of human platelets or alternatively, human plasma might degrade or inactivate AGEPC more rapidly than rabbit plasma. Indeed, washed rabbit platelets (19) are approximately 100 times more sensitive to AGEPC stimulation than is rabbit PRP probably because of plasma inactivators (22).

ACKNOWLEDGMENTS

The expert technical assistance of Mari Hecht and Richard Castillo and the typing of this manuscript by Ms. Sophie Hurst are gratefully acknowledged.

This work was supported by U. S. Public Health Service grants HL-22555 and HL-07350 and the American Heart Association, Texas Affiliate.

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