Shape Change Induced in Human Platelets by Platelet-activating Factor

CORRELATION WITH THE FORMATION OF PHOSPHATIDIC ACID AND PHOSPHORYLATION OF A 40,000-DALTON PROTEIN*

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Washed human platelets that have been separated from plasma in the presence of prostacyclin are activated by the addition of platelet activating factor (PAF). Activation (shape change, serotonin release, and aggregation) correlates closely with the formation of phosphatidic acid and the phosphorylation of a 40,000-dalton protein.

Platelet shape change, formation of phosphatidic acid, and protein phosphorylation precede aggregation and are induced at lower concentrations of PAF than those required to induce release of serotonin and platelet aggregation. Platelet shape change, formation of phosphatidic acid, and protein phosphorylation induced by PAF are not affected by trifluoperazine or indomethacin. This indicates that these responses are independent of the liberation of arachidonic acid from platelet phospholipids and the metabolism of arachidonic acid via cyclooxygenase and lipoxygenase. These responses are, however, inhibited by prostanoyl.

Platelet shape change is the first measurable physiological response to platelet agonists and may be associated with the stimulation of phospholipase C, inducing formation of 1,2-diacylglycerol and its phosphorylated product, phosphatidic acid. Transient formation of 1,2-diacylglycerol may also induce the specific activation of the protein kinase C that phosphorylates a 40,000-dalton protein.

Recently, the inability of washed human platelets to respond to stimulation with PAF was reported (1, 2). Isolation of human platelets in the presence of prostacyclin (3) prevents activation of platelets during the separation procedure and preserves their discoid shape. Human platelets isolated in this way are responsive to PAF, allowing the study of the action of PAF on various human platelet reactions.

An early response that follows activation of platelets with stimuli such as thrombin (4-10), collagen (9), ADP (11), PAF (1, 12, 13), or ionophore A23187 (4-9) is the formation of phosphatidic acid (14). Phosphatidic acid is produced by the sequential actions of phosphatidylinositol-specific phospholipase C and 1,2-diacylglycerol-kinase (14). It has been suggested that phosphatidic acid could be an intracellular mediator of platelet activation as it is produced by a wide range of platelet stimuli and has the properties of a Ca2+ ionophore and a fusogen (1, 14). Alternatively, the formation of phosphatidic acid in activated platelets may be an epiphenomenon of the generation by phospholipase C of 1,2-diacylglycerol. 1,2-diacylglycerol has recently acquired significance in transmembrane signaling by virtue of its role as a specific activator of protein kinase C (15-17).

The present study indicates that PAF induces shape change in human platelets in parallel with the formation of phosphatidic acid and phosphorylation of a 40,000-dalton protein. These changes depend on phospholipase C activation, precede platelet aggregation and release of serotonin, and are independent of the liberation and metabolism of arachidonic acid.

MATERIALS AND METHODS

Isolation of Human Platelets, Labeling with 32P Orthophosphate, and Measurement of Phosphatidic Acid and Protein Phosphorylation—Materials were obtained as previously reported (1, 8, 18). Human platelets were isolated, labeled, and stimulated as recently described (18). For measurement of protein phosphorylation, aliquots (0.1 ml) were quenched by adding 0.025 ml of 5 times concentrated Laemmli sample buffer (19) and incubated at 100 °C for 10 min. Aliquots containing 5-50 μg of protein were separated by electrophoresis through a 7.5% sodium dodecyl sulfate-polyacrylamide gel using the Laemmli buffer (19). Gels were then stained with Coomassie brilliant blue, dried, and their radioactivity determined by radiography. Estimation of radioactivity of the 40,000-dalton protein was done by cutting out the specific areas of the gel which were placed in scintillation vials and heated for 2 h at 80 °C in 30% hydrogen peroxide. Then, scintillation fluid was added and radioactivity determined by liquid scintillation counting.

**Scanning Electron Microscopy**—Platelets were fixed in 0.1 M sodium phosphate buffer (pH 7.3) containing 3% glutaraldehyde. Samples were then filtered through a 0.2-μm nucleopore filter and washed with 20 ml of prefitered distilled water using a syringe pump (1.5 ml/min flow rate). Filters were dehydrated in a graded series of ethanol, critical point dried in a Polaron drier with liquid CO2 as the transition fluid, and coated with platinum in a Polaron sputter coater (Model E5100). Platelets were examined in a JEOL JSM-35 scanning electron microscope at an accelerating voltage of 15 kV.

RESULTS

Platelet activating Factor Induces Shape Change in Washed Human Platelets—Platelet activating factor induces shape change in human platelets over a concentration range of 1 nM to 1 μM (Fig. 1). Scanning electron microscopy confirmed that the observed decrease in light transmission was due to platelet shape change.

**Fig. 2** shows the changes in platelet shape induced during a 30-s incubation of human platelets with 0.1 μM PAF. Platelets change from smooth, discoid-shaped cells to spiny spheres with protrusion of pseudopods. The release of serotonin from platelets is observed at higher concentrations of PAF than those needed for induction of shape change, occurring only at PAF concentrations over 0.1 μM.

PAF-induced Shape Change in Human Platelets Is Associ...
ated with the Formation of Phosphatidic Acid and Is Independent of the Metabolism of Arachidonic Acid—PAF-induced platelet shape change and the accompanying formation of phosphatidic acid show a similar sensitivity to inhibitors (Fig. 3). Neither response is inhibited by 50 μM trifluoperazine (a calmodulin-antagonist which inhibits phospholipases A₂, Refs. 1 and 10) nor 10 μM indomethacin (an inhibitor of cyclooxygenase). Scanning electron microscopy examination also indicates that indomethacin does not prevent PAF-induced shape change (Fig. 2). On the other hand, prostacyclin (2–4 ng/ml) prevents both PAF-induced platelet shape change (Fig. 2) and the formation of phosphatidic acid (Fig. 3).

The PAF-induced formation of phosphatidic acid is also dependent on the concentration of PAF in the range 1 nM to 1 μM with a maximal effect at 0.1 μM (data not shown). At none of these PAF concentrations does trifluoperazine and indomethacin inhibit the PAF-induced formation of phosphatidic acid. However, the formation of phosphatidic acid is sensitive to prostacyclin at all PAF concentrations tested (data not shown).

PAF Induces Simultaneous Formation of Phosphatidic Acid

Fig. 1. Shape change of washed human platelets induced by different concentrations of platelet-activating factor (PAF). Washed human platelets (0.5-ml samples) were placed into aggregometer tubes and stimulated with indicated concentrations of PAF. Platelet shape change is shown by the initial decrease of light transmission and the disappearance of the oscillations produced by discoid platelets. Values in boxes indicate the release of [³H]serotonin after stimulation with the different concentrations of PAF for 30 s expressed as a percentage of total platelet [³H]serotonin content.

Fig. 2. Scanning electron micrographs of human platelets. Samples of washed human platelets were preincubated (while stirring in the aggregometer tubes) at 37 °C for 3 min without additions, or with 10 μM indomethacin, or with 2 ng/ml of prostacyclin before stimulation with 0.1 μM PAF for 30 s. A and B, no additions; C and D, PAF; E, indomethacin plus PAF; F, prostacyclin plus PAF. A, B, C, and E are from one experiment, and D and F from a different experiment.

and Phosphorylation of a 40,000-Dalton Protein during Platelet Shape Change—Stimulation of human platelets by PAF induces shape change with simultaneous formation of phosphatidic acid and phosphorylation of a 40,000-dalton protein (Fig. 4). Both phosphorylated products reach maximal formation at about 20 s (Fig. 4).

Effect of Different Concentrations of Prostacyclin on PAF-induced Shape Change, Formation of Phosphatidic Acid, and Phosphorylation of a 40,000-Dalton Protein—Prostacyclin (4 ng/ml) inhibits PAF-induced shape change and formation of phosphatidic acid (Fig. 3). We have further studied the effect of different concentrations of prostacyclin in an attempt to differentiate these various responses. Figs. 5 and 6 show one of three experiments that gave similar results and indicate that, within the limits of experimental error, the dependence of the various responses (shape change, formation of phosphatidic acid, and phosphorylation of a 40,000-dalton protein) on prostacyclin concentration is identical. This suggests that there is a common site of action for prostacyclin in eliciting each of these three responses.

Effect of Different Concentrations of Trifluoperazine on PAF-induced Shape Change, Formation of Phosphatidic Acid, and Phosphorylation of a 40,000-Dalton Protein—Concentra-
tions of trifluoperazine ranging from 10 to 100 μM do not affect the degree of shape change, formation of phosphatidic acid, and phosphorylation of a 40,000-dalton protein (data not shown). Similarly, indomethacin (1–10 μM) does not change the PAF-induced formation of phosphatidic acid and phosphorylation of the 40,000-dalton protein (data not shown).

**DISCUSSION**

Shape change, aggregation, and release of granule contents are physiological expressions of platelet activation. PAF stimulation of human platelets induces shape change which precedes the other two responses and can be studied separately.

Phosphatidic acid formation and phosphorylation of a 40,000-dalton protein occur parallel to the induction of platelet shape change by PAF. Formation of both these phosphorylated molecules and shape change are totally independent of the metabolism of arachidonic acid. Thus, trifluoperazine, which inhibits phospholipases of the A₂ type (1, 10), does not affect PAF-induced shape change, formation of phosphatidic acid, or phosphorylation of a 40,000-dalton protein. Neither are these responses affected by indomethacin, which effectively inhibits cyclooxygenase and the consequent formation of active endoperoxides and thromboxanes. Platelet shape change and the related formation of phosphatidic acid induced by PAF are, however, inhibited by prostacyclin. Prostacyclin decreases the accumulation of phosphatidic acid in platelets by its effects on the phosphatidylinositol cycle (1, 14) and this further emphasizes the interrelationship between appearance of phosphatidic acid and activation of platelets.

We have shown that there is a correlation between conditions favoring phosphatidic acid generation, protein phosphorylation, and platelet activation, suggesting that phosphatidic acid or its precursor, 1,2-diacylglycerol, may play a direct role in platelet responses. Phosphatidic acid may participate in Ca²⁺ gating (14), an event which is associated with activation of many cell types. Alternatively, 1,2-diacylglycerol may be related to transmembrane signaling since it is an activator of...
present time to determine whether one or both of these dalton protein products are necessary for shape change.

...via the initial production of endoperoxides or thromboxanes. Was formed. Accumulation of low and high levels of phosphatidic acid produced is closely correlated with the degree of platelet activation. Furthermore, platelet activation was detected only when phosphatidic acid was formed. Accumulation of low and high levels of phosphatidic acid were associated with shape change and aggregation, respectively. In those studies, it was found that the induction of phosphatidic acid formation can follow two distinct pathways. 1) Arachidonic acid (20) and collagen (18) stimulate phospholipase C and phosphatidic acid formation via the initial production of endoperoxides or thromboxanes. 2) Thrombin, in contrast (18), stimulates phosphatidic acid formation directly by an endoperoxide-independent mechanism. The action of PAF is analogous to that of thrombin; i.e. it does not require endoperoxides or thromboxanes to trigger stimulation of phospholipase C, formation of phosphatidic acid, and platelet shape change.

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REFERENCES
1. Lapetina, E. G. (1982) J. Biol. Chem. 257, 7314–7317
2. Marcus, A. J., Safier, L. B., Ullman, H. L., Wong, K. T. H., Broekman, M. J., Weksler, B. B., and Kaplan, K. L. (1981) Blood 58, 1027–1031
3. Vargas, J. R., Radomski, M., and Moncada, S. (1982) Prostaglandins 23, 929–945
4. Lapetina, E. G., Chandrabose, K. A., and Cuatrecasas, P. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 814–822
5. Lapetina, E. G., and Cuatrecasas, P. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 121–125
6. Lapetina, E. G., and Cuatrecasas, P. (1979) Biochim. Biophys. Acta 573, 394–402
7. Lapetina, E. G., Billah, M. M., and Cuatrecasas, P. (1981) J. Biol. Chem. 256, 5037–5040
8. Lapetina, E. G., Billah, M. M., and Cuatrecasas, P. (1981) Nature (Lond.) 292, 367–369
9. Broekman, M. J., Ward, J. W., and Marcus, A. J. (1980) J. Clin. Invest. 66, 275–281
10. Walenga, R. W., Opas, E. E., and Feinstein, M. B. (1981) J. Biol. Chem. 256, 12523–12528
11. Lloyd, J. V., and Mustard, J. F. (1974) Br. J. Haematol. 26, 243–253
12. Shaw, J. O., Klusick, S. J., and Hanahan, D. J. (1981) Biochim. Biophys. Acta 663, 222–229
13. Shokla, S. D., and Hanahan, D. J. (1982) Biochem. Biophys. Res. Commun. 106, 697–703
14. Lapetina, E. G. (1982) Trends Pharmacol. Sci. 3, 115–118
15. Kawahara, Y., Takai, Y., Minakuchi, R., Sano, K., and Nishizuka, Y. (1980) Biochem. Biophys. Res. Commun. 97, 309–317
16. Takai, Y., Kihbuchi, K., Matsuura, T., and Nishizuka, Y. (1981) Biochem. Biophys. Res. Commun. 101, 61–67
17. Lapetina, E. G. (1983) Life Sci. 32, 2069–2082
18. Siess, W., Cuatrecasas, P., and Lapetina, E. G. (1983) J. Biol. Chem. 258, 4683–4686
19. Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685
20. Siess, W., Siegel, F. L., and Lapetina, E. G. (1983) J. Biol. Chem. 258, in press

![Graph](attachment:graph.png)

**Fig. 5.** Effect of different concentrations of prostacyclin on the PAF-induced platelet shape change ( ), phosphorylation of a 40,000-dalton protein ( ), and formation of phosphatidic acid ( ). Washed human platelets prelabeled with 32P (1.0 ml samples) were preincubated for 3 min in the aggregometer tubes at 37 °C while stirring with different concentrations of prostacyclin, as indicated, and then stimulated with 0.1 μM PAF. Two platelet samples (0.1 ml each) were taken just before addition of PAF and 15 s after addition of PAF for separation of proteins and phosphatidate, respectively. Shape change was recorded, and the extent of inhibition of shape change induced by prostacyclin was measured in mm in relation to samples stimulated with PAF in the absence of prostacyclin. Results in all cases are expressed as % of changes induced by PAF in the absence of prostacyclin. The extent of the stimulation of the phosphorylation of the 40,000-dalton protein and phosphatidic acid induced by 0.1 μM PAF during 15-s incubations was 194% and 195%, respectively.

![Diagram](attachment:diagram.png)

**Fig. 6.** Polyacrylamide gel electrophoresis of proteins from human platelets. Samples are from the experiment from Fig. 6. A shows proteins stained with Coomassie brilliant blue and B shows a radioautography of the same gel. 40,000-dalton protein runs just ahead of the actin band.
Shape change induced in human platelets by platelet-activating factor. Correlation with the formation of phosphatidic acid and phosphorylation of a 40,000-dalton protein.

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