THE METABOLISM OF PLATELET ACTIVATING FACTOR IN PLATELETS AND PLASMA OF VARIOUS ANIMALS

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Abstract—Metabolism of platelet-activating factor (PAF) in rabbit plasma or in rabbit platelets was studied.

1. [C\textsuperscript{3}H\textsubscript{3}] Labeled PAF was degraded into lysoPAF and choline in plasma. An agonist of PAF, NT071 was not degraded in the plasma. Albumin protects the degradation of PAF in plasma deprived of albumin but not the degradation of lysoPAF. These findings indicate that PAF may be metabolized in plasma by acetylhydrolase and then by lysophospholipase D.

2. PAF was converted to phosphatidylecholine (PC) in washed rabbit platelets. The radioactivities in PC was recovered in the fraction of lysoPC after mild alkaline treatment, suggesting that the product is 1-alkyl-2-acyl-glycerophosphocholine.

3. The binding of PAF and lysoPAF to rabbit platelets, rabbit erythrocytes and liposomal membranes were next examined. The binding of PAF to various membranes was inhibited by albumin. Albumin also suppressed the activation of platelets by PAF. A monomeric form of PAF, which is free from albumin, may react with target cell membrane and also to be degraded by catabolic enzymes.

4. The binding of lysoPAF to platelets, erythrocytes and liposomes was more effectively inhibited by albumin than that of PAF. The affinity of PAF to lipid bilayers may be higher than that of lysoPAF.

Key words: Platelet-activating factor (PAF), metabolism, albumin, platelet membrane.

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INTRODUCTION

1-o-Alkyl-2-acetyl-sn-3-glycerophosphocholine, the platelet activating factor (PAF) was first isolated and characterized (Demopoulous et al., 1979; Hanahan et al., 1980; Polonsky et al., 1980). It is released from rabbit basophils via an IgE-dependent mechanism and activates platelets (Halonen et al., 1973). PAF is released not only from basophils but also from stimulated neutrophils, macrophages and platelets (Lynch et al., 1979; Lotner et al., 1980; Mencia-Huerta and Benveniste, 1981; Chignard et al., 1980), suggesting that it may act as a potent mediator in the pathological processes such as anaphylaxis and inflammation. In fact, the compound shows not only platelet activation but also very potent hypotensive and vasoactive activities (Blank et al., 1979; Pinckard et al., 1980).

In the present paper, we will describe the binding of PAF to albumin in plasma and the metabolism of PAF in plasma and platelets.

METHODS AND MATERIALS

PAF was prepared from bovine heart phosphatidylycholine by the method of Demopoulous et al. (1979). [N-C14] PAF (13 Ci/mm mol) was prepared by introducing 3H-labeled methyl group into the choline moiety with [3H] methy iodide by the method of Stoffel et al. (1971). In brief, phosphatidylycholine was first purified from bovine heart by chromatographies on aluminum oxide neutral and Iatrobeads (silicagel). After hydrogenation, the preparation was demethylated. After purification by column chromatography, the product was again methylated with 3H-methyl iodide.

3H-labeled lysoPAF was obtained by alkali-treatment. Acetylation of lysoPAF was performed by the method of Demopoulous et al. (1979). An agonist of PAF, NT-071, was kindly gifted from Drs. H. Nomura and S. Tsushima, Takeda Chemical Ind. Co., Osaka, Japan. Radio-labeled NT-071 was prepared by the same procedure as that used for 3H-labeled PAF.

Rabbit plasma was prepared as follows: rabbit blood was citrated by the addition of 9 vols. of blood to 1 vol. of 3.8% sodium citrate and centrifuged at 1800 xg for 10 min at 4°C, then the supernatant was used as plasma.

RESULTS

Degradation of PAF in rabbit plasma

3H-labeled PAF (8 x 10^-9 M) was incubated with 20 μl of rabbit plasma at 37°C. The reaction mixture was then extracted with a Bligh-Dyer's solvent. The chloroform fraction was analyzed by silicic acid thin-layer chromatography (DC-Fortigplatten Kieselgel 60, Merck) with a solvent system of chloroform/methanol/water (65 : 35 : 8 v/v). The aqueous fraction was evaporated and the residue which was redissolved in water was analyzed by cellulose thin-layer chromatography (avicel SF, Asahi Kasei) with a solvent system of ethanol/1 M ammonium acetate (pH 7.3) (7 : 3 v/v). A new spot appeared on a thin-layer chromatogram of chloroform fraction of the reaction mixture.
Metabolism of PAF

The radioactivities recovered in the new spot, which corresponds to lysoPAF, increased linearly until 5 min (Fig. 1A). About 45% of PAF was degraded after 5 min incubation. A part of the radioactivities was recovered in the upper phase (water phase) of the extraction. The radioactive product was tentatively identified to be choline by cellulose thin-layer chromatography. No appreciable radioactivity could be observed in the region corresponding to phosphocholine (Fig. 1B). LysoPAF might be further hydrolyzed to choline and 1-alkyl-2-lysophosphatidic acid. Choline formation was also observed during incubation of lysoPAF with rabbit plasma (Fig. 1C). Time course of the production of choline from lysoPAF was rather similar to that observed with PAF. Acetylhydrolase and lysophospholipase D (or phospholipase D) may be involved in the degradation of PAF in plasma (Fig. 2). A synthetic PAF agonist, NT-071 (Fig. 3), was not sensitive to both hydrolytic activities in rabbit plasma and any appreciable formation of product corresponding to a lyso-compound was not observed upon incubation with rabbit plasma (data not shown). Any water-soluble product was also not formed (Fig. 3). These observations are consistent with the degradation pathway shown in Fig. 2. PAF may be hydrolyzed to lysoPAF, from which choline may be produced.

Acid labile factor in plasma was reported to inactivate PAF activity (Farr et al., 1980). Rabbit plasma was adjusted to pH 3.0 with 1 N HCl, incubated for 10 min at room temperature and then it was readjusted to pH 7.2 with 1 N NaOH. These treatment abolished the formation of lysoPAF and choline (data not shown). This acid labile

![Fig. 1. Degradation of PAF (A, B) and lysoPAF (C) by rabbit plasma. Rabbit plasma was prepared as described in "Methods and Materials". Experimental conditions were described in the text. (A), the formation of lysoPAF from PAF. The assay was performed with the chloroform fraction; (B), the formation of choline from PAF. Products recovered from the aqueous fraction were assayed. (C), the formation of choline from PAF.](image-url)
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\[
\begin{align*}
&\text{CH}_3\text{COO} \quad \text{O}-(\text{CH}_2)_n-\text{CH}_3 \\
&\text{choline} \quad \text{HO} \quad (\text{choline})
\end{align*}
\]

PAF $\xrightarrow{\text{1}}$ lysoPAF $\xrightarrow{\text{2}}$ *CHOLINE

**Fig. 2.** Degradation pathway of PAF in plasma.

**Fig. 3.** Insensitivity of NT071 to lysophospholipase D in plasma.

$^3$H-PAF (●) and $^3$H-NT071 (○) (8 × 10$^{-9}$ M) were incubated with rabbit plasma as described in Fig. 1 and the water-soluble product was analyzed.

factor seems to be acetylhydrolase.

**Effect of albumin on degradation of PAF and lysoPAF in rabbit plasma**

Rabbit plasma was fractionated into albumin and non-albumin fractions by Affi-Gel Blue (Bio-Rad) column chromatography according to the method of Tavis et al. (1976). Both acetylhydrolase and lysophospholipase D activities were quantitatively recovered from the fraction deprived of albumin. The addition of crystalline bovine serum albumin to the plasma deprived of albumin (500 μg as protein) suppressed the breakdown of PAF (Fig. 4A and B). The formation of lysoPAF was almost completely inhibited by the presence of 10$^{-8}$ M albumin. The formation of choline was affected more sensitively by albumin; 10$^{-6}$ M albumin suppressed the formation completely. The formation of choline from lysoPAF was not, however, affected by albumin up to 10$^{-4}$ M (Fig. 4C).

As described below, PAF forms complex with albumin. These findings indicate that PAF bound to albumin is not accessible to acetylhydrolase, whereas lysoPAF bound to albumin might be accessible to lysophospholipase D. The lack of formation of
Fig. 4. Effect of albumin on the degradation of PAF (A, B) and lysoPAF (C) by rabbit plasma deprived of albumin.
Albumin–depleted plasma was prepared according to the method of Tavis et al. (1976). ['H-PAF mixed with various amounts of bovine serum albumin (BSA) was incubated with the plasma (500 μg protein). The formation of lysoPAF (A) and choline (B) from PAF and that of choline from lysoPAF (C) were analyzed as in Fig. 1.

Choline from PAF bound to albumin again indicates that choline is formed via two successive steps, step 1 (acyethylhydrolase) and then step 2 (lyso phospholipase D).

Conversion of PAF into "phosphatidylcholine" in washed rabbit platelets

When radio-labeled PAF was incubated with washed platelets (5 × 10⁸ cells/ml) which were suspended in Tyrode solution containing 1 mM EDTA, it was converted into "phosphatidylcholine" (Fig. 5). A part of the radioactivity of PAF incubated with platelets were recovered from the spot corresponding to phosphatidylcholine on a silicic acid thin layer chromatogram, as found by Touqui et al. (1983). After mild alkaline hydrolysis, the product was changed quantitatively to a compound which showed a spot corresponding to lysophosphatidylcholine. The finding suggests that the product may be 1-alkyl-2-acyl-glycerophosphocholine. About 50% of PAF bound to platelets was changed to 1-alkyl-2-acyl-glycerophosphocholine after 120 min incubation. Neither lysoPAF nor water-soluble products could be observed during the incubation.

Metabolism of PAF in plasma and platelets of various animals

Platelets of rabbit, human, guinea pig and suncus were sensitive to PAF to form...
aggregates, whereas those of rat and mouse were not (Table 1). The observation that rat and mouse platelets were insensitive to PAF confirmed the previous report (Namm et al., 1982). We next examined the metabolism of PAF in plasma and platelets of various animals. Acetylhydrolase activity was found not only in rabbit but also in guinea pig plasma. Weak activity was also detected in human, suncus and rat plasma. In rat plasma, the acetylhydrolase activity was detectable as found by Blank

Fig. 5. Conversion of PAF to "PC" in rabbit platelet.  
$^3$H-PAF (1×10$^{-8}$ M) was incubated with washed platelets (5×10$^9$ cells/ml) suspended in a Tyrode solution containing 1 mM EDTA. The radioactivities associated with platelets were then extracted with a Bligh-Dyer's solvent. The chloroform fraction was analyzed by silicic acid thin layer chromatography; ○, PAF; ●, "phosphatidylcholine"; □, lysoPAF. The radioactivity in aqueous fraction was also measured (▲).

Table 1. Sensitivity of platelets to PAF and metabolism of PAF in various animals.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Rabbit</th>
<th>Human</th>
<th>Guinea pig</th>
<th>Rat</th>
<th>Mouse</th>
<th>Suncus</th>
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<tbody>
<tr>
<td>Platelet activation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td>Plasma</td>
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<tr>
<td>Acetylhydrolase</td>
<td>+</td>
<td>±</td>
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<tr>
<td>Lysophospholipase D</td>
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<td>+</td>
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<tr>
<td>Platelet Conversion to PC</td>
<td>+</td>
<td>(−)</td>
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Platelets of various animals were prepared as in "Methods and Materials." Their sensitivities to PAF were observed by their aggregations on a platelet aggregometer. The activities of acetylhydrolase and lysophospholipase D were measured as in Fig. 1. Conversion of PAF to phosphatidylcholine was as in Fig. 5. nd: not determined.
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et al. (1981), but it was much weaker than that observed in rabbit plasma. Conversion of PAF to 1-alkyl-2-acyl-glycerophosphocholine (PC in table 1) does not occur significantly in platelets of all animals tested except for rabbit. Change of PAF to “PC” was suggested previously by Touqui et al. (1983). The discrepancy may be due to some differences of experimental conditions used.

Effect of albumin on the activation of platelets by PAF and on the binding of PAF to platelets

Radio-labeled PAF (2.5×10⁻⁸ M) was incubated with rabbit platelets for 30 min at 37°C in the presence or in the absence of albumin and the radio-activities recovered from platelets were measured. PAF was not significantly bound to platelets in the presence of 10⁻⁴ M of bovine albumin (Fig. 6A). The binding of lysoPAF to platelets was even more severely affected by albumin; it inhibited the binding even at 10⁻¹⁰ M (Fig. 6A). Albumin suppressed not only binding of PAF to platelets but also activation of platelets by PAF (Fig. 6B). Release of pre-loaded ¹⁴C-serotonin from platelets (1×10⁸ cells/ml) was measured after 3 min incubation with 1×10⁻⁷ M of PAF mixed with various concentrations of albumin. The binding of PAF to erythrocyte membranes or liposomes was also affected by albumin (Fig. 7). Rabbit erythrocytes (1×10⁷ cells) were incubated with 2.5×10⁻⁸ M of PAF or lysoPAF at 37°C for 30 min. The amount of PAF or lysoPAF bound to erythrocytes was obtained by the method described previously (Tanaka et al., 1980). Liposomes consisting of egg phosphatidylcholine, didetylphosphate and cholesterol (molar ratio, 1:0.1:1) (12μM as phospholipid) were prepared by the method of Szoka and Papahadjopoulos (1978). The liposomes were incubated with PAF or lysoPAF (5×10⁻¹⁰ M) at 37°C for 30 min under the same experimental condition as that for erythrocytes. After centrifugation, the radio-activity recovered from liposomes was measured. Again, the presence of 10⁻⁴ M of albumin almost completely inhibited the binding of PAF to both membranes. Hemolysis of rabbit erythrocytes by PAF and lysoPAF (10⁻⁸ M) was inhibited by the presence of a certain amount of albumin (data not shown). Binding affinity of PAF to albumin, which was determined by equilibrium dialysis, was almost the same with that of lyosphatidylcholine (data not shown). These results suggest that albumin forms complex with a monomeric PAF or its analog, inhibiting the binding of PAF to membranes. PAF and lyoPAF may be incorporated into lipid bilayer of membranes in the absence of albumin, since structural analogs, lyosphatidylcholine and short-chain diacylglycerophosphocholines were previously shown to be incorporated into lipid bilayers (Utsumi et al., 1978; Mashino et al., 1983). The incorporation was also inhibited by albumin. Albumin could also remove PAF from liposomal membranes under some conditions. When liposomes prepared from egg yolk phosphatidylcholine, didetylphosphate, cholesterol and PAF (molar ratio, 1:0.1:1:10⁻⁴), which contained 4×10⁻⁷ M PAF, were incubated with 10⁻⁸ M albumin, about 50% of PAF was transferred from liposomes to albumin (data not shown). Albumin could not release PAF significantly at 10⁻⁸ M. PAF has affinity both to albumin and to lipid bilayers. The final distribution of PAF to lipid bilayers and to albumin may be determined both by the
Fig. 6. Effect of bovine serum albumin (BSA) on binding of PAF to rabbit platelet (A) and activation of platelet by PAF (B).

(A) $^3$H-PAF (○) and $^3$H-lysoPAF (●) ($2.5 \times 10^{-4}$ M) were incubated at 37°C for 30 min with rabbit platelets ($4 \times 10^8$ cells/ml) in the presence of various amounts of bovine serum albumin and the radioactivities associated with the platelets were determined. (B) Release of $^{14}$C-serotonin from platelets was measured as in the text.

amount of albumin and that of membranes. The rate of association and dissociation of PAF with albumin and lipid bilayers may be another important factor regulating the distribution of PAF.

It is noteworthy here that the sensitivity of lysoPAF to albumin is much higher than that of PAF. Binding of lysoPAF to platelets, erythrocytes and liposomes was completely inhibited by $10^{-4}$ M of albumin (Fig. 6A and 7A, B). At the concentration, albumin showed no appreciable effect on the binding of PAF. The difference between PAF and lysoPAF may be due to stronger affinity of PAF to lipid bilayers than that of lysoPAF.
Fig. 7. Binding of PAF to rabbit erythrocyte (RBC) (A) and to liposome (B). Experimental conditions were as in the text. (A) Binding of PAF (○) or lysoPAF (●) to rabbit erythrocytes. (B) Binding of PAF (○) or lysoPAF (●) to liposome.

**Effect of liposomes on the activation of platelets by PAF**

Rabbit platelets were first mixed with liposomes (small unilamellar vesicles prepared from egg yolk phosphatidylcholine). To the mixture, $10^{-7}$ M PAF was added and the release of $^{14}$C-serotonin was measured after 2 min incubation. The coexistence of egg yolk phosphatidylcholine liposomes (3 mM) inhibited the release of serotonin almost completely (data not shown). The amount of liposomes required for the inhibition is calculated to be about 100 folds of that of platelet lipids. PAF should have stronger affinity to specific receptors on platelet membranes than to non-specific receptors, lipid bilayers.

**DISCUSSION**

PAF is produced by various cells such as basophils (Halonen et al., 1973), and neutrophils (Lynch et al., 1979) when cells were stimulated. Though the site of synthesis and intracellular transport of PAF have not been clarified yet, it is plausible that PAF is released from cells to media as a monomer (Fig. 8). Monomeric form of PAF released into plasma may directly react with specific receptors on target cell surface or
may be incorporated into lipid bilayers of not only of target cell but also of many other cell membranes such as erythrocytes. Some of PAF may be degraded by acetylhydrolase and then successively by lysophospholipase D. Since lysoPAF has little biological activity, the enzymes may be involved in detoxication of PAF. All these reactions were inhibited by serum albumin, indicating that complex formed between albumin and PAF may play a role as a reservoir or a carrier of PAF.

In rabbit plasma, $5 \times 10^{-4}$ M of albumin is contained (Geinitz, 1954). Although the concentration of albumin in plasma seems to be enough for inhibiting the reactivity of PAF, it turns out that the break down of PAF as well as its binding to platelets and activation of platelets occurs even in fresh plasma. Addition of plasma (more than $10^{-4}$ M as albumin) did not affect the activation of washed platelets by PAF. The discrepancy may be due to the different conformational structure of albumin in plasma from purified albumin. Alternatively, some other proteins in plasma may interact with albumin, modifying the conformational structure of the protein.

REFERENCES

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