PLATELET ACTIVATING FACTOR ANALOGUES: LACK OF CORRELATION BETWEEN THEIR ACTIVITIES TO PRODUCE HYPOTENSION AND ENDOTHELIUM-MEDIATED VASODILATION

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Hypotensive activities of 11 synthetic derivatives of platelet activating factor (PAF) were examined and compared with their activities to produce endothelium-related relaxation of isolated rat thoracic aorta. The derivatives showed variety of hypotensive activities; some of the derivatives were more potent than natural PAF and some were virtually inactive compared to PAF. However, all of the compounds tested exhibited exactly the same activities to produce endothelium-related vasodilation. These results confirmed our previous view that the PAF-induced hypotension is not solely due to the endothelium-related vascular relaxation observed in vitro.

Keywords—platelet activating factor (PAF); platelet activating factor (PAF) analogue; hypotension; rat thoracic aorta; vasodilation; vascular endothelium; phospholipid

INTRODUCTION

Platelet activating factor (PAF, 1-\(O\)-alkyl-2-acetyl-\(sn\)-3-glycerophosphocholine) has been shown to have potent hypotensive activity.\(^1\)\(^,\)\(^2\) We have previously reported systematic studies on the hypotensive action of PAF with various animal species. We have shown that it produced strong hypotension in all of the animal species tested and suggested that PAF may produce hypotension mainly by acting on peripheral arterial blood vessels to produce vasodilation.\(^3\) In the next step, we have examined the mechanism of vasodilation by PAF using isolated arterial preparations. As a result, we have obtained some evidences indicating the involvement of endothelial cells in the vasodilation by PAF,\(^4\) similarly to the case of acetylcholin (ACh), which was originally hypothesized by Furchgott et al.\(^5\)\(^,\)\(^6\) However, we have concluded that PAF-induced hypotension may not be solely due to the endothelium-related vasodilation because relatively high concentrations of PAF were needed to produce the endothelium-mediated relaxation of isolated arterial smooth muscle.

In the present study, to further confirm the above conclusion, we examined the hypotensive activities of various synthetic derivatives of PAF and compared them with their activities to produce endothelium-related vasodilation. Preliminary studies on the hypotensive activities of some of the present derivatives were already reported elsewhere.\(^7\)

MATERIALS AND METHODS

1) Blood Pressure Measurements in Anesthetized Rats—Male Wistar strain rats (250–300 g) were anesthetized with urethane (600 mg/kg, i.p.) and alpha-chloralose (Tokyo-Kasei, 60 mg/kg, i.p.). Arterial blood pressure was measured from the left carotid artery by means of a pressure transducer (Nihon Kohden, MPU-0.5) and recorded on an ink-writing oscillograph (Nihon Kohden, WI-681-C). PAF and its derivatives were injected intravenously through the...
femoral vein. In some of the experiments, blood pressure changes were similarly examined in spontaneously hypertensive rats (SHR).

2) Measurements of Endothelium-Related Vascular Relaxation — Male Wistar strain rats (300 g) were decapitated, and their thoracic aortae were removed and cut into spiral strips (approximately 2 mm × 25 mm). Throughout the procedure for making the spiral strips, care was taken to avoid rubbing of the intimal surface. Strips were mounted in organ bath containing physiological salt solution which was gassed with 95% O₂-5% CO₂ at 37 °C and had the following composition (mM): NaCl, 135; KCl, 5; CaCl₂, 2; MgCl₂, 1; NaHCO₃, 15; glucose, 5.5. Resting tension of 1.0 g was applied and the developed tension was measured isometrically with force displacement transducer (Nihon Kohden, TB-611T). Tissues were allowed to equilibrate for 60–90 min prior to the addition of any agent. Responses to 10⁻⁷ M norepinephrine were elicited, followed by the addition of PAF or its derivatives.

The intimal surface of some of the strips was rubbed gently with a disposable cotton applicator which removed the endothelial layer; care was taken not to overstretch the preparations during the rubbing procedure. Following equilibration, subsequent contractile responses to 10⁻⁷ M norepinephrine were unaffected by the rubbing procedure.

3) PAF and PAF Derivatives — PAF and its derivatives were synthesized and supplied by Ohno, et al. The structure of PAF and its derivatives are shown in Figs. 1–3. Phospholipids, dipalmitoyl phosphatidyl choline, dilauryl phosphatidyl choline, and phosphatidyl ethanolamine (egg), are the gift of Prof. Nojima. These compounds were stored in chloroform solution at -20 °C. Chloroform was evaporated under vacuum and distilled water was added; in the case of blood pressure experiments, physiological saline was used. Sonication was made for 30 s to
dissolve them completely. The other drugs used were L-norepinephrine bitartrate (Wako) and papaverine hydrochloride (Wako).

RESULTS

1) Hypotensive Activities of PAF and Its Derivatives

In Fig. 4 are summarized hypotensive activities of PAF and its derivatives. In panel A, the lowest blood pressures produced by the test compounds are plotted as a function of their dosages, which indicates the degree of the hypotension produced by these compounds. In panel B are plotted time required to obtain 50% recovery of the blood pressure from the maximum decrease as a function of the dosages; it is considered to reflect the duration of the hypotension produced by these compounds. As shown, the results are in good accordence either when the degree of the hypotension is plotted or when the duration is plotted. When comparing the hypotensive potencies of these PAF derivatives on the bases of the results illustrated in both panels, the following conclusion can be drawn. P-3 and P-4 were more potent than PAF, and the potencies of P-1 and P-8 were about the same as that of PAF. P-7, P-6, P-2, P-5 and PAF enantioomer were less potent than PAF; potencies of P-9, P-10 and P-11 were negligible compared to PAF. The hypotensive activities of the compounds from P-1 to P-5 have been reported elsewhere in more detail.89

2) Endothelium-Related Vasodilation

PAF derivatives whose hypotensive potencies were higher than, same as, or less than PAF were selected and their activities to produce the endothelium-related vasodilation were examined; with respect to hypotension, P-4 was more potent, P-1 and P-8 were equipotent, P-5 was less potent, and the potencies of P-10 and lyso PAF were substantially negligible. In addition, a typical phospholipid, lyssolecithin, was tested. In Fig. 5 are shown the dose-response curves for the relaxing effects of these compounds on the endothelium-containing aortic strips precontracted with 10^{-7} M norepinephrine. As is clearly shown, the dose-response curves were almost identical for all of the compounds tested in spite of the great differences between their hypotensive activities. In other words, even the compounds with substantially no hypotensive activities exhibited about the same degree of endothelium-related vasodilating potencies as those with the extremely strong hypotensive activities. To confirm that these vasodilation is related to endothelium, P-1 was tested with the endothelium-denuded preparations similarly precontracted with 10^{-7} M norepinephrine; as a result, no relaxation was obtained in these preparations, which is shown in Fig. 5.

Since it was suspected that most of the phospholipids may be more or less active to produce the endothelium-related vasodilation, 3 natural phospholipids, dipalmitoyl phosphatidyl choline, diararyl phosphatidyl choline and phosphatidyl ethanolamine mixture obtained from egg, were tested in the other series of experiments. The obtained dose-response curves are
shown in Fig. 6, which are superimposed with that of PAF on the molar basis. As shown, all of these phospholipids did produce the endothelium-related vasodilation, although they were less potent than PAF.

DISCUSSION

The present study clearly demonstrated the lack of the correlation between the activities of various PAF derivatives to produce hypotension and endothelium-related vasodilation. Among the derivatives tested in the present study, two were more potent than natural PAF in their hypotensive activities. This fact was already emphasized in our previous report, in which the hypotensive activities of the derivatives listed in Fig. 1 were studied. In the present study, we have newly examined the hypotensive activities of the compounds listed in Figs. 2 and 3. As a result, it was found that they showed a variety of hypotensive activities as shown in Fig. 4; some were more potent than PAF, some equipotent, some less potent, and some were almost inactive compared to PAF.

FIG. 4. Hypotensive Activities of PAF Derivatives

A. The lowest blood pressure produced by PAF derivatives are plotted as a function of their dosages.

B. Duration of the blood pressure decrease is plotted as a function of dosages of PAF derivatives. Ordinate: time required to obtain 30% recovery of the blood pressure from the maximum decrease. PAF+ indicates the results obtained in SHR. "enant." indicates PAF- enantiomer. Number of experiments were 4–6 for each compound. Since standard error was very small, it was deleted from the figure for the simplicity.

FIG. 5. Endothelium-Related Relaxation Produced by PAF Derivatives and Phospholipids

Relaxation was expressed as % of the 10^{-4} M papaverine-induced maximum relaxation. Rat aortic spiral strips were precontracted with 10^{-2} M norepinephrine. P-1 was also tested with endothelium-denuded preparations; no relaxation was produced in this preparation ( ). Number of preparations are 5 for each curve. Because standard error of mean was very small, it was deleted from the figure for the simplicity.

○ PAF; ● Lyso-PAF; △ P-4; ▲ P-5; □ P-8; ■ P-10; ▽ lysolecithin (palmitoyl); ◆ P-I (w/ endothelium); ♦ P-I (w/o endothelium).
Thus, we have selected some compounds showing various hypotensive activities and examined whether their potencies to produce endothelium-related vasodilation might be parallel to their hypotensive potencies. As a result, surprisingly enough, it was found that all of the compounds tested exhibited the same activities to produce the endothelium-related vasodilation. Moreover, some of the typical phospholipids, dipalmitoyl phosphatidyl choline, dilauryl phosphatidyl choline, and phosphatidyl ethanolamine, also showed the endothelium-related vasodilatation activities, though they were slightly less potent than PAF. From these results, it can be concluded that the PAF-induced hypotension is not, at least solely, caused by the endothelium-related vasodilation, confirming our previous view.\(^4\)

It seemed in the present study that most of the phospholipids were able to produce endothelium-related vasodilation somewhat non-specifically. The mechanism for this is not clear at present. Phospholipids might penetrate into the endothelial cell membrane and produce some structural changes, leading to the leakage of some relaxing substance(s). However, this hypothesis remains to be elucidated. We are now planning to examine the structural changes in the endothelial cell membrane produced by PAF and other phospholipids by means of electron microscopy.

Extensive studies have been performed on the role of endothelium in the ACh-induced vasodilation mainly by the research group of Furchgott. His group demonstrated that ACh stimulates endothelial cells to produce and release some factor which diffuses to the smooth muscle cells and activates their relaxation. The factor is now referred to as endothelium-derived relaxing factor (EDRF).\(^9\) Although it has not yet been identified chemically, Furchgott group speculated that the factor might be a liable hydroperoxide or free radical intermediate product. However, in a recent report by another study group, it was proposed that EDRF is not a lipoxygenase derivative or free radical but an unstable compound with a carbonyl group at or near its active site.\(^10\)

On the other hand, the role of endothelium in the relaxation of pre- contracted blood vessel preparations has been demonstrated for various substances other than ACh. These substances include adenosine triphosphate (ATP), adenosine diphosphate (ADP), bradykinin, substance P, histamine, thrombin, angiotensin II, arachidonic acid, and calcium ionophore A23187, etc. However, detailed mechanisms for their endothelium-related vasodilation have not yet been clarified.

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**FIG. 6. Endothelium-Related Relaxation Produced by some Phospholipids**

Relaxation was expressed as \(\%\) of \(10^{-4}\) \(M\) papaverine-induced maximum relaxation. Rat aortic spiral strips were precontracted with \(10^{-7}\) \(M\) norepinephrine. DLPC: dilauryl phosphatidyl choline, DPPC: dipalmitoyl phosphatidyl choline, PE: phosphatidyl ethanolamine (egg). Number of preparations are 6 for each curve.

- PAF
- DLPC
- DPPC
- PE

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**10.** A recent report by another study group, it was proposed that EDRF is not a lipoxygenase derivative or free radical but an unstable compound with a carbonyl group at or near its active site.
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The only evidence is that the vasodilation produced by them was abolished in the endothelium-denuded preparations. Therefore, the involvement of some non-specific factor can not be excluded in their endothelium-related vasodilation, similarly to the case of the present study with PAF derivatives and phospholipids.

In conclusion, the results in the present study further support our previous view that the PAF-induced hypotension is not solely due to the endothelium-related vascular relaxation. With respect to the mechanisms for PAF-induced hypotension, extensive pharmacological studies in our previous paper excluded many possibilities, and the only remaining possibility was arterial vasodilation. However, it is impossible at present to speculate the true mechanism(s) involved in the vasodilation, and further studies are now in progress. Another suggestion drawn by the present findings was that some smooth muscle relaxing substance(s) might be released from the endothelial cells in a somewhat non-specific manner by the action of phospholipids; one possibility for this might be changes in membrane fluidity.

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REFERENCES


