POSSIBLE ROLE OF ENDOTHELIUM IN THE VASODILATOR RESPONSE OF RAT THORACIC AORTA TO PLATELET ACTIVATING FACTOR (PAF)

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As the endothelium has an important role in the vasodilating effect of acetylcholine, we investigated the possible role of the endothelium in the vasodilating effect of platelet activating factor (PAF). Experiments were done on spirally cut rat thoracic aorta either containing or denuded of endothelial cells. It was demonstrated that relaxation by PAF and acetylcholine of pre-contracted strips required the presence of endothelial cells. The result strongly suggested the possible involvement of endothelium in the vasodilation produced by PAF as well as by ACh.

Keywords — platelet-activating factor; PAF; rat thoracic aorta; vasodilation; vascular endothelium; acetylcholine

INTRODUCTION

1-O-Alkyl-2-acetyl-sn-3-glycerophosphocholine, platelet activating factor (PAF), was first isolated and characterized by Demopoulos, et al., Hanahan, et al., and Polonsky, et al. In addition to the platelet activating action, it has been shown to have potent hypotensive activity. The present authors have previously studied the hypotensive action of PAF with various animal species and shown that it produced strong hypotension in all of the animal species tested. We have suggested that PAF may produce hypotension mainly by acting on peripheral arterial blood vessels to produce vasodilation.

Recently, Furchgott and his colleagues have shown that the vasodilation produced by acetylcholine may be mediated by some unknown substance released from endothelial cells in blood vessels. Therefore, we examined the possibility that PAF may produce the vasodilation through the same mechanism and obtained some preliminary evidences indicating the involvement of endothelial cells in the vasodilation produced by PAF, which will be described in this report.

MATERIALS AND METHODS

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 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 drug.

Responses to 10⁻⁷M norepinephrine were elicited, followed by the addition of acetylcholine or PAF. The intimal surface of some of the

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strips was rubbed gently with a disposable cotton applicator which removed the endothelial layer; care was taken not to overstretched the preparations during the rubbing procedure. Following equilibration, subsequent contractile responses to $10^{-7}$M norepinephrine were unaffected by the rubbing procedure. Acetylcholine or PAF then was added.

In some experiments, a technique was used in which rubbed vessel preparations were suspended in close contact with normal vessel strips. This “sandwich” technique is according to Furchgott, et al. A spirally cut strip, freed of endothelial cells by rubbing, was brought into contact with the endothelial surface of a longitudinal vessel strip of the same width.

PAF used was 1-O-hexadecyl-2-O-acetyl-sn-glyceryl-3-phosphorylcholine, which was synthesized and supplied by Ohno, et al. PAF was stored in chloroform solution at $-20^\circ$C. Chloroform was evaporated under vacuum and distilled water was added. Sonication was made for 30 s to dissolve completely. The drugs used were $L$-norepinephrine bitartrate (Wako), acetylcholine chloride (Daiichi), papaverine hydrochloride (Wako), and atropine sulfate (Wako).

RESULTS AND DISCUSSION

Fig. 1 shows the typical records illustrating the responses to acetylcholine (ACh) and PAF of normal aortic strips, endothelium denuded strips, and “sandwich” strips. In control endothelium-containing preparations pre-

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**FIG. 1. Effects of Increasing Concentrations of ACh and PAF on Spirally Cut Rat Aortic Strips Precontracted with Norepinephrine ($10^{-7}$ M)**

Drug concentrations are expressed as minus logarithms of cumulative concentrations (M) for ACh and as $\mu$g/ml for PAF, and W indicates washout of the chamber. A and A’: Spirally cut strips with endothelium intact. B and B’: Spirally cut strips with endothelium removed by rubbing. C and C’: “Sandwich” strips made from the spirally cut strips without endothelium and longitudinal strips with endothelium mounted together with their entire intimal surfaces apposed. D and D’: Spirally cut strips after removing the endothelium-containing longitudinal strips from the preparations in C and C’.
contracted by norepinephrine (NE, $10^{-7}$M), the addition of both ACh and PAF elicited concentration-dependent relaxation. On the contrary, the endothelium-denuded preparations similarly precontracted by NE showed a complete loss of relaxation in response to consecutive concentrations of ACh and PAF. In the next step, the endothelium-denuded strip was brought into contact with the endothelial surface of a longitudinal strip ("sandwich" preparation, see Methods). With the strip thus mounted, both ACh and PAF again elicited relaxations in the rubbed preparation. The relaxations induced by both agonists (especially PAF) occurred more slowly and were less pronounced than in control preparations. Removal from these preparations of the endothelium-containing longitudinal vessels produced again the complete loss of relaxation.

The above described results with ACh are identical with those described by Furchgott, et al. Thus, confirmed was the involvement of endothelial cells in the aortic vasodilator response to ACh. In addition, the present results clearly showed that PAF produced concentration-dependent relaxation in endothelium-containing preparations, though it was slow compared to ACh-induced relaxation. The relaxation completely disappeared in the endothelium-free preparations. Moreover, the relaxation by PAF recovered, though partially, in the "sandwich" preparations. These results strongly suggest the possibility that endothelial cells play some role in PAF-induced aortic relax-

FIG. 2. Dose-Response Curves for the Relaxing Effects of PAF on Spirally Cut Aortic Strips Precontracted Either with Norepinephrine ($10^{-7}$M, A) or Increased External K$^+$ (30 mM, B)

Open circles: responses of strips with endothelium intact. Solid circles: responses of strips with endothelium removed by rubbing. Abscissa: concentrations of PAF ($\mu$g/ml). Ordinate: relaxation expressed as % of the maximum relaxation produced by papaverine ($10^{-4}$M). Each point and vertical bar indicate the mean of 5 experiments (A) or 6 experiments (B) and standard error of the mean.
Endothelium-related Vasodilation by PAF

...ation similarly to ACh-induced vasodilation.

The results were analogous when a 30 mM K+ solution was used as pre-contracting agent. In Fig. 2 are shown dose-response curves for PAF with control and endothelium-denuded preparations pre-contracted either with NE or 30 mM K+. As is shown clearly, PAF elicited dose-dependent relaxation in control preparations regardless of the pre-contracting agents. However, the relaxing responses to PAF of the preparations contracted by high K+ were less pronounced than those contracted by NE; maximum relaxation by PAF of K+-contracted preparation was about half of that of NE-contracted preparation. In other words, contractions produced by increasing the K+ concentration were less sensitive to PAF. The reason for this is unknown at present. However, Furchgott, et al. showed the similar results with respect to ACh-induced aortic relaxation, and suggested that relaxation by ACh can occur independently of membrane potential changes of the smooth muscle cells.4) In contrast to the endothelium-intact preparations, PAF did not produce significant relaxation at any concentration tested in the endothelium-free preparations regardless of which agents were used for precontraction, as is shown by flat lines in Fig. 2.

The role of the endothelium in the relaxation of pre-contracted blood vessel preparations has also been demonstrated for various physiological substances other than ACh, e.g., ATP and ADP,10) thrombin,11) bradykinin,12) and histamine.13) The study presented here strongly suggested that the presence of endothelium is also essential for the relaxing effect of PAF on isolated rat thoracic aorta preparations pre-contracted by NE or high external K+. However, it may be difficult to consider that PAF-induced hypotension is solely due to the endothelium-related vascular relaxation, because relatively high concentrations of PAF were needed to produce the relaxation of isolated arterial smooth muscle.

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