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Effects of platelet-activating factor on conversion of angiotensin I to II

Hideaki Kawaguchi and Hisakazu Yasuda

Department of Cardiovascular Medicine, Hokkaido University, School of Medicine, Sapporo 060 Japan

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We have studied the effects of platelet-activating factor (alkyl-acetyl-GPC) on conversion of angiotensin I to II. Platelet-activating factor (PAF) had a novel effect on angiotensin I conversion. Pulmonary artery endothelial cells converted 30% of [^{125}I]angiotensin I to angiotensin II in the absence of PAF, but their activity was greatly stimulated in its presence. When PAF was added to pulmonary artery endothelial cells, the conversion of angiotensin I to II was enhanced up to 68%. Maximal stimulation without cellular damage was achieved at 10^{-5} M PAF. LysoPAF did not stimulate the conversion of angiotensin I to angiotensin II at any concentration used.

Platelet-activating factor; Angiotensin converting enzyme; (Pulmonary endothelial cell)

1. INTRODUCTION

Platelet-activating factor (PAF) is released from a number of mammalian cells upon stimulation [1,2], and appears to be involved in several important cellular events. For example, it causes the aggregation and degranulation of platelets [3] and stimulates arachidonic acid release and subsequent thromboxane synthesis [4].

Our series of experiments revealed that PAF directly acted on Swiss mouse 3T3 fibroblasts, releasing arachidonic acid from the cell membrane [5], and stimulated the release of prostacyclin from aortic smooth muscle cells [6]. In renal epithelial cells PAF stimulated PGE_2 release and calcium membrane permeability [7]. These results showed that there was a possibility that PAF directly acted on renal and vascular cells, and regulated vascular tone. In this study experiments have been done to elucidate the mechanisms of PAF's vasoactive effects, and to determine the effect of PAF on conversion of angiotensin I to II in pulmonary artery endothelial cells.

Correspondence address: H. Kawaguchi, Department of Cardiovascular Medicine, Hokkaido University, School of Medicine, Sapporo 060, Japan

2. MATERIALS AND METHODS

2.1. *Materials*

3-[^{125}I]Angiotensin I (2000 Ci/mmol) and [^{125}I]-angiotensin II (2000 Ci/mmol) were obtained from Radiochemical Centre, Amersham (England). Angiotensin I, 1-*O*-hexadecyl-2-acetyl-phosphorylcholine (C16-PAF) and lysophosphorylcholine-1-*O*-hexadecyl (C16-lysoPAF) were obtained from Sigma (St. Louis, MO). 1-*O*-Octadecyl-2-acetyl-phosphorylcholine (C18-PAF) and lysophosphorylcholine-1-*O*-octadecyl (C18-lysoPAF) were purchased from Bachen Feinchemikalien AG (Bubendorf, Switzerland). All other materials were of reagent purity.

2.2. *Cell culture*

Bovine pulmonary artery endothelial cells (PAEC) were obtained from Flow Laboratory (England), and maintained in Eagle's Minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). The dishes were in-

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cubated at 37°C in 5% CO_2/95% air atmosphere [8]. Cells grown to confluence were subcultured at 2×10^5 cells/dish in 3 ml of MEM containing 10% FBS in 60-mm dishes [9]. Cultures established in this manner with cells between passages 20 and 50 were used throughout the study.

2.3. *Angiotensin I conversion*

Studies into the conversion of angiotensin I (AI) to angiotensin II (AII) were done using PAEC. Triplicate cultures of 2×10^5 cells were plated in 60-mm dishes with 10% FBS supplemented medium, and incubated for 1 week to reach confluence. Confluent cultures were used for all assays. Cells were washed with fresh MEM and then incubated with 0.3% FBS supplemented MEM for 24 h at 37°C. After washing the cells again, [^{125}I]AI (0.1 μCi/50 nmol) was added to the dishes and incubated for up to 60 min at 37°C with or without PAF. [^{125}I]AI was separated from [^{125}I]AII on ODS columns (Sep Pak, Waters Associates, Milford, MA) [9], which had been previously equilibrated with 10 ml of Krebs-Henselites buffer, followed by 20 ml of 0.1 M sodium phosphate buffer, pH 5.7. The sample was loaded on to the column and washed with 2 ml of 0.1 M sodium phosphate buffer. A solution (8.0 ml) of 80% phosphate buffer, 20% ACN (pH 5.7), was then passed through the column, and the fractions collected in tubes for measurement of radioactivity. This was followed by 5 ml of 0.1 M phosphate buffer (25%), 75% ACN (pH 5.7), which was also collected in fractions and counted. The columns were regenerated by washing with 5 ml of 100% ACN.

2.4. *Cytotoxicity assay*

Cells were cultured in the same manner as described for the assay of AI converting study except that no [^{125}I]AI was used. Cell viability was assessed by the ability to exclude 0.2% nigrosin dye for 20 min at room temperature using an inverted microscope with phase optics [10].

3. RESULTS

3.1. *Stimulation of AI conversion to AII*

Fig.1. shows the elution pattern of AI and AII. They were separated by the stepwise ACN gradient system.

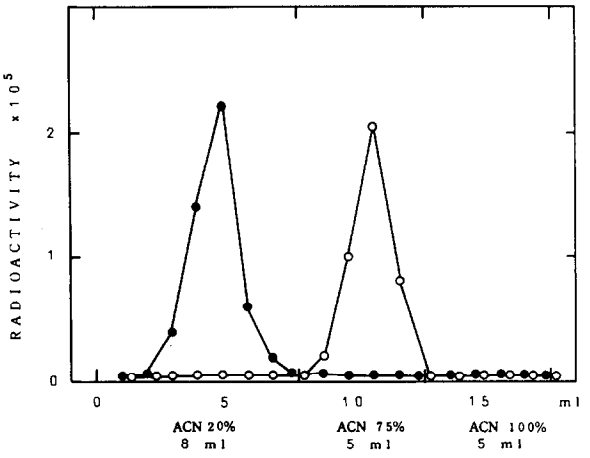

Fig.1. Elution profiles for AI (○) (0.1 μCi) and AII (●) (0.1 μCi). AI and AII were eluted by a stepwise solvent system of acetonitril described in section 2.

The effect of PAF on conversion of AI to AII was determined by the addition of [^{125}I]AI to endothelial cells, and incubating with the indicated concentration of PAF for 20 min. About 30% of AI was converted to AII in PAF's absence. Addi-

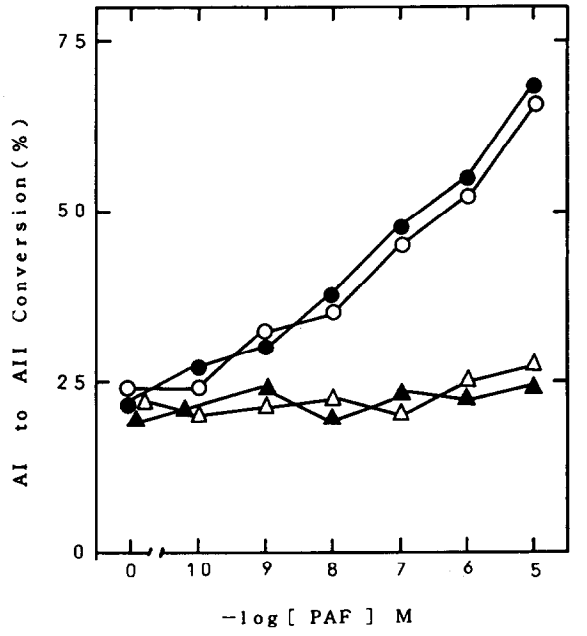

Fig.2. Effect of PAF on angiotensin conversion. Endothelial cells were plated and assayed as described in section 2. Points represent means ± SE. C16-PAF (●), C18-PAF (○), C16-lysoPAF (▲), C18-lysoPAF (△).

tion of either C16-PAF or C18-PAF markedly enhanced the conversion dose-dependently (fig.2), with the two forms of PAF showing no difference in their enhancing ability. The maximum conversion of AI to AII by PAF was 68% at 10^{-5} M. Neither C16-lysoPAF nor C18-lysoPAF had any effect on AI conversion to AII. As shown in fig.3, the angiotensin conversion reaction was linear for 20 min both with and without PAF. Thus, both C16- and C18-PAF (1 μM) stimulated the conversion of AI to AII from the beginning of the reaction (fig.3).

3.2. *Cytotoxicity*

At higher concentrations of PAF cells were killed. The addition of PAF (10^{-3}–10^{-2} M) to a cell population caused profound changes in cellular morphology to occur. At this time, the cells were found to stain strongly with 0.2% nigrosin (fig.4).

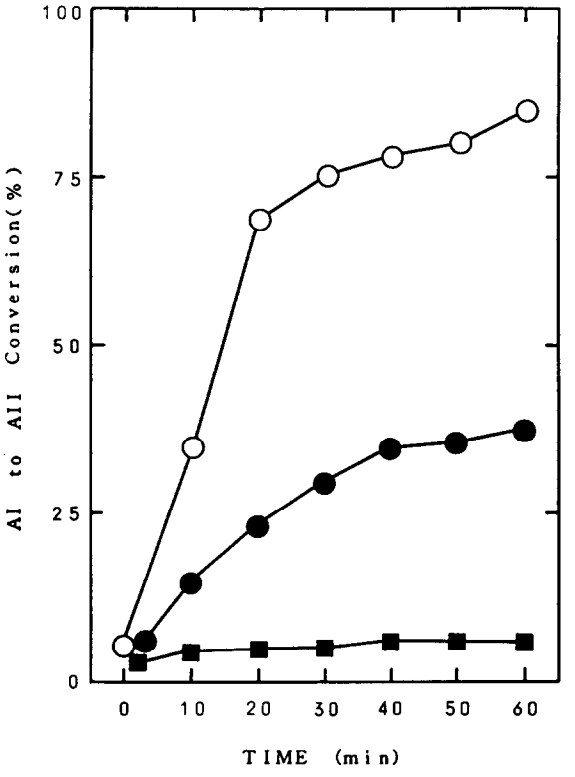

Fig.3. Time course of angiotensin conversion. Cells were cultured as described in section 2. Then AI was added and incubated for the indicated period (○) with or without 1 μCi PAF (●). Points represent means ± SE. (■) Cell-free dishes with PAF.

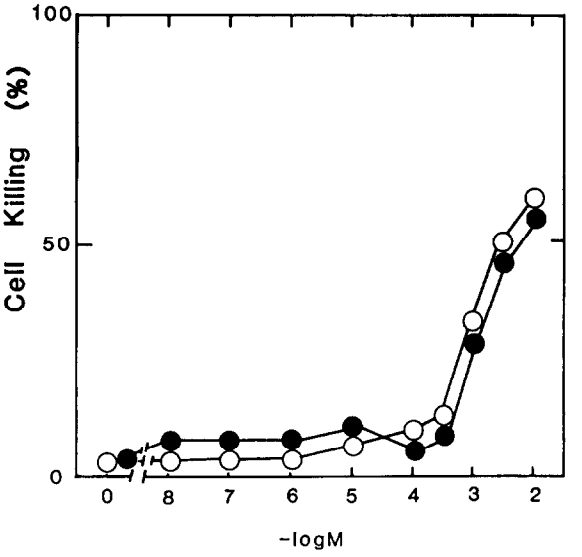

Fig.4. Cytotoxicity. Parallel cultures were established as in fig.2. Cells were killed at 20 min after treatment by failure to exclude 0.2% nigrosin dye for 20 min at room temperature. A total of 250 cells on each dish were counted and averages (mean ± SE) plotted. C16-PAF (●), C18-PAF (○).

4. DISCUSSION

PAF is a potent lipid mediator that is strongly implicated in the pathogenesis of acute allergic and inflammation reaction [2,3], and in vasodilation [11,12].

In the present study, we found a novel PAF effect on AI conversion to AII. It is well-known that bradykinin, a potent blood pressure-lowering substance, and angiotensin are hydrolyzed by the same enzyme [13]. Hydrolysis of AI yields AII, the most potent of known mammalian vasopressor hormones, which is released into the systemic arterial circulation. The net result is that a blood pressure-raising substance is formed and a blood pressure-lowering substance is inactivated [13]. From this point of view, stimulation of angiotensin conversion by PAF may play an important role for blood pressure regulation. Further investigations on the mechanism of the effects of PAF on angiotensin converting enzyme are under way.

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