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Angiotensin peptides attenuate platelet-activating factor-induced inflammatory activity in rats

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ABSTRACT

Angiotensin (Ang)—a peptide that is part of the renin-angiotensin system-induces vasoconstriction and a subsequent increase in blood pressure; Ang peptides, especially AngII, can also act as potent pro-inflammatory mediators. Platelet-activating factor (PAF) is a potent phospholipid mediator that is implicated in many inflammatory diseases. In this study, we investigated the effects of Ang peptides (AngII, AngIII, and AngIV) on PAF-induced inflammatory activity. In experiments using a rat hind-paw oedema model, AngII markedly and dose-dependently attenuated the paw oedema induced by PAF. The inhibitory effects of AngIII and AngIV on PAF-induced paw oedema were lower than that of AngII. Two Ang receptors, the AT_1 and AT_2 receptors, did not affect the AngII-mediated attenuation of PAF-induced paw oedema. Moreover, intrinsic tyrosine fluorescence studies demonstrated that AngII, AngIII, and AngIV interact with PAF, and that their affinities were closely correlated with their inhibitory effects on PAF-induced rat paw oedema. Also, AngII interacted with metabolite/precursor of PAF (lyso-PAF), and an oxidized phospholipid, 1-palmitoyl-2-(5′-oxo-valeroyl)-*sn*-glycero-3-phosphocholine (POVPC), which bears a marked structural resemblance to PAF. Furthermore, POVPC dose-dependently inhibited AngII-mediated attenuation of PAF-induced paw oedema. These results suggest that Ang peptides can attenuate PAF-induced inflammatory activity through binding to PAF and lyso-PAF in rats. Therefore, Ang peptides may be closely involved in the regulation of many inflammatory diseases caused by PAF.

1. Introduction

Angiotensin (Ang)—a peptide of the renin-angiotensin system-induces vasoconstriction and a subsequent increase in blood pressure. So far, four Ang peptides (AngI, AngII, AngIII, and AngIV) and four receptor subtypes (AT_1, AT_2, AT_3, and AT_4) have been identified [1]. AngI is inactive and is converted to AngII by angiotensin-converting enzyme. AngII is converted to AngIII, which is converted to AngIV by aminopeptidase. AngII and AngIII are full agonists for the AT_1 and AT_2 receptor, while AngIV binds with low affinity to the AT_1 and AT_2 receptors, but with high affinity and specificity to the AT_4 receptor. The AT_3 receptor was discovered in cultured neuroblastoma cells, though its existence is still controversial [2]. Among the Ang peptides, AngII, the most important effecter peptide, plays an important role in cardiovascular homeostasis by regulating blood volume, blood pressure, and peripheral vascular tone [3,4]. Recent studies have provided experimental evidence that Ang peptides participate in inflammation-related processes, such as vascular permeability and atherogenecity [3]. For example, AngII at doses without causing inflammation facilitates carrageenan- and dextran-induced inflammatory oedema through induction of mast cell degranulation via the AT_2 receptor [5,6], and the AT_2 receptor is involved in pulmonary oedema induced by AngII itself (at edematogenic doses) in rats [7].

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), a potent phospholipid mediator, plays an important role in several physiological events [8]. It has been implicated in the pathogenesis of many inflammatory diseases including asthma, anaphylaxis, and atherosclerosis [9,10]. The bioactivities of PAF are mediated via its binding to a specific PAF receptor [10,11]. PAF is rapidly degraded to biologically inactive 1-*O*-alkyl-*sn*-glycero-3-phosphocholine (lyso-PAF) by PAF acetylhydrolase (PAF-AH), and the conversion of lyso-PAF into PAF is catalyzed by lyso-PAF acetyltransferase (lyso-PAFAT) [12,13]. However, with respect to inflammatory activity, the relationship between Ang peptides and PAF is poorly understood yet.

This study aimed to investigate the effects of Ang peptides (AngII, AngIII, and AngIV) on PAF-induced inflammatory activity in rats. The results indicate that Ang peptides can attenuate

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AngII	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
AngIII	Arg-Val-Tyr-Ile-His-Pro-Phe
AngIV	Val-Tyr-Ile-His-Pro-Phe

Fig. 1. Amino acid sequences of AngII, AngIII, and AngIV. Each amino acid is represented by the three-letter code.

PAF-induced inflammatory activity through binding to PAF and lyso-PAF.

2. Materials and methods

2.1. Materials

PAF (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, C16) was purchased from Enzo Life Sciences Inc. (Plymouth Meeting, PA, USA). AngII was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). AngIII and AngIV were purchased from Peptide Institute, Inc. (Osaka, Japan). The amino acid sequences of AngII, AngIII, and AngIV were shown in Fig. 1. Losartan potassium salt, 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-methanol, monopotassium salt, was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Lyso-PAF (1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine, C16), POVPC (1-palmitoyl-2-(5′-oxo-valeroyl)-*sn*-glycero-3-phosphocholine), and PD123319 trifluoroacetate salt, (6*S*)-1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(2,2-diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid, di(2,2,2-trifluoroacetate) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Bovine serum albumin (BSA; Fraction V RIA grade, A-7888) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless otherwise noted.

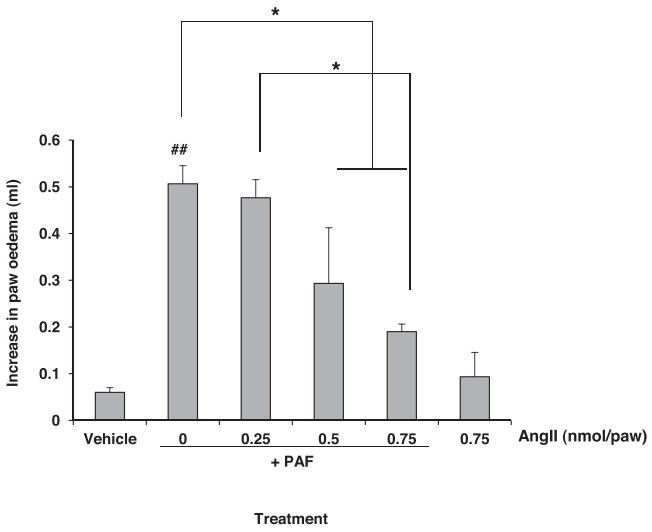

Fig. 2. Effects of AngII on PAF-induced rat paw oedema. Rats were treated with by intraplantar injection with PAF (2 nmol/paw) in the absence or presence of AngII (0–0.75 nmol/paw). Forty-five minutes after the injection, the paw oedema was quantified by measuring the increase in paw oedema (mL). Each value represents the mean ± S.D. (n = 3–4). $^{\#\#}P < 0.05$ compared to vehicle-treated rats and $^{*}P < 0.05$ (analysis using ANOVA followed by post-hoc test).

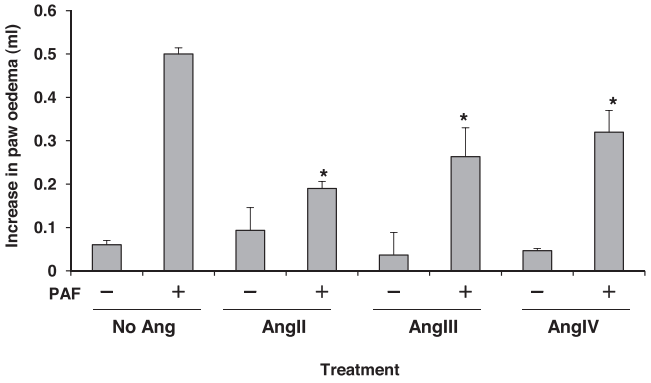

Fig. 3. Effects of Ang peptides on PAF-induced rat paw oedema. Rats were treated with by intraplantar injection with PAF (2 nmol/paw) with AngII, Ang III, or AngIV (0.75 nmol/paw). Forty-five minutes after the injection, the paw oedema was quantified by measuring the increase in paw oedema (ml). Each value represents the mean ± S.D. (n = 3–4). $^{*}P < 0.05$ compared to PAF alone-treated rats (analysis using the Student's t-test).

2.2. Measurement of PAF-induced rat paw oedema

Animal care and experimental procedures complied with the principles and guidelines of the Japanese Council on Animal Care and they were also approved by the Animal Care and Use Committee for Iwaki Meisei University (approval no 14-3).

Male Wistar rats (200–250 g body weight) were obtained from CLEA Japan, Inc. (Tokyo, Japan). Measurements of PAF-induced rat hind paw oedema were conducted as described in previous studies [14–16]. All injections were performed with sterile 1-mL syringes with 27-gauge needles (Terumo Corporation, Tokyo, Japan) under ether anaesthesia. The subplantar surface of the hind paw of the rat was injected with PAF (100 μL; 2 nmol/paw), which was dissolved in sterile solution (vehicle) containing 0.25% BSA, 10 mM tris(hydroxymethyl) aminomethane (Tris; pH 7.5), and 150 mM sodium chloride, in the absence or presence of AngII (0.25, 0.5, and 0.75 nmol/paw), AngIII (0.75 nmol/paw), and AngIV (0.75 nmol/paw), and then sonicated for 5 min. After 45 min of the PAF stimulus (the time of peak response of the paw oedema induced by PAF), paw oedema was quantified by measuring the increase in paw volume (mL) using a water displacement method. The effect of AngII on PAF-induced paw oedema was also investigated in the presence of one of the major oxidized phospholipids, POVPC (2 and 4 nmol/paw).

To examine whether two Ang receptors, AT_1 and AT_2 receptors, are related in the effect of Ang peptides on PAF-induced inflammation, the mixture of a selective AT_1 receptor antagonist (losartan; 100 nmol/paw) and a selective AT_2 receptor antagonist (PD123319; 100 nmol/paw) was injected into the subplantar surface of the hind paw 45 min prior to PAF (2 nmol/paw) with AngII (0.75 nmol/paw) injection [5]. After 45 min of the PAF stimulus (the time of peak response of the paw oedema induced by PAF), paw oedema was quantified. The reported IC_{50} values of AngII, losartan, and PD123319 for AT_1 receptor against ^{125}I-[Sar^1, Ile^8] AngII binding *in vitro* are 7.92 nmol/L, 8–50 nmol/L, and >10 μmol/L, respectively, while those for AT_2 receptor are 0.52 nmol/L, >10 μmol/L, and 5.6 nmol/L, respectively [17,18].

2.3. Tyrosine fluorescence spectroscopy

To examine the interaction of Ang peptides with various phospholipids, we investigated the changes in the intrinsic tyrosine fluorescence of the peptide in the presence of lipids. PAF C16, lyso-PAF C16, or POVPC (each 0–30 μmol/L) was incubated for 30 min at 37 °C in PBS (10 mM phosphate and 150 mM sodium chloride, pH