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Similar coronary vascular effects in the rat perfused heart of platelet-activating factor structural analogues with agonist and antagonist properties

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1 Selective blockade of platelet-activating factor (PAF) receptor subtypes by PAF receptor antagonists has been demonstrated. However, selective activation of PAF receptor subtypes by PAF receptor agonists has not been reported.

2 When structural analogues of PAF that have been shown to possess either agonist or antagonist effects were administered by a bolus injection in the rat perfused heart, they all showed agonist effects. Lower amounts produced vasodilatation while higher amounts produced vasodilatation followed by vasoconstriction. These coronary vascular effects were typical of that observed with PAF. Lyso-PAF did not show the same typical pattern of coronary vascular effect, confirming that the detergent effect of PAF structural analogues did not play a role in the coronary vascular effects. Other PAF antagonists, CV-6209 and WEB 2170, also did not produce the PAF-like response in the rat perfused heart.

3 The coronary vascular effects of hexanolamine-PAF (H-PAF, putative antagonist) and ethanolamine-PAF (E-PAF, agonist) were further studied. Pretreatment with FR-900452 (a PAF receptor antagonist) or MK-886 (a leukotriene synthesis inhibitor) significantly reduced the vasodilator and vasoconstrictor effects of H-PAF and E-PAF.

4 Pretreatment of rat perfused hearts with low concentrations of H-PAF and E-PAF blocked the response to PAF administration in a dose- and time-dependent manner. However, the pretreatment with either H-PAF or E-PAF did not result in a coronary vascular effect expected of a PAF receptor agonist. These results were compatible with H-PAF and E-PAF behaving as PAF receptor antagonists.

5 In summary, our results demonstrate that several PAF structural analogues possess agonist action in the rat perfused heart. Like the coronary vascular effects of PAF, the effects of H-PAF and E-PAF were blocked by a PAF antagonist (FR-900452) and a leukotriene synthesis inhibitor (MK-886). This suggests that both H-PAF and E-PAF mediate their effect through activation of PAF receptors with a subsequent release of leukotrienes that produced vasodilatation and vasoconstriction. Furthermore, pretreatment of perfused hearts with these compounds blocked the response to PAF in these hearts. Thus these compounds can also behave like a PAF receptor antagonist. This latter action may be due to a gradual receptor inactivation or desensitization by the pretreatment of H-PAF and E-PAF through a PAF receptor agonist effect rather than being a PAF receptor antagonist.

Keywords: Platelet-activating factor structural analogues; agonist; antagonist; rat perfused heart

Introduction

Platelet-activating factor (PAF) is a phospholipid which has been shown to possess a wide range of biological actions (Braquet *et al.*, 1987). PAF showed specific binding suggestive of the presence of a specific receptor site (Valone *et al.*, 1982; Hwang *et al.*, 1983). The existence of a PAF receptor was confirmed by the molecular cloning and sequencing of the receptor (Honda *et al.*, 1991). PAF has potent vascular effects in the heart (Piper & Stewart, 1986; 1987). Depending on the experimental conditions and the amount of PAF, it has been shown to produce vasodilatation or vasodilatation followed by vasoconstriction in the rat perfused heart (Man *et al.*, 1990). It has been demonstrated that the functionally opposite vasodilator and vasoconstrictor effects of PAF could be selectively abolished by different PAF antagonists in the rat perfused heart. Selectivity for blocking the vasodilator effect of PAF was observed with CV-6209 and selectivity for blocking the vasoconstrictor effect was observed with FR-900452, WEB 2086 and BN-50739 (Hu & Man, 1991). These results are compatible with the concept of the existence of PAF receptor subtypes. The activation of the first PAF receptor subtype produces vasodilatation and the activation of the second PAF receptor subtype produces vasoconstriction.

Although selectivity for blocking PAF receptor subtypes by antagonists was demonstrated in our previous study (Hu & Man, 1991), selectivity for activating PAF receptor subtypes by different agonists in the heart has not been reported. Several PAF structural analogues were screened for their agonist effects in the rat perfused heart. During the course of this study, we observed that the putative PAF receptor antagonist possibly with partial agonist effect, hexanolamine-PAF (H-PAF, Grigoriadis & Stewart, 1991), demonstrated significant agonist action. The present study was designed to investigate the coronary vascular effects of PAF structural analogues that have been reported to possess agonist and antagonist actions in the rat perfused heart. The mechanism(s) of action of these PAF analogues in the rat perfused heart was also examined.

Methods

Heart perfusion

Male Sprague-Dawley rats (250-300 g) were killed by cervical dislocation a minimum of 15 min after heparin (1000 units

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kg^{-1}, i.p.) and diazepam (5 mg kg^{-1}, i.p.) were administered. Hearts were rapidly excised and the Langendorff technique was used to perfuse hearts with Krebs-Henseleit solution maintained at 37 ± 0.5°C and gassed with 95% O_2/5% CO_2. The composition of Krebs-Henseleit solution was as follows (mM): NaCl 120, KCl 4.7, NaH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 1.25, $NaHCO_3$ 25 and glucose 5.5. The heart was allowed to beat spontaneously and was perfused at a constant flow rate. The perfusion pressure was measured by a pressure transducer attached to a side arm of the aortic cannula. Changes in perfusion pressure were recorded on a chart recorder and were also monitored with a digital display.

Drug preparation and administration

All hearts were equilibrated for 20 min in Krebs-Henseleit solution and the flow rate was gradually adjusted to obtain a perfusion pressure of 65–75 mmHg. The flow rate was then maintained constant for the rest of the experiment. Under the experimental conditions, vasodilatation resulted in a decrease in perfusion pressure and vasoconstriction resulted in an increase in perfusion pressure (Man *et al.*, 1990).

All test solutions were made fresh daily. PAF, PAF structural analogues, lyso-PAF and CV-6209 were dissolved in saline (0.9% NaCl) containing 0.25% bovine serum albumin (BSA). WEB 2170 was dissolved in ethanol then diluted with Krebs-Henseleit solution (final concentration, 0.1% ethanol). A bolus injection of these compounds was given in a volume of 0.1 ml over a 1 s period into the perfusion line 5–6 cm proximal to the aortic cannula. The maximum changes in coronary perfusion pressure as well as the time at which these changes occurred were monitored. Only one test compound was administered to each heart for the assessment of its coronary vascular effects. In separate experiments, the effects of bolus injections of PAF structural analogues were tested after pretreating the hearts with FR-900452 (a PAF receptor antagonist, Okamoto *et al.*, 1986) or MK-886 (a leukotriene synthesis inhibitor, Gillard *et al.*, 1989). In these experiments, an aliquot of FR-900452 or MK-886 stock solution was added to the perfusate 10 min before testing the effects of the bolus injection. FR-900452 and MK-886 were dissolved in ethanol, and the final concentration of ethanol in the perfusate was 0.1%. This amount of ethanol has previously been shown not to affect the perfusion pressure and the response to PAF (Hu & Man, 1991). The ability of PAF structural analogues to block the coronary vascular effects of PAF was examined by pretreatment with these compounds. The appropriate amount of PAF structural analogue stock solution was added to the perfusate and the heart was then perfused with this solution for 5–20 min. The effect of a bolus injection of 100 pmol PAF was measured after the appropriate period of pretreatment.

Materials

PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), carbamyl-PAF (C-PAF, 1-*O*-hexadecyl-2-N-methyl-carbamyl-*sn*-glycero-3-phosphocholine), methyl-PAF (M-PAF, 1-*O*-hexadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine), lyso-PAF (1-*O*-alkyl-*sn*-glycero-3-phosphocholine) and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). Hexanolamine-PAF (H-PAF, 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phospho (N,N,N-trimethyl) hexanolamine) was obtained from Biomol Research Laboratories Inc. (Plymouth Meeting, PA). Pyrrolidino-PAF (P-PAF,1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phospho (N-methylpyrroilidino) ethanolamine) and ethanolamine-PAF (E-PAF, 1-*O*-hexadecyl-2-*O*-ethyl-*sn*-glycero-3-phosphocholine) were obtained from Cascade Biochem Ltd. (Reading, Berkshire, England). CV-6209 (2-[N-acetyl-N-(2-methoxy-3-octadecylcarba-moyloxypropoxycarbonyl)-aminomethyl]-1-ethylpyridinium chloride) was provided by Takeda Chemical Industries (Osaka, Japan). WEB 2170 ((5-(2-chloro-phenyl)-3,4-dihydro-10-methyl-3-((4-morpholinyl) carbonyl)-2H,7H-cyclopenta(4,5)thieno [3,2-f] [1,2,4]triazolo-[4,3-a][1,4]diazepine) was provided by Boehringer Ingelheim KG (Federal Republic of Germany). FR-900452 ((1-methyl-3-(1-(5-methylthiomethyl-6-oxo-3-(2-oxo-3-cyclopenten-1-ylidene)-2-piperazinyl)ethyl)-2-indolinone) was provided by Fuji-sawa Pharmaceutical Co. Ltd. (Tsukuba, Japan). MK-886 (3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2,2-dimethylpropanoic acid) was supplied by Merck Frosst Canada Inc. (Montreal, Canada). All other chemicals were of reagent grade and were obtained from Mallinckrodt Specialty Chemicals Canada Inc. (Mississauga, Canada).

Statistical analyses

Data were analyzed by analysis of variance (ANOVA) followed by Student-Newman-Keuls test where appropriate. Bonferroni correction was used for multiple comparisons with Student's *t* test. Significant changes in perfusion pressure were determined by the 95% confidence interval and the Bonferroni correction was used when multiple doses of the same compound were tested. Values are expressed as mean ± standard deviations (s.d.) and $P < 0.05$ was considered to be statistically significant.

Results

Effects of PAF analogues on the coronary perfusion pressure of the rat perfused heart

Figure 1 shows the effects of various amounts of PAF structural analogues administered by a bolus injection on the perfusion pressure in rat perfused hearts. All the compounds had the same qualitative effect as PAF. Lower amounts of these PAF structural analogues produced vasodilatation (decrease in perfusion pressure) while higher amounts produced vasodilatation followed by vasoconstriction (increase in perfusion pressure). In addition, M-PAF also produced significant vasodilatation and vasoconstriction (−19.8 ± 8.5 and 15.8 ± 9.7 mmHg decrease and increase in perfusion pressure respectively by 10 nmol, $n = 17$ and −15.5 ± 7.4 and 31.2 ± 13.4 mmHg by 100 nmol, $n = 6$). P-PAF was the most potent among all the PAF structural analogues tested. Vasodilatation was produced by 1 pmol of P-PAF and vasodilatation followed by vasoconstriction was initiated by 10 pmol or higher (Figure 1). This is similar to the potency of PAF reported in

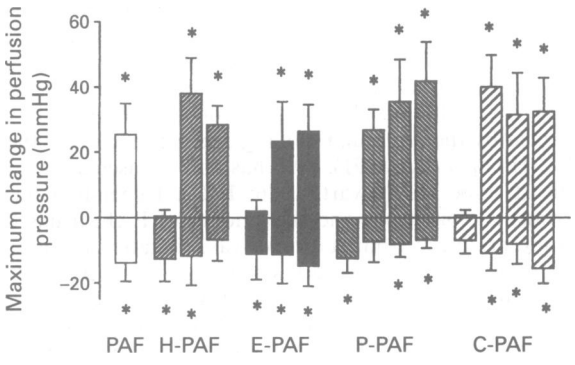

Figure 1 The effect of PAF, H-PAF, E-PAF, P-PAF and C-PAF on the maximum change in perfusion pressure in rat perfused hearts. PAF and PAF structural analogues were administered by a bolus injection and amounts used were: PAF 100 pmol, $n = 10$; H-PAF 100 pmol, 1 nmol and 10 nmol, $n = 13$, 12 and 5 respectively; E-PAF 100 pmol, 1 nmol and 10 nmol, $n = 7$, 11 and 5 respectively; P-PAF 1 pmol, 10 pmol, 100 pmol and 1 nmol, $n = 5$, 6, 10 and 8 respectively; C-PAF 10 pmol, 100 pmol, 1 nmol and 10 nmol, $n = 5$, 7, 8 and 5 respectively. Values represent mean ± s.d. *Significant ($P < 0.05$) decrease or increase in perfusion pressures produced by the bolus injection of the respective PAF structural analogues.

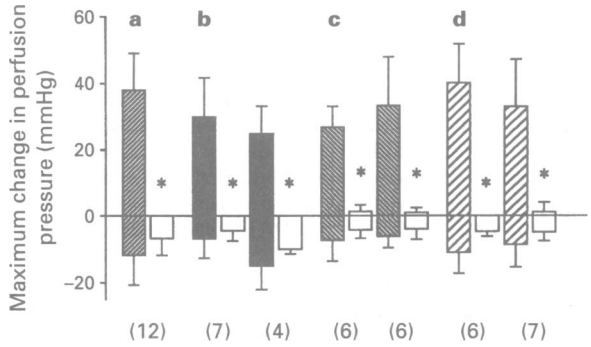

Figure 2 The effect of a second injection of PAF structural analogue on the perfusion pressure 10 min after the first injection with the same compound. (a) Shows the effect of a first injection of 1 nmol H-PAF (left hatched columns) and the effect of a second injection (adjacent open columns); (b) shows the effect of a first injection of 1 and 10 nmol E-PAF (solid columns) and the effect of a second injection (corresponding adjacent open columns); (c) shows the effect of a first injection of 10 and 100 pmol P-PAF (right hatched columns) and the effect of a second injection (corresponding adjacent open columns); (d) shows the effect of a first injection of 100 pmol and 1 nmol C-PAF (wide left hatched columns) and the effect of a second injection (corresponding adjacent open columns). Values represent mean ± s.d. and values in parentheses represent the number of hearts used in each group. $*P<0.05$ when compared to the effect of the corresponding first injection by Student's t test for paired data. Bonferroni correction was used for data in panels (b), (c) and (d).

our previous study (Man *et al.*, 1990). Based on the minimum amount sufficient to produce both vasodilatation and vasoconstriction, C-PAF was 10 times, and E-PAF and H-PAF were 100 times less potent than P-PAF and PAF.

After the first injection of PAF to the perfused heart, the coronary vascular response to a second injection of PAF was reduced or abolished (Piper & Stewart 1986; 1987; Man *et al.*, 1990). The effects of a second injection of a PAF structural analogue were examined 10 min after the first injection of the same PAF structural analogue. The vasoconstrictor effect was nearly eliminated while the vasodilator effect was attenuated in most cases (Figure 2). This reduction of the coronary vascular effect after a first injection of PAF structural analogues in the rat perfused heart was similar to that observed previously with PAF (Man *et al.*, 1990; Hu *et al.*, 1991a).

PAF and PAF structural analogues have a significant detergent property at high concentrations. When large amounts of PAF structural analogues are used, the coronary vascular effects can be produced by a combination of the direct effect on the PAF receptor and the non-specific effect. To assess this non-specific action, a compound with no direct effect on the PAF receptor such as lyso-PAF but with similar structural and detergent properties can be used. When a very large amount of lyso-PAF (100 nmol, $n=6$) was administered by a bolus injection, an immediate but transient vasoconstriction followed by vasodilatation was occasionally observed ($n=3$). This effect of 100 nmol lyso-PAF, an amount 10 times more than the highest amounts used for H-PAF, E-PAF and C-PAF, did not result in the typical pattern of vasodilatation nor vasodilatation followed by vasoconstriction produced by PAF and the PAF structural analogues used in this study. The effects of bolus injections of CV-6209 and WEB 2170 (PAF receptor antagonists with and without structural similarity to PAF, respectively, Terashita *et al.*, 1987; Meade & Heuer, 1990) were also examined. Bolus injection of 1 nmol CV-6209 had little effect on perfusion pressure (-1.5 ± 1.4 mmHg and no detectable increase in perfusion pressure, $n=6$) and 10 nmol CV-6209 often resulted in cardiac arrhythmias (2 of 6). Bolus injection of 1 nmol WEB 2170 also had little effect on perfusion pressure (data not shown, $n=4$).

Effects of FR-900452 and MK-886 on the vasodilatation and vasoconstriction produced by H-PAF and E-PAF in the rat perfused heart

Since H-PAF had been previously reported to be a PAF receptor antagonist, it is interesting that it produced the same qualitative response on the coronary perfusion pressure of the rat heart as PAF. If the coronary vascular effects of H-PAF observed in the present experiments are mediated by the activation of PAF receptors, then its effect should be blocked by a PAF receptor antagonist. In addition, we have also shown that the vasodilator and vasoconstrictor effects of PAF in the rat perfused heart are mediated through leukotrienes and can be blocked by a leukotriene synthesis inhibitor (Hu *et al.*, 1991b). To determine whether the effects of H-PAF were mediated directly through PAF receptors, the effects of bolus injections of H-PAF were investigated after hearts were pretreated with 50 μM FR-900452 or 10 μM MK-886. These results are illustrated in Figure 3. In this study, 10 nmol H-PAF which produced consistent vasodilatation and vasoconstriction was first used. Pretreatment with FR-900452 blocked only the vasoconstrictor effect of H-PAF and had no effect on the vasodilator effect (Figure 3). However, the vasodilatation and vasoconstriction produced by a smaller amount of H-PAF (1 nmol) were significantly attenuated by pretreatment with 50 μM FR-900452 (decrease and increase in perfusion pressure were: -15.2 ± 4.0 and 26.4 ± 14.0 mmHg, with no pretreatment, and -1.8 ± 1.8 and 3.0 ± 1.9 mmHg, after pretreatment with 50 μM FR-900452, $n=5$ and 4 respectively). Pretreatment with 10 μM MK-886 abolished the vasodilatation and vasoconstriction produced by 10 nmol H-PAF (Figure 3). E-PAF, another PAF structural analogue that produced a biphasic effect in the rat heart, was also examined for comparison with that observed with H-PAF. Pretreatment with 50 μM FR-900452 or 10 μM MK-886 significantly reduced the vasodilator and vasoconstrictor effects of 1 nmol E-PAF (Figure 3).

H-PAF and E-PAF pretreatment on the coronary vascular effects of PAF

The ability of H-PAF to serve as a PAF receptor antagonist was investigated in the rat perfused heart. The vasodilatation and vasoconstriction produced by bolus injections of 100 pmol PAF were examined under control conditions (no pretreatment) or after 10 min pretreatment with 1×10^{-7} or 3×10^{-7} M

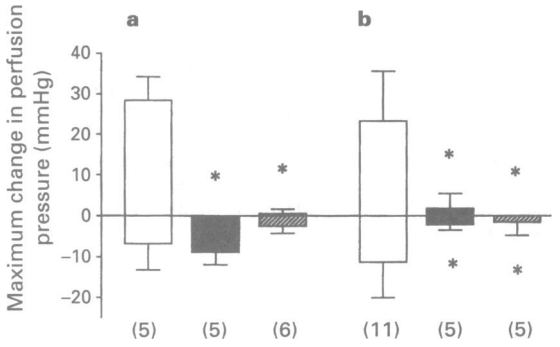

Figure 3 The effects of pretreatment with FR-900452 (50 μM) and MK-886 (10 μM) on the vasodilator and vasoconstrictor effects of 10 nmol H-PAF and 1 nmol E-PAF. (a) Shows the changes in perfusion pressure induced by 10 nmol H-PAF in controls (no pretreatment, open columns) and after pretreatment with FR-900452 (solid columns) and MK-886 (hatched columns); (b) shows the changes in perfusion pressure induced by 1 nmol E-PAF in controls (no pretreatment, open columns) and after pretreatment with FR-900452 (solid columns) and MK-886 (hatched columns). Values represent mean ± s.d. and values in parentheses represent the number of hearts used in each group. Individual differences were identified by ANOVA followed by the Student-Newman-Keuls method. $*P<0.05$ when compared to the respective controls.

H-PAF. For comparison, E-PAF which produced similar agonist effects to H-PAF as described in the previous section was also examined. The results are summarized in Figure 4. Pretreatment with either concentrations of H-PAF or E-PAF were able to significantly attenuate the vasodilatation and vasoconstriction produced by PAF. Although not statistically significant, the higher concentration appeared to reduce the effect of PAF to a greater extent. The vasodilatation and vasoconstriction produced by bolus injections of 100 pmol PAF were reduced by the pretreatment with 1×10^{-7} M H-PAF and E-PAF in a time-dependent manner (Figure 5).

During the pretreatment with 3×10^{-7} M H-PAF and E-PAF, an initial fall in perfusion pressure (vasodilatation) was observed (Table 1). In 2 of 7 hearts perfused with H-PAF and 1 of 8 hearts perfused with E-PAF, subsequent increases in perfusion pressure were observed. In contrast, perfusion of hearts containing 1×10^{-8} M PAF resulted in vasodilatation followed by vasoconstriction typical of a bolus injection of PAF (Table 1). Pretreatment for 10 min with this very low concentration of PAF significantly reduced the coronary vascular effect of PAF (-1.3 ± 2.8 and 2.7 ± 2.3 mmHg decrease and increase in perfusion pressure produced by a bolus injection of 100 pmol PAF, $n=6$).

Discussion

In the present study, all PAF structural analogues exhibited PAF-like coronary vascular effects in the rat perfused heart. Lower amounts produced vasodilatation while higher amounts produced vasodilatation followed by vasoconstriction. As with the effects of PAF reported in our previous study (Man *et al.*, 1990), maximum vasodilatation occurred after 10–20 s after a bolus injection and maximum vasoconstriction occurred at 60–90 s. Although differing in potency (i.e. different amounts may be required to elicit vasodilatation or vasodilatation followed by vasoconstriction), a full coronary vascular effect similar to that achieved by PAF was observed with all compounds tested. Hence all PAF structural analogues used in this study can be considered to be full agonists. Regarding the potency of the PAF structural analogues, P-PAF was reported to be more potent than PAF (Coeffier *et al.*, 1986). C-PAF was 5 times less potent than PAF (O'Flaherty *et al.*, 1987). E-PAF was about 100 times less potent than PAF in releasing 5-hydroxytryptamine from rabbit platelets and required 10 times more in degradation and desensitization of human neutrophils (Wykle *et al.*, 1981). These potency ratios are similar to that observed in the perfused heart.

H-PAF also demonstrated the typical agonist response in this study but H-PAF is considered to be a PAF receptor antagonist. It should be noted that the PAF structural analogues often referred to as H-PAF here and elsewhere (1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phospho(N,N,N-trimethyl)-hexanolamine) has a small difference in structure from the other PAF structural analogue, U66985 (1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phospho(N,N,N-trimethyl)hexanolamine), first reported to be an effective PAF receptor antagonist (Tokumura *et al.*, 1985). Since H-PAF is commercially available

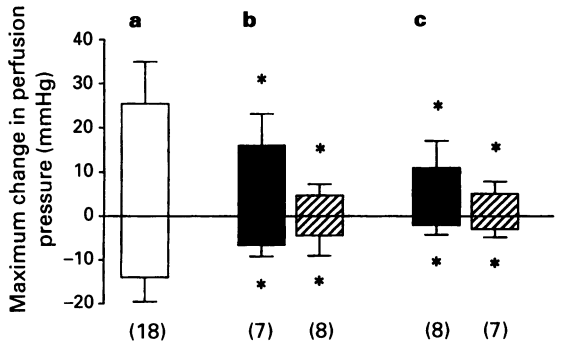

Figure 4 The effect of pretreatment of rat perfused hearts with H-PAF and E-PAF on the vasodilator and vasoconstrictor effects of 100 pmol PAF: (a) shows the response to PAF under control conditions (no pretreatment, open columns); (b) and (c) show the response to PAF after pretreatment for 10 min with 1×10^{-7} M (solid columns) and 3×10^{-7} M (hatched columns) H-PAF and E-PAF respectively. Values represent mean ± s.d. and values in parentheses represent the number of hearts used in each group. Individual differences were identified by ANOVA followed by the Student-Newman-Keuls method. *$P<0.05$ when compared to the value with no pretreatment (a).

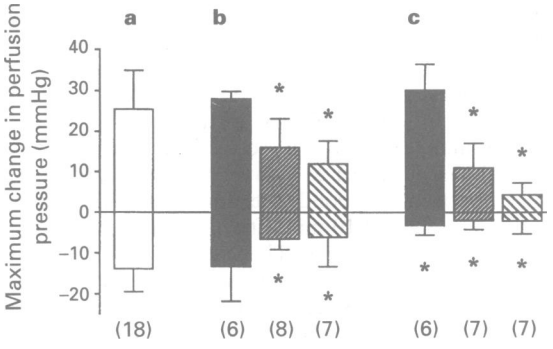

Figure 5 Time-dependent effects of pretreatment with H-PAF and E-PAF on the vasodilatation and vasoconstriction produced by 100 pmol PAF in the rat perfused heart: (a) shows the response to PAF under control conditions (no pretreatment, open columns); (b) and (c) show the response to PAF after pretreatment for 5 min (solid columns), 10 min (narrow hatched columns) and 20 min (wide hatched columns) with 1×10^{-7} M H-PAF and E-PAF respectively. Values represent mean ± s.d. and values in parentheses represent the number of hearts used in each group. Individual differences were by ANOVA followed by the Student-Newman-Keuls method. *$P<0.05$ when compared to the value with no pretreatment (a).

Table 1 Changes in perfusion pressure during 10 min of pretreatment of hearts with a perfusate containing H-PAF, E-PAF and PAF

	Decrease in perfusion pressure		*Increase in perfusion pressure*		
	Maximum (mmHg)	*Time* (s)	*Maximum* (mmHg)	*Time* (s)	n
H-PAF (3×10^{-7} M)	-10.3 ± 6.3*	166 ± 135	22, 44[a]	–	7
E-PAF (3×10^{-7} M)	-12.0 ± 4.3*	70 ± 13	12[b]	–	8
PAF (1×10^{-8} M)	-9.3 ± 5.0*	58 ± 6	25.5 ± 13.8*	158 ± 46	6

For abbreviations, see text.
Time represents the average time at which maximum change occurred. Values represent mean ± s.d and *n* the number of experiments in each group.
*$P<0.05$ for the decrease or increase in perfusion pressure.
[a]Only 2 of 7 hearts had a detectable increase in perfusion pressure; the average time for the maximum constriction to occur was not calculated, [b]Only 1 of 8 hearts had a detectable increase in perfusion pressure; the average time for the maximum constriction to occur was not calculated.

while U66985 is not, H-PAF has been used in many investigations and assumed to have the same properties. Indeed many studies and catalogues referring to H-PAF as a PAF receptor antagonist, cited the paper by Tokumura *et al.* (1985) and Buxton *et al.* (1986) where U66985 was used instead of H-PAF. Indeed, it has been reported that H-PAF showed a partial agonist effect (Grigoriadis & Stewart, 1991). It was pointed out in this study that the difference in the structure (1-*O*-hexadecyl and 1-*O*-octadecyl form of hexanolamine PAF, i.e. H-PAF and U66985 respectively) may be responsible for being a partial agonist or an antagonist. Since H-PAF was only able to produce 50% of the maximum PAF response, H-PAF was considered to be a partial agonist (Grigoriadis & Stewart, 1991). This is in contrast to the full agonist response demonstrated by H-PAF in the present study. The differences may be due to the use of platelet aggregation assay and superoxide anion generation in macrophages in the previous study and the use of rat perfused hearts in this study to assess the agonist effects of H-PAF.

In addition to the similarity of the coronary vascular effects of H-PAF, E-PAF, P-PAF and C-PAF to that produced by PAF in the rat perfused heart, our results also provided additional evidence that the coronary vascular effects of H-PAF and E-PAF are mediated via activation of the PAF receptor. This conclusion is confirmed by the ability of 50 μM FR-900452, a PAF receptor antagonist (Okamoto *et al.*, 1986), to block the effect of H-PAF and E-PAF in the heart. This concentration of FR-900452 has been shown to be capable of blocking both the vasodilatation and vasoconstriction produced by 100 pmol PAF (Hu & Man, 1991) although a lower concentration of FR-900452 (5 μM) was effective only in blocking the vasoconstrictor effect of PAF (Hu *et al.*, 1991b; Hu & Man, 1991). In this study, the effect of 1 nmol H-PAF was blocked by FR-900452 more effectively than the effect of 10 nmol H-PAF. This suggested competition for the PAF receptor sites between FR-900452 and H-PAF. The activation of PAF receptors in the heart is followed by the synthesis and release of leukotrienes which are responsible for both the vasodilatation and vasoconstriction (Hu *et al.*, 1991b). The use of a leukotriene synthesis inhibitor, MK-886, also enabled us to confirm that the action of these PAF structural analogues is through PAF receptor activation. Since MK-886 blocked the synthesis of leukotrienes, the ability of MK-886 to block the effect of PAF or PAF structural analogues does not involve competition for the PAF receptor sites. This mechanism of action predicts that larger amounts of the agonist cannot overcome the effect of pretreatment with MK-886. Indeed the ability of MK-886 to block the effect of H-PAF was not affected even when higher amounts of H-PAF (10 nmol) were used.

Although the putative PAF receptor antagonist, H-PAF, showed full agonist effects in the present study, this was not so for two other PAF antagonists tested. CV-6209 and WEB 2170, PAF receptor antagonists with and without structural similarity to PAF (Terashita *et al.*, 1987; Meade & Heuer, 1990), were examined in this study. When administered in the same manner as that used for the PAF structural analogues, CV-6209 and WEB 2170 did not exhibit any agonist effect in the perfused heart. The results with lyso-PAF indicated that the indirect detergent effect can produce a small effect on coronary vessels, but this action is different from the effect through PAF receptor activation. Vasoconstriction was detected first after a bolus injection of lyso-PAF and this effect was transient lasting only several seconds. In contrast, the typical response due to PAF receptor activation was vasodilatation first followed by a prominent vasoconstriction that peaked at about 60–90 s. Hence it is unlikely that the detergent effect associated with the use of a rather high amount of PAF structural analogues in this study may contribute to the coronary vascular effects in the perfused heart.

If H-PAF and E-PAF are full agonists, then the ability of pretreatment with H-PAF and E-PAF to block the effect of PAF may seem contradictory. This intriguing discrepancy can be explained on the following basis. The mechanism of H-PAF and E-PAF pretreatment in blocking the PAF receptor is via subthreshold activation of the PAF receptor leading to PAF receptor inactivation and/or desensitization. This will lead to blockade of the PAF receptor and inhibition of subsequent response to PAF in the heart. The same will be expected in repeated injections of PAF in the perfused rat heart (as reported in previous studies). The ability of PAF structural analogues to diminish the subsequent response after a single bolus injection of these compounds is also confirmed in this study. Indeed our data also demonstrated that the presence of a low concentration of PAF in the perfusate can lead to inactivation and/or desensitization of the PAF receptors in the heart. The main difference is that the interventions with PAF were always associated with a significant coronary vascular effect in the heart with subsequent blocking of the PAF effects. In contrast, the pretreatments with H-PAF and E-PAF were not associated with a consistent vasoconstriction (Table 1) although a vasodilator response was observed. The key may be the ability of H-PAF and E-PAF to produce a gradual subthreshold activation, since we did not observe the typical vasodilatation and vasoconstriction when PAF is given by bolus injection or by continuous infusion that can then block the subsequent PAF response.

In summary, we demonstrated that several PAF structural analogues produce a full agonist response in the rat perfused heart. However, pretreatment with a low concentration of these compounds can inactivate and/or desensitize the PAF receptor without producing prominent vascular effects. These compounds may therefore give the apparent appearance of PAF receptor antagonists. Our results suggested that before they can be classified as agonist or antagonist, their effects should be studied with an appropriate model. Since both agonist and antagonist will compete for the binding with the PAF receptor, receptor binding studies will not be sufficient to distinguish between whether the PAF structural analogues have PAF receptor agonist or antagonist properties.

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References

BRAQUET, P., TOUQUI, L., SHEN, T.Y. & VARGAFTIG, B.B. (1987). Perspectives in platelet-activating factor research. *Pharmacol. Rev.*, **39**, 97–145.

BUXTON, D.B., HANAHAN, D.J. & OLSON, M.S. (1986). Specific antagonists of platelet activating factor-mediated vasoconstriction and glycogenolysis in the perfused rat liver. *Biochem. Pharmacol.*, **35**, 893–897.

COEFFIER, E., BORREL, M.C., LEFORT, J., CHIGNARD, M., BROQUET, C., HEYMANS, F., GODFROID, J.J. & VARGAFTIG, B.B. (1986). Effects of PAF-acether and structural analogues on platelet activation and bronchoconstriction in guinea-pigs. *Eur. J. Pharmacol.*, **131**, 179–188.

GILLARD, J., FORD-HUTCHINSON, A.W., CHAN, C., CHARLESON, S., DENIS, D., FOSTER, A., FORTIN, R., LEGER, S., MCFARLANE, C.S., MORTON, H., PIECHUTA, H., RIENDEAU, D., ROUZER, C.A., ROKACH, J., YOUNG, R., MACINTYRE, D.E., PETERSON, L., BACH, T., EIERMANN, G., HOPPLE, S., HUMES, J., HUPE, L., LUELL, S., METZGER, J., MEURER, R., MILLER, D.K., OPAS, E. & PACHOLOK, S. (1989). L-663,536 (MK-886) (3-[1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can. J. Physiol. Pharmacol.*, **67,** 456–464.

GRIGORIADIS, G. & STEWART, A.G. (1991). 1-*O*-hexadecyl-2-acetyl-*sn*-glycer-3-phospho(N,N,N trimethyl)hexanolamine: an analogue of platelet-activating factor with partial agonist activity. *Br. J. Pharmacol.*, **104,** 171–177.

HONDA, Z., NAKAMURA, M., MIKI, I., MINAMI, M., WATANABE, T., SEYAMA, Y., OKADO, H., TOH, H., ITO, K., MIYAMOTO, T. & SHIMIZU, T. (1991). Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. *Nature*, **349,** 342–346.

HU, W., CHOY, P.C. & MAN, R.Y.K. (1991a). Characterization of the coronary vascular responses to platelet-activating factor in the isolated perfused heart. *Lipids*, **26,** 700–704.

HU, W., KINNAIRD, A.A.A. & MAN, R.Y.K. (1991b). Mechanisms of the coronary vascular effects of platelet-activating factor in the rat perfused heart. *Br. J. Pharmacol.*, **103,** 1097–1102.

HU, W. & MAN, R.Y.K. (1991). Differential actions of platelet-activating factor (PAF) receptor antagonists on the vasodilator and vasoconstrictor effects of PAF in the rat perfused heart. *Br. J. Pharmacol.*, **104,** 773–775.

HWANG, S.-B., LEE, C.-S.C., CHEAH, M.J. & SHEN, T.Y. (1983). Specific receptor sites for 1-*O*-alkyl-1-*O*-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor) on rabbit platelet and guinea pig smooth muscle membranes. *Biochemistry*, **224,** 4756–4763.

MAN, R.Y.K., HU, W. & KINNAIRD, A.A.A. (1990). Coronary vascular response to platelet-activating factor in the perfused rat heart. *J. Lipid Mediators*, **2,** 75–83.

MEADE, C.J. & HEUER, H.O. (1990). Hetrazepines as PAF antagonists. In *Platelet-activating Factor Antagonists. New Developments for Clinical Application.* ed. O'Flaherty, J.T. & Ramwell, P.W. pp. 47–80. Texas: Portofolio Publishing.

O'FLAHERTY, J.T., REDMAN JR, J.F., SCHMITT, J.D., ELLIS, J.M., SURLES, J.R., MARX, M.H., PIANTADOSI, C. & WYKLE, R.L. (1987). 1-*O*-alyky-2-*N*-methylcarbamyl-glycerophosphocholine: a biologically potent, non-metabolizable analog of platelet-activating factor. *Biochem. Biophys. Res. Commun.*, **147,** 18–24.

OKAMOTO, M., YOSHIDA, K., NISHIKAWA, M., ANDO, T., IWAMI, M., KOHSAKA, M. & AOKI, H. (1986). FR-900452, a specific antagonist of platelet activating factor (PAF) produced by Streptomyces Phaeofaciens. *J. Antibiotics*, **39,** 198–204.

PIPER, P.J. & STEWART, A.G. (1986). Coronary vasoconstriction in the rat, isolated perfused heart induced by platelet-activating factor is mediated by leukotriene C4. *Br. J. Pharmacol.*, **88,** 595–605.

PIPER, P.J. & STEWART, A.G. (1987). Antagonism of vasoconstriction induced by platelet-activating factor in guinea-pig perfused hearts by selective platelet-activating factor receptor antagonists. *Br. J. Pharmacol.*, **90,** 771–783.

TERASHITA, Z., IMURA, Y., TAKATANI, M., TSUSHIMA, S. & NISHIKAWA, K. (1987). CV-6206, a highly potent antagonist of platelet activating factor *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.*, **242,** 263–268.

TOKUMURA, A., HOMMA, H. & HANAHAN, D.K. (1985). Structural analogs of alkylacetylglycerophosphocholine inhibitory behaviour on platelet activation. *J. Biol. Chem.*, **260,** 12710–12714.

VALONE, F.H., COLES, H.E., REINHOLD, V.R. & GOETZL, E.J. (1982). Specific binding of phospholipid platelet-activating factor by human platelets. *J. Immunol.*, **129,** 1637–1641.

WYKLE, R.L., MILLER, G.H., LEWIS, J.C., SCHMITT, J.D., SMITH, J.A., SURLES, J.R., PIANTADOSI, C. & O'FLAHERTY, J.T. (1981). Stereospecific activity of 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphocholine and comparison of analogs in the degradation of platelets and neutrophils. *Biochem. Biophys. Res. Commun.*, **100,** 1651–1658.

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