Partial agonist effect of the platelet-activating factor receptor antagonists, WEB 2086 and WEB 2170, in the rat perfused heart

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1 WEB 2086 and WEB 2170 are potent platelet-activating factor (PAF) receptor antagonists and have been used widely as pharmacological tools to investigate the actions of PAF in a variety of biological systems.

2 Low concentrations of WEB 2086 and WEB 2170 blocked the vasoconstrictor action of PAF in the rat perfused heart. In this study, we observed that moderate concentrations of WEB 2086 and WEB 2170 increased the perfusion pressure in rat isolated hearts under constant flow perfusion. The vasoconstrictor actions of WEB 2086 and WEB 2170 were not observed with a structurally different PAF receptor antagonist, FR-900452.

3 To determine whether this vasoconstrictor action of WEB 2086 involved non-specific effects or was via the activation of PAF receptors, hearts were pretreated with 1000 pmol PAF or 50 μ M FR-900452. These pretreatments attenuated the vasoconstrictor action of 1 μ M WEB 2086, suggesting that the action of WEB 2086 may be mediated via PAF receptors. Pretreatment with the leukotriene receptor antagonist (L-649,923, 5 μ M) and the leukotriene synthesis inhibitor (MK-886, 10 μ M) that are known to block the vasoconstrictor action of PAF receptor activation also attenuated the vasoconstrictor action of WEB 2086. Pretreatment with PAF or MK-886 attenuated the vasoconstrictor action of 0.5 μ M WEB 2170.

4 When PAF receptors were activated by PAF in the perfused heart, significant amounts of leukotriene C₄ and leukotriene C₄/D₄/E₄ were detected in the coronary effluent. However, no significant amount of these leukotrienes was detected in the coronary effluent when hearts were perfused with 1 μ M WEB 2086 or 0.5 μ M WEB 2170.

5 In summary, our results indicate that WEB 2086 and WEB 2170 possess partial agonist effects in the rat perfused heart where they produced vasoconstriction via the activation of PAF receptor. This action could be attenuated by PAF pretreatment or a PAF receptor antagonist. The vasoconstrictor action of WEB 2086 and WEB 2170 involved the production of leukotrienes. But unlike the vasoconstrictor action of PAF, no significant amount of leukotrienes was detected in the effluent suggesting that the vasoconstrictor action of WEB 2086 and WEB 2170 may be explained on the basis of intracellularly or locally produced leukotrienes.

Keywords: Platelet-activating factor receptor antagonists; WEB 2086; WEB 2170; FR-900452; partial agonist; rat perfused heart

Introduction

Platelet-activating factor (PAF) is a lipid mediator that has a variety of biological actions (Braquet *et al.*, 1987) it has been demonstrated to bind to the cell membrane with high affinity suggesting that its action is mediated by the activation of specific receptors located on the cell membrane (Valone *et al.*, 1982; Hwang *et al.*, 1983). The PAF receptor has now been cloned and sequenced (Honda *et al.*, 1991) thus confirming the existence of a specific receptor for PAF. To examine the biological effects of PAF, a number of PAF receptor antagonists have been developed. These antagonists have been useful in demonstrating many pathophysiological roles of PAF including platelet activation and thrombosis, shock, allergy and immune responses (Braquet *et al.*, 1987). Using receptor binding assays, several studies show that PAF receptor antagonists compete with PAF for the same high affinity site and prevent the activation of the receptor by PAF (Braquet *et al.*, 1987; Saunders & Handley, 1987). In the rat perfused heart, PAF produces a vasodilatation followed by a vasoconstriction (Man *et al.*, 1990). Leukotriene B₄ (LTB₄) may be involved in the vasodilator effect while LTC₄ and/or LTD₄ may be involved in the vasoconstrictor

effect in the perfused heart (Hu *et al.*, 1991b). We have recently examined the selectivity of a number of PAF receptor antagonists for their ability to block the vasodilator and vasoconstrictor actions of PAF in the rat perfused heart (Hu & Man, 1991a). During the course of this study, we observed that WEB 2086 showed a small vasoconstrictor action during the 10 min pretreatment period. This effect may be due to a non-specific effect of WEB 2086 on the coronary blood vessels. Alternatively, WEB 2086 may produce vasoconstriction if it possesses a partial agonist action on the PAF receptor. Although there is no report on commonly used PAF receptor antagonists with a partial agonist effect, there is a recent paper indicating that a PAF structural analogue (hexanolamine PAF, a putative PAF receptor antagonist) showed partial agonist activity (Grigoriadis & Stewart, 1991). The present study was designed to investigate if WEB 2086 and its structural analogue WEB 2170 possess partial agonist action.

Methods

Rat heart perfusion

Following cervical dislocation, hearts from Sprague-Dawley rats (250–350 g) were rapidly excised and placed in cool

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Krebs-Henseleit solution (4°C) oxygenated with 95% O₂: 5% CO₂. The solution had the following composition (in mM): NaCl 120, NaH₂PO₄ 1.18, MgSO₄ 1.18, KCl 4.76, CaCl₂ 1.25, NaHCO₃ 25.0, and glucose 5.5. The aorta was cannulated for coronary perfusion. The heart was allowed to beat spontaneously. The temperature of the perfusate was maintained at 37 ± 0.5°C and the coronary flow was controlled by a roller pump. The perfusion pressure was measured by a pressure transducer attached to a side arm of the aortic cannula. The perfusion pressure was recorded on a Gould chart recorder and monitored with a digital display of the perfusion pressure. The detailed methodology of the isolated heart perfusion system has been described previously (Man *et al.*, 1990). All stock solutions to be used in subsequent sections were made fresh daily and kept at 4°C between experiments. In these experiments, only one concentration of the drug was used in each heart.

Vasoactive effect of PAF receptor antagonists

Hearts were equilibrated with Krebs-Henseleit solution for 20 min. During the equilibration period, the flow rate was adjusted to obtain a control perfusion pressure of 65–75 mmHg. The vasoactive actions of PAF receptor antagonists were determined by the perfusion of the heart with Krebs-Henseleit solution containing the appropriate amount of the PAF receptor antagonist for 10 min under constant flow perfusion. The vasoactive effect was measured as changes in the perfusion pressure (Man *et al.*, 1990). WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]-diazepine-2-yl]-1-(4-morpholinyl)-1-propanone, Boehringer Ingelheim, KG) and WEB 2170 (5-(2-chlorophenyl)-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, Boehringer Ingelheim, KG) were dissolved in Krebs-Henseleit solution. FR-900452 (1-methyl-3-(1-(5-methylthiomethyl-6-oxo-3-(2-oxo-3-cyclopenten-1-ylidene)-2-piperazinyl)ethyl)-2-indolinone, Fujisawa Pharmacological Co., Japan) was dissolved in ethanol then diluted in Krebs-Henseleit solution to a final concentration of 50 µM in 0.1% ethanol. This amount of ethanol has been shown not to affect the coronary vascular effects of PAF (Hu *et al.*, 1991b).

Effect of WEB 2170 on the vasodilator and vasoconstrictor effects of PAF

Following a 20 min equilibration period, hearts were pretreated with a solution containing the appropriate amount of WEB 2170 for 10 min. PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, prepared from bovine heart, Sigma Chemical Co.) was prepared in saline (0.9% NaCl) containing 0.25% bovine serum albumin (Sigma Chemical Co.). A bolus injection of PAF (100 pmol) was given in a volume of 0.1 ml and over a 1 s period, into the perfusion line 5–6 cm proximal to the aortic cannula. Changes in perfusion pressure were recorded. To serve as controls, the effect of PAF was determined in hearts with no WEB 2170 pretreatment.

Mechanism of vasoconstrictor action of WEB 2086 and WEB 2170

To determine the involvement of PAF receptors in the action of WEB 2086 and WEB 2170, PAF receptors were blocked by PAF pretreatment or FR-900452. For pretreatment with PAF, a bolus injection of 1000 pmol of PAF was given in a volume of 0.1 ml. The perfusion pressure returned to baseline level with 5 min and pretreatment with PAF has been shown to abolish any subsequent response to PAF in the perfused heart (Piper & Stewart, 1986; Hu *et al.*, 1991a). Changes in perfusion pressure induced by 1 µM WEB 2086 were determined after the PAF pretreatment. In separate experiments, the effect of 0.5 µM WEB 2170 on the perfusion pressure was examined after the same PAF pretreatment. The effect of

WEB 2086 was also determined after a 10 min pretreatment with 50 µM FR-900452.

The leukotriene receptor antagonist, L-649,923 (sodium (βS*, R*)-4-(3-(4-(acetyl-4-hydroxy-2-propylphenoxy)-propylthio)-γ-hydroxy-β-methylbenzenebutanoate, Merck Frosst Canada Inc., Jones *et al.*, 1986) was dissolved in distilled water and the leukotriene synthesis inhibitor, MK-886 (3-[1-(4-chlorobenzyl)-3-*t*-butylthio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid, Merck Frosst Canada Inc., Gillard *et al.*, 1989) was dissolved in ethanol, and then diluted in Krebs-Henseleit solution to a concentration of 5 µM and 10 µM respectively. The effects of WEB 2086 and WEB 2170 were examined in separate experiments after the heart had been pretreated for 10 min with either L-649,923 or MK-886. The cyclo-oxygenase inhibitor, indomethacin, was added to the perfusion solution at the start of the stabilization period and the vasoactive action of the PAF receptor antagonist was tested after 45 min of perfusion in the presence of indomethacin.

Radioimmunoassay for leukotrienes

Leukotrienes were determined by a competitive binding radioimmunoassay using a commercially available kit from Amersham International (U.K.). Samples of effluent from rat perfused isolated heart were collected into test tubes for periods of 0–2, 2–5 and 5–10 min after various protocols. Samples were capped and stored at –20°C. The concentration of leukotrienes in the effluent from rat perfused heart was determined without prior extraction or purification.

LTC₄: effluent (100 µl) from perfused rat heart was mixed with 100 µl of [5, 6, 8, 9, 11, 12, 14, 15 (n)-³H]-LTC₄, followed by 100 µl of specific antiserum to LTC₄ and 100 µl of assay buffer. The mixture was incubated overnight at 4°C. Then 500 µl of dextran-coated charcoal was added to the mixture in an ice-bath for 10 min to remove unbound leukotrienes. After centrifugation at 2,000 g for 10 min at 4°C, the supernatant was decanted into 10 ml of aqueous scintillation fluid and the radioactivity measured in a β-scintillation counter (Beckmann). The concentration of LTC₄ in each sample was determined from the standard curve. The sensitivity of the LTC₄ assay was 8 pg/tube.

LTC₄/D₄/E₄: effluent (100 µl) from perfused rat heart was mixed with 100 µl of [14, 15 (n)-³H]-LTC₄, followed by 100 µl of antiserum to the leukotrienes and 100 µl of assay buffer. The mixture was incubated overnight at 4°C. Then 250 µl of dextran-coated charcoal was added to the mixture in an ice-bath for 15 min to remove unbound leukotrienes. After centrifugation at 2,000 g for 15 min at 4°C, the supernatant was decanted into 10 ml of aqueous scintillation fluid and the radioactivity measured in a β-scintillation counter. The concentration of leukotrienes in each sample was determined from a standard curve. The sensitivity for LTC₄ in this assay was 12.5 pg/tube. Compared to LTC₄ (100%), the cross-reactivity for LTD₄ was 181.8% and the cross-reactivity for LTE₄ was 92.7%.

Statistical analyses

Data were analysed by the Student's *t* test and analysis of variance (ANOVA) followed by Duncan's test where appropriate. Values are expressed as means ± standard deviations (s.d.) and *P* < 0.05 was considered statistically significant.

Results

Effects of WEB 2086 and WEB 2170 on the perfusion pressure of rat perfused hearts

In our previous study, we demonstrated that pretreatment with a low concentration of WEB 2086 (0.5 µM) blocked the vasoconstrictor action of 100 pmol PAF in the rat perfused

heart while a higher concentration of WEB 2086 (100 μM) blocked both the vasodilator and vasoconstrictor actions of PAF (Hu & Man, 1991a). However a gradual increase in the perfusion pressure with some concentrations of WEB 2086 was observed during the pretreatment period with this PAF receptor antagonist. This time course of perfusion pressure change was much slower than that observed with PAF (Figure 1). Since a 10 min pretreatment period was used in our previous study, the same time period was used to determine changes in perfusion pressure after 10 min of perfusion in the presence of WEB 2086. Statistically significant increases in perfusion pressure were observed with 1 μM of WEB 2086, a moderate concentration of WEB 2086 (65.5 ± 0.7 and 80.9 ± 5.7 mmHg before and after 10 min of perfusion, $n = 10$, $P < 0.001$). This concentration of WEB 2086 was chosen for subsequent experiments. For comparison, WEB 2170, a structurally similar PAF receptor antagonist was also studied. A gradual increase in perfusion pressure in the presence of WEB 2170 was also observed (Figure 1). This is in contrast to the much faster time course for the coronary vascular effect of PAF (Figure 1). The increase in perfusion pressure by various concentrations of WEB 2170 is shown in Figure 2. Concentrations of 0.02–1.0 μM WEB 2170 produces consistent vasoconstriction in the rat perfused heart. In order to determine the ability of WEB 2170 to block the vasodilator and vasoconstrictor effects of PAF, experiments

were conducted with a 10 min perfusion period with various concentrations of WEB 2170 prior to testing the coronary vascular effect of 100 pmol PAF. A low concentration of WEB 2170 (0.02 μM) did not affect the vasodilator nor vasoconstrictor effects of 100 pmol PAF while 0.05, 0.1 and 0.5 μM WEB 2170 blocked the vasoconstrictor effect of PAF but had no effect on the vasodilator effect of PAF (Table 1). WEB 2170 (1 μM) appeared to block both the vasodilator and vasoconstrictor effects of PAF. Since an equivalent concentration of WEB 2170 to WEB 2086 (1 μM) would be more potent than WEB 2086 in blocking the vasodilator effect of PAF in the perfused heart, 0.5 μM of WEB 2170 was used for further studies.

Unlike WEB 2086 and WEB 2170, FR-900452, a PAF receptor antagonist with a different chemical structure (Okamoto *et al.*, 1986), did not affect the perfusion pressure at a concentration that blocked both the vasodilatation and vasoconstriction produced by 100 pmol PAF (66.0 ± 0.9 and 68.3 ± 4.6 mmHg before and after 10 min of perfusion with a solution containing 50 μM FR-900452, $n = 8$, $P > 0.05$).

The mechanism for the vasoconstrictor action of WEB 2086 and WEB 2170

Pretreatment with PAF or FR-900452 had been shown to be effective in blocking the coronary vascular effect of PAF in the rat heart (Piper & Stewart, 1986; Hu *et al.*, 1991a). These pretreatments attenuated the vasoconstrictor action of 1 μM WEB 2086 (Figure 3). These results suggested that the action of WEB 2086 may be mediated via PAF receptors. Pretreatment with the leukotriene receptor antagonist L-649,923 (5 μM) or the leukotriene synthesis inhibitor MK-886 (10 μM) has been shown to block the vasoactive actions of PAF (Hu *et al.*, 1991b). These pretreatments also significantly attenuated the vasoconstrictor action of WEB 2086 (Figure 4). Pretreatment with 2.8 μM indomethacin had no significant effect on the vasoconstrictor action of WEB 2086 (14.8 ± 7.3 mmHg, $n = 4$). Pretreatment with 1000 pmol PAF or MK-886 significantly attenuated the vasoconstrictor action of 0.5 μM WEB 2170 (Figure 5).

Determination of leukotrienes in the coronary effluent

The vasoconstrictor action of PAF in the rat perfused heart has been shown to be due to the release of leukotrienes (Piper & Stewart, 1986; Hu *et al.*, 1991b; Hu & Man, 1991b).

Figure 1 Time course of typical perfusion pressure changes by 1 μM WEB 2086 (a), 0.5 μM WEB 2170 (b) and 100 pmol PAF (c). The time scale is 2 min for (a) and (b) and 1 min for (c). Vertical scale represents the perfusion pressure in mmHg. Arrows indicate the time of application of the various treatments. Values in each panel represent the baseline perfusion pressure and are highlighted by the dotted lines.

Figure 2 Effect of various concentrations of WEB 2170 on the perfusion pressure of rat perfused hearts. Changes in perfusion pressure were recorded at the end of a 10 min perfusion period in the absence or presence of various concentrations of WEB 2170. Values represent mean $\pm$ s.d., n = number of hearts used in each group.

Table 1 Effect of WEB 2170 on the vasodilator and vasoconstrictor effects of 100 pmol PAF in the rat perfused heart^a

Concentration of WEB 2170 (μM)	Peak vasodilator effect (mmHg) ^b	Peak vasoconstrictor effect (mmHg) ^b	n
0	-8.6 ± 5.5	27.8 ± 4.8	8
0.02	-14.6 ± 5.0	21.3 ± 9.7	5
0.05	-14.2 ± 3.7	NC	5
0.1	-10.6 ± 1.1	NC	5
0.5	-9.5 ± 4.6	NC	5
1.0	$-3.4 \pm 2.3^*$	NC	5

Values represent mean $\pm$ s.d., n = number of experiments. NC denotes no detectable change (increase) in perfusion pressure. Statistical analysis were performed by ANOVA followed by Duncan's test.

* $P < 0.05$ when compared to the appropriate data in the absence of WEB 2170.

^aHearts were perfused for 10 min with various concentrations of WEB 2170; 100 pmol PAF was administered as a single bolus injection. Each heart received only one concentration of WEB 2170 and one injection of PAF.

^bWith constant flow perfusion, decrease in perfusion pressure represents vasodilatation (negative value) and increase in perfusion pressure represents vasoconstriction.

Figure 3 Effect of pretreatment with 1000 pmol PAF bolus injection and 50 μ M FR-900452 on perfusion pressure changes by 1 μ M WEB 2086 in rat perfused hearts. Values represent mean $\pm$ s.d., n = number of hearts used in each group. Control indicates no pretreatment. * P < 0.05 and ** P < 0.01 when compared to the corresponding control value.

Figure 4 Effect of pretreatment with 10 μ M MK-886 and 5 μ M L-649,923 on perfusion pressure changes by 1 μ M WEB 2086 in rat perfused hearts. Values represent mean $\pm$ s.d., n = number of hearts used in each group. Control indicates no pretreatment. * P < 0.05 when compared to the corresponding control value.

Figure 5 Effect of pretreatment with 1000 pmol PAF bolus injection and 10 μ M MK-886 on perfusion pressure changes by 0.5 μ M WEB 2170 in rat perfused hearts. Values represent mean $\pm$ s.d., n = number of hearts used in each group. Control indicates no pretreatment. ** P < 0.01 when compared to the corresponding control value.

The amount of LTC_4 and the combined amount of $\text{LTC}_4/\text{D}_4/\text{E}_4$ in the effluent were measured by radioimmunoassay. Significant amounts of leukotrienes were detected in the coronary effluent during the initial 2 min after 1000 pmol PAF was administered (Table 2). This time interval corresponded to the maximal effect of PAF after a bolus injection. Since the effects of WEB 2086 and WEB 2170 on perfusion pressure were gradual, effluents were collected at 0–2, 2–5 and 5–10 min intervals. No detectable or very small amounts of

Table 2 The concentration of leukotrienes in the coronary effluent after a bolus injection of PAF, and perfusion with 1 μ M WEB 2086 and 0.5 μ M WEB 2170 in the rat perfused heart^a

	PAF (1000 pmol)	WEB 2086 (1 μ M)	WEB 2170 (0.5 μ M)
<i>LTC₄</i> (pg ml ⁻¹ effluent)			
0–2 min	1183 $\pm$ 293 (3)	10 $\pm$ 20 (4)	30 $\pm$ 52 (3)
2–5 min		– (4)	17 $\pm$ 21 (3)
5–10 min		– (4)	30 $\pm$ 52 (3)
<i>LTC₄/D₄/E₄</i> (pg ml ⁻¹ effluent)			
0–2 min	1700 $\pm$ 360 (3)	66 $\pm$ 31 (4)	43 $\pm$ 49 (4)
2–5 min		70 $\pm$ 50 (4)	– (4)
5–10 min		53 $\pm$ 41 (4)	23 $\pm$ 45 (4)

Values represent mean $\pm$ s.d. Number in parentheses indicated the number of experiments.

– denotes no detectable amount.

^aLeukotrienes were measured by radioimmunoassay and each sample was determined in duplicate. Background amounts of LTC_4 and $\text{LTC}_4/\text{D}_4/\text{E}_4$ (perfusion with normal buffer only) were 45 $\pm$ 17 and 78 $\pm$ 73 pg ml⁻¹ effluent respectively (n = 4).

leukotrienes were found in the effluent from hearts perfused with 1 μ M WEB 2086 or 0.5 μ M WEB 2170 at all intervals examined (Table 2).

Discussion

The major finding of this study was the moderate coronary vasoconstrictor actions of the PAF receptor antagonist, WEB 2086 and its structural analogue, WEB 2170. We propose that this action is mediated by the partial agonist effect of WEB 2086 and WEB 2170. The evidence in support of a partial agonist action of WEB 2086 and WEB 2170 is as follows: pretreatment with a bolus injection of PAF that would abolish subsequent response to PAF in the perfused heart, significantly attenuated the vasoconstriction produced by WEB 2086 and WEB 2170; FR-900452, a PAF receptor antagonist (Okamoto *et al.*, 1986) that we showed to be devoid of any significant coronary vascular effect in the heart, also attenuated the vasoconstriction produced by WEB 2086; the activation of PAF receptors by PAF in the heart led to the production of vasoactive leukotrienes and the vasoconstriction could be blocked by leukotriene receptor antagonists and a leukotriene synthesis inhibitor (Piper & Stewart, 1986; Hu *et al.*, 1991b). Our results also demonstrated that MK-886 and L-649,923 decreased the vasoconstrictor action of WEB 2086 and WEB 2170.

It should be noted that the vasoconstrictor action of WEB 2086 was not completely abolished by pretreatment with PAF, FR-900452, MK-886 or L-649,923 at concentrations that had been shown to block completely the coronary vascular effects of PAF in our previous studies (Hu *et al.*, 1991b; Hu & Man, 1991a). Previous studies showed that PAF may block subsequent responses to PAF in the heart by receptor desensitization or depletion of vasoactive leukotrienes release (Piper & Stewart, 1986; Man *et al.*, 1990; Hu *et al.*, 1991a). Since the coronary vascular effects of PAF involve the release of leukotrienes (Hu *et al.*, 1991b), the leukotriene synthesis inhibitor MK-886 and the leukotriene receptor antagonist L-649,923 should be effective in blocking the effect of PAF. It has been suggested that cyclo-oxygenase products are involved in mediating the vasoconstrictor action of PAF in the heart (Piper & Stewart, 1986; 1987). However, our results indicated that the partial agonist action of WEB 2086 did not involve vasoactive cyclo-oxygenase products since indomethacin had no effect on the vasoconstrictor action of WEB 2086 under our experimental conditions. This is compatible with our previous data showing that the coronary

vascular effects of PAF via the activation of PAF receptors in the heart did not involve vasoactive cyclo-oxygenase products directly although cyclo-oxygenase products were present in the coronary effluent (Hu & Man, 1991b). Our results therefore suggest that a component of the vasoconstrictor action of WEB 2086 may be due to non specific action on the coronary blood vessels.

It is intriguing that activation of PAF receptors by PAF was accompanied by the release of leukotrienes into the coronary effluent while the partial agonist action of WEB 2086 and WEB 2170, though involving leukotrienes and attenuated by a leukotriene synthesis inhibitor or a leukotriene receptor antagonist, does not result in significant amounts of leukotrienes in the coronary effluent. This suggests that the vasoconstrictor action of WEB 2086 and WEB 2170 may be mediated by intracellularly or locally produced leukotrienes. Hence no significant amount of leukotrienes can be found in the coronary effluent in the presence of WEB 2086 and WEB 2170.

An alternate hypothesis to explain the present results may be that WEB 2086 and WEB 2170 release PAF. Pretreatment with PAF and FR-900452 would therefore be effective in attenuating the vasoconstrictor effect. Since the coronary vascular effect of PAF is mediated by leukotrienes, pretreatment with L-649,923 and MK-886 would also be expected to have an effect. One difficulty with this hypothesis is that if PAF is released by WEB 2086 and WEB 2170, then detectable amounts of leukotrienes in the coronary effluent should be observed as a result of PAF released. Moreover, PAF produces an initial vasodilatation. However, vasodilatation was not observed with WEB 2086 and WEB 2170 in the present study.

In our previous study (Hu & Man, 1991a) and this study, low concentrations of WEB 2086 and WEB 2170 blocked the vasoconstrictor effect of PAF and at higher concentrations blocked the vasodilator effect of PAF in the rat perfused heart. Thus WEB 2086 and WEB 2170 should be considered to possess antagonist action under these experimental conditions. Since a partial agonist can occupy the PAF receptors and prevent further activation of PAF receptors by a full agonist, WEB 2086 and WEB 2170 with partial agonist actions would be capable of blocking the actions of PAF. However, we were not able to define the partial agonist action of WEB 2086 and WEB 2170 as a percentage of a full agonist such as PAF since the time course of pressure changes initiated by WEB 2086 and WEB 2170 were very different from that of the agonist PAF. In contrast to the slow time course of WEB 2086 and WEB 2170 on perfusion pressure changes (peak effect not observed even after 5 min, Figure 1), the effect of PAF was much faster. Peak effect was observed within 1 to 2 min with PAF and the perfusion pressure gradually returned to baseline level by 5 min even with a continuous infusion of PAF (Man *et al.*, 1990).

Currently, there has been no report of a partial agonist action of most commonly used PAF receptor antagonists. WEB 2086 is considered to be a PAF receptor antagonist devoid of agonist action in most systems tested (Meade & Heuer, 1990). However, the results from the present study suggest a partial agonist effect of WEB 2086 in the rat heart. If there are PAF receptor subtypes as suggested by recent reports (Lambrecht & Parnham, 1986; Hwang, 1988; Stewart & Dusting, 1988; Kroegel *et al.*, 1989; Hu & Man, 1991a), then it is possible that WEB 2086 only has partial agonist action on one receptor subtype and is a full antagonist on the other receptor subtype. If the PAF receptor subtype where WEB 2086 is a full antagonist is predominant in most systems, then it will be difficult to notice the partial agonist action of WEB 2086 on the other PAF receptor subtype that is not dominant. Since our previous data suggested that the rat heart contained both PAF receptor subtypes, we would expect to observe both the full antagonist and the partial agonist actions of WEB 2086. However, the presence of PAF receptor subtypes remains to be fully elucidated.

Recently hexanolamine PAF, a structural analogue of PAF with putative PAF receptor antagonist action, had been shown to exhibit partial agonist action (Grigoriadis & Stewart, 1991). Although the 1-*O*-hexadecyl form of hexanolamine PAF showed partial agonist activity, the 1-*O*-octadecyl form (U66985) was devoid of agonist activity (Tokumura *et al.*, 1985). It is also of interest that U66983, with 3 methylene residues between the phosphate and choline nitrogen, showed agonist activity while U66982, with 10 methylene residues, had weak antagonist activity (Tokumura *et al.*, 1985). The possible sites for the partial agonist and antagonist actions of WEB 2086 and WEB 2170 are currently not known.

WEB 2170, a structural analogue of WEB 2086, has been shown to be slightly more potent than WEB 2086 and has similar properties (Meade & Heuer, 1990). Our results also suggested that WEB 2170, like WEB 2086, has partial agonist action and produced coronary vasoconstriction. In contrast, FR-900452, a structurally different PAF receptor antagonist, does not share this property with WEB 2086 and WEB 2170. It remains to be determined if other commonly used PAF receptor antagonists behave as full receptor antagonists or under certain circumstances act as partial agonists as in the case of WEB 2086 and WEB 2170.

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