Br. J. Pharmacol. (1991), **104**, 773–775 **SPECIAL REPORT**

Differential actions of platelet-activating factor (PAF) receptor antagonists on the vasodilator and vasoconstrictor effects of PAF in the rat perfused heart

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Selectivity for blocking the coronary vasodilator and vasoconstrictor effects of platelet-activating factor (PAF) in the rat perfused heart was observed with different PAF antagonists. CV-6209 showed selectivity for blocking the vasodilator effect of PAF and a higher concentration (10 fold) was required to block the vasoconstrictor effect. The remaining PAF antagonists (FR-900452, WEB 2086 and BN-50739) showed selectivity for blocking the vasoconstrictor effect of PAF (10, 200 and 1000 fold respectively). A combination of low concentrations of CV-6209 (10 nM) with FR-900452 (5 μM) or WEB 2086 (0.5 μM) was effective in blocking both the vasodilator and vasoconstrictor effects of PAF. CV-6209 and WEB 2086 did not affect the vasodilator action of leukotriene B_4 (LTB_4) and the vasoconstrictor action of LTC_4 and LTD_4. Our results support the hypothesis that the functionally opposite effects of PAF in the rat perfused heart may be mediated by different PAF receptor subtypes.

Keywords: PAF receptor subtypes; vasodilatation; vasoconstriction; PAF antagonists

Introduction Platelet-activating factor (PAF) has been shown to possess a variety of biological activities and is a potent activator of platelet aggregation (Braquet *et al.*, 1987). The predominant coronary vascular effect of PAF is vasoconstriction (Piper & Stewart, 1986). However, vasodilatation, vasoconstriction and the combination of both responses could be observed in the rat perfused heart depending on the experimental conditions and the amount of PAF (Man *et al.*, 1990). These results may be explained by the existence of two PAF receptor subtypes (Hu *et al.*, 1991). The activation of the first subtype of PAF receptor produces vasodilatation while the activation of the second subtype of PAF receptor produces vasoconstriction. In the present study, the ability of different PAF antagonists to block the coronary vasodilator and vasoconstrictor effects of PAF selectively in the rat perfused heart were examined.

Methods Following cervical dislocation, hearts from Sprague-Dawley rats (250–350 g) were excised and the aorta was cannulated for coronary perfusion. The Krebs-Henseleit solution had the following composition (mM): NaCl 120, NaH_2PO_4 1.18, $MgSO_4$ 1.18, KCl 4.76, $CaCl_2$ 1.25, $NaHCO_3$ 25.0 and glucose 5.5, and was oxygenated with 95% O_2:5% CO_2. A 10 min equilibration period with normal Krebs-Henseleit solution was followed by 10 min of pretreatment with a solution containing a PAF antagonist. The flow rate was adjusted to maintain a perfusion pressure of 65–70 mmHg before the injection of PAF and the flow rate was kept constant for the rest of the experiment (Man *et al.*, 1990).

CV-6209 (2[N-acetyl-N-(2-methoxy-3-octadecylcarbamoyloxypropoxycarbonyl)-aminomethyl]-1-ethylpyridinium chloride) was dissolved in saline (0.9% NaCl) containing 0.25% bovine serum albumin (BSA, Sigma Chemical Co.). FR-900452 (1-methyl-3-(1-(5-methylthiomethyl-6-oxo-3-(2-oxo-3-cyclopenten-1-ylidene)-2-piperazinyl)ethyl)-2-indolinone) was dissolved in ethanol then diluted in Krebs-Henseleit solution to a final concentration of 1–50 μM in 0.1% ethanol. 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) was dissolved in saline. BN-50739 (tetrahydro-4,7,8,10 methyl-(chloro-2-phenyl)6[(dimethoxy-3,4-phenyl)thio] methythiocarbonyl-9 pyrido[4'3'-4,5]thieno[3,2-f] triazolo-1,2,4[4,3-a] diazepine-1,4) was dissolved in dimethyl sulphoxide (DMSO) and slowly added to warm (50°C) Krebs-Henseleit solution while stirring constantly to a maximum final concentration of 10 μM in 0.4% DMSO. PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (prepared from bovine heart, Sigma Chemical Co.), was prepared fresh daily in saline with 0.25% BSA. Only one injection of PAF was given to each heart. Bolus injections of 100 pmol PAF were 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. This amount of PAF elicited a reproducible vasodilatation followed by a vasoconstriction (Man *et al.*, 1990). LTB_4, LTC_4 and LTD_4 were prepared daily and were administered by continuous infusion (300 pmol min^{-1}).

Results Pretreatment with low concentrations of the PAF antagonist CV-6209 (10 and 50 nM) were effective in blocking the vasodilator effect of 100 pmol PAF and a higher concentration (100 nM) was required to abolish the vasoconstrictor effect (Table 1). Pretreatment with the other PAF antagonists showed a reverse pattern. The vasoconstrictor effect of PAF was abolished by a low concentration of FR-900452 (5 μM), WEB 2086 (0.5 μM) and BN-50739 (0.01 μM) while higher concentrations (50 μM FR-900452, 100 μM WEB 2086 and 10 μM BN-50739) were required to completely abolish the vasodilator effect (Table 1). When the vasodilator effect of PAF was abolished by a low concentration of FR-900452, WEB 2086 or BN-50739, a significantly greater vasodilator effect was observed. Pretreatment with 0.1% ethanol and 0.4% DMSO (the vehicle for FR-900452 and BN-50739 respectively) did not affect the effects of 100 pmol PAF. Bolus injections of 0.1 ml saline did not show any detectable coronary vascular effect. A combination of low concentrations of CV-6209 (10 nM) with FR-900452 (5 μM) or WEB 2086 (0.5 μM) was effective in blocking both the vasodilator and vasoconstrictor effects of 100 pmol PAF in the rat perfused heart (6 and 5 experiments, data not shown). Using the data from Table 1, approximately 10 fold selectivity for blocking the vasodilator effect of PAF than for the vasoconstrictor effect of PAF was observed for CV-6209 while a 10, 200 and 1000 fold selectivity for blocking the vasoconstrictor effect were observed for FR-900452, WEB 2086 and BN-50739. The effects of PAF antagonists on leukotriene responses were evaluated in hearts with and without pretreatment with CV-6209 and WEB 2086. With no pretreatment, infusion of LTB_4 (300 pmol min^{-1}) produced vasodilatation (peak decrease in perfusion pressure,

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Table 1 Effects of the PAF antagonists, CV6209, FR-900452, BN-50739 and WEB 2086, on the vasodilator and vasoconstrictor effects of 100 pmol PAF in the rat perfused heart

	Concentration	*Peak vasodilator effect* (mmHg)[a]	*Peak vasoconstrictor effect* (mmHg)[a]	n
CV-6209	0	−8.4 ± 2.2	29.2 ± 5.8	5
	10 nM	−2.2 ± 1.3**[b]	36.0 ± 9.7	5
	50 nM	−1.2 ± 2.1**	31.0 ± 15.0	5
	100 nM	−2.4 ± 1.9**	1.4 ± 1.9**[b]	5
FR-900452	0	−7.8 ± 1.7	26.3 ± 9.0	6
	1 μM	−13.6 ± 4.7*	23.8 ± 11.8	5
	3 μM	−15.3 ± 2.7*	20.8 ± 5.8	6
	5 μM	−14.5 ± 7.6*	NC[b]	6
	30 μM	−7.5 ± 4.9	NC	6
	50 μM	−1.6 ± 1.5*[b]	0.8 ± 2.0**	5
WEB 2086	0	−9.8 ± 3.9	24.3 ± 4.5	6
	0.1 μM	−15.0 ± 1.4	9.0 ± 3.6**	4
	0.5 μM	−18.4 ± 3.9*	NC[b]	5
	1 μM	−24.8 ± 6.0**	NC	6
	10 μM	−26.6 ± 5.5**	NC	4
	30 μM	−11.0 ± 2.9	0.3 ± 0.5**	5
	50 μM	−12.7 ± 4.0	0.3 ± 0.6**	4
	100 μM	−2.5 ± 2.1*[b]	0.2 ± 0.4**	6
BN-50739	0	−9.8 ± 4.6	23.4 ± 8.1	5
	0.001 μM	−11.7 ± 3.2	31.3 ± 2.1**	5
	0.01 μM	−15.4 ± 4.0*	0.8 ± 1.8**[b]	5
	0.1 μM	−18.0 ± 4.5**	0.3 ± 0.7**	8
	1 μM	−7.0 ± 1.4	NC	5
	5 μM	−3.0 ± 0.4**	NC	5
	10 μM	−0.3 ± 0.6**[b]	0.3 ± 0.5**	5

Values represent mean ± standard deviation, n = number of experiments. NC denotes no detectable change in perfusion pressure. Statistical analyses were performed by analysis of variance followed by Duncan's test. * $P < 0.05$ and ** $P < 0.01$ when compared to the appropriate data in the absence of the PAF antagonist.

[a] With constant flow perfusion, decrease in perfusion pressure represents vasodilatation (negative value), and increase in perfusion pressure represents vasoconstriction.

[b] Since PAF antagonists did not produce a progressive transition of blocking the vasodilator or vasoconstrictor effects of PAF, the lowest concentration that reduced each effect completely was chosen for the calculation of selectivity for the vasodilator and vasoconstrictor effects.

-11.5 ± 2.2 mmHg, $n = 6$) while LTC_4 and LTD_4 produced vasoconstriction (peak increase in perfusion pressure, 27.8 ± 4.5 and 26.3 ± 1.5 mmHg, $n = 6$ and 6 respectively) in the rat perfused heart. CV-6209 (100 nM) and WEB 2086 (100 μM) did not significantly affect the vasodilator effect of LTB_4 (-11.0 ± 1.8 and -10.2 ± 1.1 mmHg, $n = 4$ and 5 respectively) and the vasoconstrictor effect of LTC_4 (20.6 ± 4.4 and 35.6 ± 10.5 mmHg, $n = 5$ and 5 respectively) and LTD_4 (24.0 ± 1.6 and 31.8 ± 2.1 mmHg, $n = 4$ and 5 respectively).

Discussion The present study has demonstrated that functionally opposite vasodilator and vasoconstrictor effects of a single concentration of PAF could be selectively abolished by different PAF antagonists in the perfused heart. These results are compatible with the concept of existence of PAF receptor subtypes. 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. The presence of PAF receptor subtypes can also explain the different time course and the threshold amount of PAF for the PAF-induced vasodilatation and vasoconstriction in the heart (Man *et al.*, 1990).

The existence of PAF receptor subtypes has been postulated in a number of studies using different cell types in the same or different animal species. Based on the lower affinity of kadsurenone for pig peripheral leukocyte aggregation than for PAF-induced chemiluminescence of guinea-pig peritoneal macrophages (91 fold difference in pA_2 values), Lambrecht & Parnham (1986) suggested the presence of PAF receptor subtypes (named PAF_1 and PAF_2 receptors). Differences in potency (6–10 times) of ONO-6240 on PAF receptor binding and PAF-induced aggregation in human leukocytes and platelets also supported the presence of PAF receptor subtypes (Hwang, 1988). A dissociation between PAF-induced superoxide anion generation (high concentration of PAF, μM range), and intracellular degranulation and peroxidase release (low concentration of PAF, nM range) in guinea-pig eosinophils were observed (Kroegel *et al.*, 1989). High and low concentrations of WEB 2086 were also required to block these PAF-induced changes. However, different ionic conditions were present in the test systems because PAF-induced superoxide production was Mg^{2+}-dependent while PAF-induced peroxidase release was Ca^{2+}-dependent. Hence, they concluded that their results could be explained on the basis of two PAF receptor subtypes or a single receptor that can exist in low and high affinity states (Kroegel *et al.*, 1989). Indeed, Hwang *et al.* (1989) demonstrated that the conformation and affinity of the PAF receptor in rabbit platelet membranes could be regulated by the ionic environment, including Ca^{2+} and Mg^{2+} concentrations.

However, PAF receptors conformational change cannot fully explain data that showed selectivity of PAF antagonists on receptor binding in similar ionic environments (Hwang, 1988). Furthermore, the effects of the PAF antagonists, WEB 2086, L-652,731 and BN-52021, on the PAF-induced chemiluminescence and prostacyclin generation by guinea-pig resident peritoneal macrophages and on pig peripheral blood leukocyte and platelet aggregation also showed significant differences in pA_2 values and the rank order of potency of PAF antagonists between macrophages, and platelets and leukocytes (Stewart & Dusting, 1988). In this study, PAF antagonists showed selectivity for blocking the vasodilator and vasoconstrictor effects of PAF in the perfused heart and thereby provided further evidence for the existence of PAF receptor subtypes. Unlike previous studies where receptor subtypes exist in different cell types, our data suggest that receptor subtypes can exist in the same organ and initiate opposite effects although findings with different concentra-

tions of PAF need to be evaluated. However, definitive proof for the presence of PAF receptor subtypes awaits the purification and sequencing of these receptors. Moreover, the role of these putative PAF receptor subtypes in the heart and the functionally opposite vasodilator and vasoconstrictor effects in the regulation of coronary flow remains to be elucidated.

It has been proposed that the coronary vasodilator effect of PAF is mediated by LTB_4 whereas the vasoconstrictor effect of PAF is mediated by LTC_4 and LTD_4 (Hu *et al.*, 1991). It is possible that non-specific actions of the PAF antagonists may influence the leukotrienes-mediated vasodilator and vasoconstrictor effects of PAF. However, our results showed that the coronary vascular effects of leukotrienes were not significantly altered by CV-6209 and WEB 2086. Hence, the selectivity of PAF antagonists on the vasodilator and vasoconstrictor effects of PAF is not likely due to non-specific actions.

Our results showed that if the action of PAF involved multiple receptor subtypes, much higher concentrations of a single PAF antagonist would be required. However, our data suggest that it is possible to combine low concentrations of two selective PAF antagonists to produce the same effect, thus eliminating the need for high concentrations. This may be particularly noteworthy if high concentrations of PAF antagonists are associated with undesirable side effects.

The generous supplies of the PAF antagonists, CV-6209 by Takeda Chemical Industries, Japan, FR-900452 by Fujisawa Pharmaceutical Co., Ltd., Japan, WEB 2086 by Boehringer Ingelheim KG, Federal Republic of Germany and BN-50739 by Institut Henri Beaufour, France are greatly appreciated. We also wish to thank Dr. Ford-Hutchinson of Merck Frosst Canada Inc. for the supply of LTB_4, LTC_4 and LTD_4. This study was supported by the MRC of Canada.

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(Received September 20, 1991
Accepted September 27, 1991)