Br. J. Pharmacol. (1988), **94**, 1225–1233

Characterization of receptors for platelet-activating factor on platelets, polymorphonuclear leukocytes and macrophages

A.G. Stewart & G.J. Dusting

Department of Physiology, University of Melbourne, Parkville, Victoria 3052, Australia

1 We have compared the potency of the putative platelet-activating factor (Paf) receptor antagonists (WEB 2086, L-652,731 and BN 52021) against Paf-induced aggregation of rabbit and guinea-pig platelets, aggregation of rabbit polymorphonuclear leukocytes (PMNLs) and prostacyclin generation by guinea-pig resident peritoneal macrophages.

2 On rabbit washed platelets and PMNLs WEB 2086, L-652,731 and BN 52021 each antagonized competitively Paf-induced aggregation. The rank order of potency was WEB 2086 $\simeq$ L-652, 731 > BN 52021 and was the same for the two cell types.

3 The pA_2 values for each of the three antagonists were similar on rabbit washed platelets and PMNLs. Moreover, the pA_2 for WEB 2086 on rabbit platelets (7.58) did not differ significantly from that on guinea-pig platelets (7.69).

4 On guinea-pig resident peritoneal macrophages WEB 2086 was 10 fold less potent for receptors mediating increased generation of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) than for those mediating platelet aggregation.

5 The potencies of L-652,731 and BN 52021 were also markedly less (2 log units) for the macrophage receptors than for platelet or PMNL receptors and BN 52021 was more potent than L-652, 731 in the macrophages.

6 WEB 2086 and L-652,731 significantly reduced basal 6-oxo-$PGF_{1\alpha}$ produced by macrophages, but none of the antagonists affected 6-oxo-$PGF_{1\alpha}$ production during stimulation by A23187.

7 These data raise the possibility that there may be a Paf receptor-subtype mediating prostacyclin generation in macrophages that is different from that on the platelet and PMNL. Hence, the potency of Paf antagonists against platelet aggregation would not be a good predictor of antagonist potency in disorders involving macrophages.

Introduction

Platelet-activating factor (Paf) is one of a family of phospholipids unique in their ability to elicit biological effects at sub-nanomolar concentrations. Paf is released from and activates platelets, polymorphonuclear leukocytes (PMNLs) and macrophages in a concentration-dependent manner (Benveniste, 1985). These actions, together with marked effects on the cardiovascular and respiratory systems have raised considerable interest in its role in both anaphylaxis and inflammation (see Braquet *et al.*, 1987 for a review).

The recent availability of a number of inhibitors of Paf-induced effects has led to further investigation of its proposed roles in models of anaphylaxis and inflammation. Furthermore, on the basis of structural requirements for agonist activity, it has been suggested that Paf acts at specific receptor sites (Hwang *et al.*, 1983). Compounds such as BN 52021 (Braquet *et al.*, 1985; Nunez *et al.*, 1986), L-652,731 (Hwang *et al.*, 1985) and WEB 2086 (Casals-Stenzel *et al.*, 1987) have been described as selective, competitive Paf receptor antagonists for Paf-induced platelet aggregation and have been used to implicate Paf in cardiac anaphylaxis (Piper & Stewart, 1986a), endotoxin shock (Terashita *et al.*, 1985; Adnot *et al.*, 1986; Wu *et al.*, 1986; Casals-Stenzel, 1987a), pulmonary anaphylaxis (Touvay *et al.*, 1985; Vilain *et al.*, 1986) and allergic inflammation (Hellewell & Williams, 1986).

Despite the large number of putative receptor

© The Macmillan Press Ltd 1988

antagonists and the growing evidence of the probable importance of Paf in human disease, there have been few rigorous studies directed towards classification of Paf receptors. In many such studies only single concentrations of Paf have been used which preclude a comparison of antagonist potencies in the same system (usually aggregating platelets), and these studies also preclude comparisons of potency with other systems such as PMNL aggregation. However, there is evidence based on estimates of pA_2 values for kadsurenone that the macrophage possesses a receptor subtype for Paf not shared by platelets or PMNLs (Lambrecht & Parnham, 1986). Furthermore, the insensitivity of the PMNL receptor mediating shape changes to L-652,731 (Voelkel *et al.*, 1986) suggests that these receptors may be distinct from those mediating aggregation.

The present experiments were carried out to examine systematically pA_2 values for BN 52021, L-652,731 and WEB 2086 in platelets, PMNLs and guinea-pig resident peritoneal macrophages. We suggest that the Paf receptor on the macrophage is a distinct receptor subtype which shows lower affinity for Paf receptor antagonists than do platelet or PMNL receptors.

Methods

Isolation of platelets

Adult male rabbits (2–4 kg) were anaesthetized by intravenous administration of propanidid. Blood was collected via a cannula placed in the right carotid artery and immediately mixed with trisodium citrate (0.38% w/v, final concentration). Citrated platelet-rich plasma (PRP) was obtained by centrifugation (20 min at 150 *g*) at ambient temperature. Prostacyclin (PGI_2) was added to a final concentration of 300 ng ml^{-1} as an anticoagulant during the washing procedure (Vargas *et al.*, 1982) which involved isolating platelets by centrifugation (10 min at 1000 *g*) and resuspending in HEPES-buffered Tyrode solution (see below for composition). The platelets were washed twice, counted using an improved Neubauer chamber, resuspended in buffer (containing 1.8 mM Ca^{2+}) at a concentration of 2×10^8 ml^{-1} and kept at ambient temperature for 3 h during which time the effects of PGI_2 subsided. The HEPES-buffered Tyrode solution was of the following composition (mM): HEPES (N-2-hydroxyethyl piperazine-N-2-ethane sulphonic acid) 10, NaCl 137, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, KCl 2.7, $MgCl_2$ 0.26, D(+)-glucose 11, bovine serum albumin (BSA) 0.25% w/v, pH 7.4; solutions used for final resuspension contained $CaCl_2$ 1.8 mM). Platelets were isolated from male guinea-pigs (400–600 g) by the same method.

Isolation of polymorphonuclear leukocytes

Rabbit polymorphonuclear leukocytes (PMNLs) were isolated from peripheral blood (Haslett *et al.*, 1985) which was obtained as described above. Following the aspiration of citrated PRP from 50 ml whole blood, the pellet was mixed with 5 ml 6% (w/v) dextran (mol. wt., 5×10^5), the volume was adjusted to 50 ml with isotonic saline and the mixture was allowed to stand at ambient temperature for approximately 1 h during which time the red blood cells (RBCs) sedimented. The supernatant was aspirated and cells were sedimented by centrifugation (5 min at 750 *g*). The resulting pellet was resuspended in 10 ml isotonic saline: platelet poor plasma (4 : 1), underlayered with 3 ml Ficoll-Paque solution and subjected to centrifugation at 1000 *g* for 25 min. The pellet, containing PMNLs and RBCs was resuspended in HEPES buffer containing isotonic ammonium chloride (10 mM HEPES, 150 mM NH_4Cl, pH 7.4) and incubated at 37°C for 10 min to lyse contaminating RBCs. Following RBC lysis the PMNLs were sedimented by centrifugation (1000 *g*, 10 min) and washed twice using HEPES-buffered Tyrode (0.25% BSA, 0 Ca^{2+}). Cells were resuspended in this buffer and the concentration was adjusted to 5×10^6 ml^{-1} after counting using a haemocytometer. Cell viability, assessed by exclusion of trypan blue, exceeded 90%. Calcium (1.8 mM) was added and PMNLs were used for aggregation studies (Cunningham *et al.*, 1980) within 3 h.

Platelet and polymorphonuclear leukocyte aggregation

Aggregation studies were carried out at 37°C in a Chronolog 540 aggregometer. The reference cells contained a 1 in 10 dilution of platelets or cells and the data are presented as percentage increases in light transmission. Antagonists or the vehicle (1% dimethylsulphoxide, DMSO) were added 1 min before the addition of Paf. Aggregation responses were allowed to develop until a maximum change in light transmission occurred.

Guinea-pig peritoneal macrophages

Resident macrophages were obtained by peritoneal lavage of male guinea-pigs (400–500 g). A 50 ml volume of sterile, heparinized (50 u ml^{-1}), phosphate-buffered saline was injected (i.p.) and the peritoneal cavity was gently massaged for 1 min. The peritoneal fluid was aspirated and the cells were isolated by centrifugation (1000 *g*, 10 min). Following resuspension in RPMI 1640 (20% foetal calf serum,

Table 1 The potency of Paf on rabbit platelet and polymorphonuclear leukocyte (PMNL) aggregation and guinea-pig macrophage prostacyclin (6-oxo-prostaglandin $F_{1\alpha}$) production

	n	*−log EC₅₀*	*Max.*
Platelet	8	9.66 ± 0.14	62.6[1] ± 2.1
PMNL	9	8.56* ± 0.03	12.1[1] ± 3.1
Macrophage	17	9.02* ± 0.10	3.28[2] ± 0.41

The data are presented as mean ± s.e.mean.
* $P < 0.01$, unpaired Student's t test, compared with $-\log EC_{50}$ on platelets.
[1] Maximum responses for aggregation are expressed as percentage increase in light transmission.
[2] Maximum response for macrophage 6-oxo-$PGF_{1\alpha}$ generation is expressed as pmol per 10^6 cells h^{-1}. By comparison, A23187 (10 μM) stimulated 6-oxo-$PGF_{1\alpha}$ generation to 7.34 ± 0.63 pmol per 10^6 cells h^{-1}.

FCS) the cell concentration was adjusted to $1 \times 10^6 ml^{-1}$. The resulting cell suspension was added in 0.5 ml aliquots per well to linbro 24 well plastic culture plates and allowed to adhere for a 2 h period. The adherent cells were then washed once with RPMI 1640 without FCS and incubations were carried out in RPMI 1640 without FCS or BSA. The Paf antagonists were added in a volume of 5 μl dimethylsulphoxide (DMSO) and allowed to equilibrate for 5 min at 37°C before the addition of Paf (0.1–100 nM, 5 μl in 0.25% BSA). The incubation continued for 60 min at which time an aliquot of the supernatant was removed for radioimmunoassay (RIA) of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) without prior extraction according to the method of Salmon (1978). None of the agents used in this study interfered with the RIA of 6-oxo-$PGF_{1\alpha}$. Ninety-eight % of the adherent cells showed positive non-specific esterase staining indicating a relatively pure population of macrophages.

Analysis of Paf receptors

Logarithmic concentration-response relationships for Paf were established in platelets, PMNLs and macrophages in the absence and presence of 3 concentrations of the Paf-receptor antagonists. The data were first examined for differences in maximum responses and departure from parallelism and linearity by established methods (Diem, 1962). When the antagonist had no effect on the maximum response and the concentration-response lines did not depart from parallelism or linearity, the log concentration-response lines in individual experiments were subjected to regression analysis and

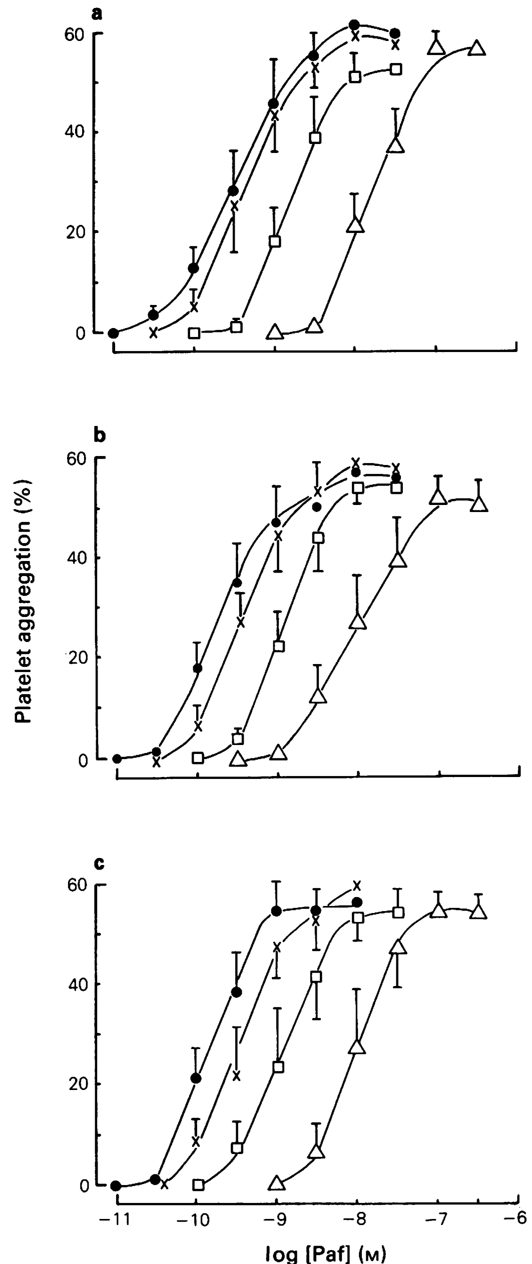

Figure 1 Antagonism of Paf-induced aggregation of rabbit washed platelets by (a) WEB 2086 (×, 10^{-8}; □, 10^{-7} and △, 10^{-6} M), (b) BN 52021 (×, 10^{-7}; □, 10^{-6} and △, 10^{-5} M) and (c) L-652-731 (×, 10^{-8}; □, 10^{-7} and △, 10^{-6} M). (●) Control responses to Paf. Ordinate scales: peak increase in percentage light transmission. Abscissa scales: logarithm of the molar concentration of Paf. Data are presented as the means of 6–7 observations and vertical lines indicate s.e.mean.

Table 2 Schild plot analysis for BN 52021, L-652,731 and WEB 2086 on rabbit washed platelets, polymorphonuclear leukocytes (PMNLs) and guinea-pig peritoneal macrophages

	Platelet			*PMNL*			*Macrophages*		
Antagonist	pA_2	*Slope*	(n)	pA_2	*Slope*	(n)	pA_2	*Slope*	(n)
WEB 2086	7.58 ± 0.21	1.02 ± 0.06	(7)	7.57 ± 0.08	0.96 ± 0.04	(7)	6.62** ± 0.12	0.96 ± 0.12	(6)
L-652,731	7.51 ± 0.16	0.91 ± 0.08	(7)	7.15 ± 0.09	0.98 ± 0.05	(7)	<5.00[1]	—	(7)
BN 52021	6.85 ± 0.18	0.90 ± 0.06	(7)	6.31* ± 0.17	1.01 ± 0.08	(7)	ND[2]	0.68 ± 0.11	(4)

The data are presented as mean ± s.e.mean.
* $P < 0.05$, ** $P < 0.01$, unpaired Student's t test. Compared with pA_2 value obtained on platelet.
[1] pA_2 value less than 5.00 since 10^{-5} M failed to cause a significant rightward shift.
[2] pA_2 value not determined since slope of Schild plot differed significantly from unity.

equi-effective concentration ratios for Paf were obtained for the 3 antagonist concentrations. These data were analysed by the method of Arunlakshana & Schild (1959). The values of pA_2 and slope of the Schild plot are presented as the means and s.e.mean obtained from at least 6 separate experiments.

Differences were examined by analysis of variance and two-tailed unpaired Student's t test using corrections for multiple comparisons.

Materials

All reagents and solvents used in this study were of analytical or higher grade. RPMI 1640 and foetal calf serum were obtained from CSL (Australia) and Flow Laboratories, respectively. Chemicals were obtained from the following sources: A23187; bovine serum albumin (BSA), grade 5, essentially fatty acid free; dextran, clinical grade C; indomethacin (Sigma Chemical Co.); dextran T500; Ficoll-Paque (Pharmacia); hexadecyl platelet-activating factor (Paf, Bachem); prostacyclin (PGI_2; Na salt, Wellcome); 6-oxo-$PGF_{1\alpha}$ antiserum (Dr J. Salmon, Wellcome Research Laboratories); HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid; BDH chemicals); WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6H-thieno (3,2-f) (1,2,4)-triazolo (4,3-G) (1,4)-diazepine-2-yl)-1-(4-morpholinyl)-1-propanone; Boehringer Ingelheim, FRG); L-652,731 (*trans*-2,5-(3,4,5-trimethoxyphenyl) tetrahydrofuran; Merck, Sharp & Dohme, U.S.A.); BN 52021 (9H-1,7a-(epoxymethano)-1H,6a-H-cyclopenta [c] furo [2,3-6] furo [3′,2′:3,4] cyclopenta [1,2-a] furan -5,9,12-(4H)-trione, 3-tert-butylhexahydro-4,7b,-11-hydroxy-8-methyl; IHB Laboratories, France).

Results

Antagonism of platelet aggregation

Paf caused aggregation of washed rabbit platelets at a threshold concentration of 0.03 nM and an EC_{50} of 0.22 nM (Table 1). BN 52021, L-652,731 and WEB 2086 each caused concentration-dependent and parallel rightward shifts of Paf-induced aggregation without altering the maximum responses (Figure 1). Schild plots for each of the antagonists further supported competitive antagonism yielding slopes not significantly different ($P > 0.05$, paired t test) from unity (Table 2). WEB 2086 and L-652,731 were approximately equipotent in rabbit platelets and both compounds were significantly ($P < 0.05$, Student's t test) more potent than BN 52021. WEB 2086 also caused a concentration-dependent, parallel rightward shift of Paf-induced aggregation of guinea-pig platelets giving a pA_2 value of 7.69 ± 0.14 ($n = 3$).

Antagonism of polymorphonuclear leukocyte aggregation

Paf caused aggregation of washed rabbit PMNLs with an EC_{50} value of 2.75 nM (Table 1) indicating that Paf is approximately 10 fold less active than on platelet aggregation. In addition, the maximum increase in light transmission for platelets (60%) was considerably greater than that for PMNLs (14%). Each of the antagonists caused concentration-dependent and rightward parallel shifts without altering the maximum responses (Figure 2).

Furthermore, the slope of the Schild plots did not differ significantly from unity ($P > 0.05$, paired t test) suggesting competitive antagonism (Table 2). The calculated pA_2 values for WEB 2086 (7.57) and L-652,731 (7.51) did not differ from those obtained on the platelet (7.58 and 7.15, respectively). On the other hand, the pA_2 for BN 52021 on PMNL aggregation was significantly less ($P < 0.05$, Student's t test) than that on the platelet.

Antagonism of guinea-pig macrophage prostacyclin production

Guinea-pig resident peritoneal macrophages isolated by lavage and short-term (2 h) culture showed

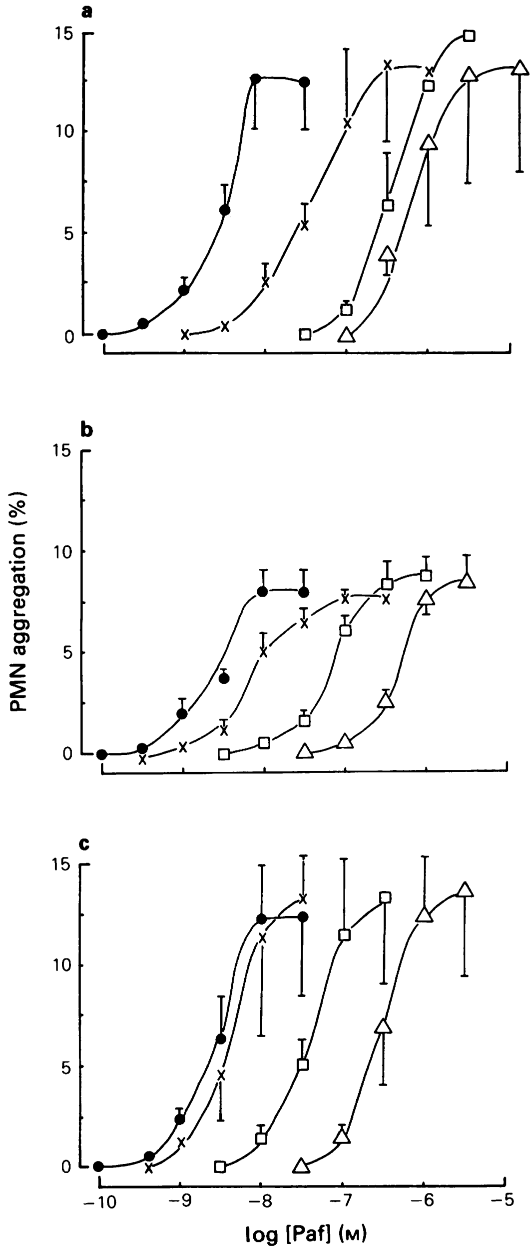

Figure 2 Antagonism of Paf-induced aggregation of rabbit washed polymorphonuclear leukocytes (PMNL) by (a) WEB 2086 (×, 10^{-7}; □, 10^{-6} and △, 10^{-5} M), (b) BN 52021 (×, 10^{-6}; □, 10^{-5} and □, 10^{-4} M) and (c) L-L-652,731 (×, 10^{-7}; □, 10^{-6} and △, 10^{-5} M). (●) Control responses to Paf. Ordinate scales: peak increase in percentage light transmission. Abscissa scales: logarithm of the molar concentration of Paf. Data are presented as the means of 6–7 observations and vertical lines indicate s.e.mean.

increased generation of prostacyclin (measured as 6-oxo-$PGF_{1\alpha}$) in response to Paf during a 1 h incubation (Figure 3). The potency of Paf in this system is intermediate between the platelet and the PMNL with an EC_{50} value of 1 nM (Table 1). The potencies of the antagonists were markedly lower on the macrophage than on the platelet or PMNL. The pA_2 value for WEB 2086 on the macrophage was approximately 1 log-unit less than that on either the platelet or the PMNL (Table 2). WEB 2086 appears to act as a competitive antagonist on the macrophage, since it produced parallel rightward shifts of the Paf concentration-effect curve without a depression of the maximum response (Figure 3) and the slope of the Schild plot did not differ significantly ($P > 0.05$, paired t test) from unity (Table 2). A pA_2 value for L-652,731 could not be obtained since only the highest concentration (10^{-4} M) produced a significant rightward shift, and the pA_2 value is therefore clearly less than 5.00. BN 52021 produced a concentration-dependent rightward shift of macrophage 6-oxo-$PGF_{1\alpha}$ generation without depressing the maximum response. However, a pA_2 value could not be calculated from these data because the slope of the Schild plot differed significantly ($P < 0.05$, paired t test) from unity (Table 2). The minimum concentration of BN 52021 required to produce a significant rightward shift in the concentration-response curve (10^{-5} M) was two orders and one order of magnitude higher than that required in either platelets (10^{-7} M) or PMNLs (10^{-6} M), respectively. However, in contrast to relative potencies on the platelet or the PMNL, BN 52021 was more potent than L-652,731 on macrophages since a significant rightward shift was obtained with 10 μM BN 52021 but not with 10 μM L-652,731. Thus all three antagonists were clearly less potent on the macrophage than on platelets or PMNLs, and their order of potency was also different in the macrophage.

WEB 2086 and L-652,731 produced a concentration-dependent reduction in the resting generation of 6-oxo-$PGF_{1\alpha}$ (Table 3). Basal 6-oxo-$PGF_{1\alpha}$ generation (1.28 ± 0.16 pmol per 10^6 cells h^{-1}) was increased by A23187 10 μM (7.34 ± 0.63 pmol per 10^6 cells h^{-1}) and this A23187-stimulated PGI_2 generation was markedly inhibited by indomethacin 2.8 μM (1.01 ± 0.09 pmol per 10^6 cells h^{-1}). None of the Paf antagonists had any significant effects ($P > 0.05$, Student's t test) on A23187-induced generation of 6-oxo-$PGF_{1\alpha}$ (Table 3).

Discussion

The results of this study strongly indicate the existence of a previously undescribed subclass of Paf receptors on the guinea-pig peritoneal macrophage.

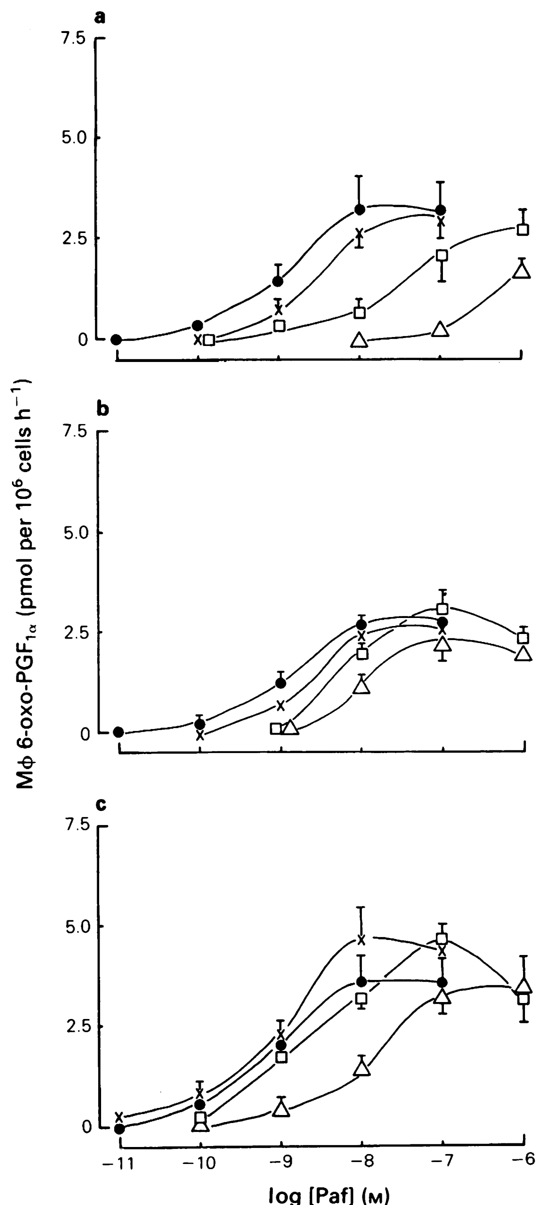

Figure 3 Antagonism of Paf-induced prostacyclin production (measured as 6-oxo- prostaglandin$F_{1\alpha}$;6-oxo-$PGF_{1\alpha}$) by guinea-pig resident peritoneal macrophages (Mϕ) by (a) WEB 2086 (×, 10^{-6}; □, 10^{-5} and △, 10^{-4} M), (b) BN 52021 (×, 10^{-5}; □, 10^{-4} and △, 10^{-3} M) and (c) L-652,731 (×, 10^{-6}; □, 10^{-5} and △, 10^{-4} M). (●) Control responses to Paf. Ordinate scales: increase (over basal) in 6-oxo-$PGF_{1\alpha}$ production (pmol) per 10^6 cells h^{-1}. Abscissa scales: logarithm of the molar concentration of Paf. Data are presented as the means of 4–7 observations; vertical lines indicate s.e.mean.

Furthermore, these results suggest that the potency of Paf antagonists on aggregation of rabbit platelets is not a reliable indicator of antagonist activity in other *in vitro* systems or in pathology.

WEB 2086, L-652,731 and BN 52021 each behaved as a competitive receptor antagonist on both platelet and PMNL aggregation. The pA_2 value for L-652,731 (7.51) is similar to the K_b value (2×10^{-8} M) determined by Hwang *et al.* (1985) using either ligand-binding studies or inhibition of rabbit platelet aggregation. In addition, these authors established the selectivity of L-652,731 for Paf versus collagen, arachidonic acid, adrenaline, thrombin, A23187 and adenosine diphosphate (ADP) at a concentration of 24 μM. This latter concentration of L-652,731 also induced a substantial rightward shift in the Paf concentration-response curve for aggregation of human PMNLs, to an extent consistent with that observed in the present study. These observations indicate a similarity in Paf receptors stimulating aggregation of platelets and PMNLs. However, Voelkel *et al.* (1986) suggested that the rabbit PMNL possesses a distinct Paf receptor that mediates adherence and shape change, since these effects were resistant to a number of putative Paf antagonists including L-652,731 (Voelkel *et al.*, 1986).

WEB 2086 is a potent antagonist of Paf on both human platelet and PMNL aggregation (Casals-Stenzel *et al.*, 1987) with little difference between these systems. This observation is in agreement with the pA_2 values observed in the present study. The selectivity of WEB 2086 for Paf on platelets has been previously established using ADP, adrenaline, collagen, 5-hydroxytriptamine and arachidonic acid. The similarity between platelet and PMNL receptors is further supported by the lack of difference in the potencies of brotizolam and triazolam in inhibition of aggregation in either system (Casals-Stenzel, 1987b) and by the similarity of pA_2 values for kadsurenone on the PMNL (Lambrecht & Parnham, 1986) and the platelet (Shen *et al.*, 1985).

Lambrecht & Parnham (1986) showed that the macrophage Paf receptor has significantly higher affinity for kadsurenone than do those on the platelet or PMNL. In our study, WEB 2086, L-652,731 and BN 52021 showed markedly lower affinities for macrophage Paf receptors mediating an increase in PGI_2 generation compared with those associated with aggregation of platelets or PMNLs. The difference for WEB 2086 was about 10 fold, whereas those for L-652,731 and BN 52021 were at least 100 fold, strongly suggesting the existence of a different subclass of Paf receptors on macrophages. In addition, BN 52021 had a significant inhibitory effect at 10 μM whereas L-652,731 did not, thus indicating a difference in the rank order of potency compared with

Table 3 Effects of Paf receptor antagonists on basal and A23187 (10 μM)-stimulated generation of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-PGF$_{1\alpha}$) by guinea-pig resident peritoneal macrophages ($n = 3$)

Antagonist	*Antagonist concentration* (M)				
	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}
	6-oxo-PGF$_{1\alpha}$ (pmol per 10^6 cells h^{-1})				
	Basal				
WEB 2086	1.28 ± 0.32	0.60 ± 0.19	0.47 ± 0.13	0.48 ± 0.14	—
L-652,731	1.41 ± 0.43	1.31 ± 0.37	0.98 ± 0.27	0.75 ± 0.12	—
BN 52021	1.10 ± 0.34	—	1.16 ± 0.24	0.67 ± 0.27	1.21 ± 0.46
	A23187-stimulated				
WEB 2086	6.03 ± 0.89	5.78 ± 0.73	5.43 ± 0.96	6.65 ± 0.81	—
L-652,731	5.64 ± 0.54	7.35 ± 1.66	4.72 ± 0.88	4.48 ± 1.39	—
BN 52021	6.49 ± 1.46	—	9.55 ± 2.64	8.27 ± 1.50	6.98 ± 0.41

The data are presented as mean ± s.e.mean.

that for platelet or PMNL aggregation. It seems likely that this subclass differs from that previously described on guinea-pig peritoneal macrophages because kadsurenone shows higher affinity (Lambrecht & Parnham, 1986), whereas the related antagonist, L-652,731, shows lower affinity. The difference between this study and that on kadsurenone may be explained by the difference in measured responses (chemiluminescence versus PGI_2 generation), indicating that different receptor subtypes may mediate distinct functional responses of the same cell type, as appears to be the case for PMNL aggregation and adherence. In addition, the present study used resident peritoneal macrophages and that with kadsurenone used elicited macrophages, raising the possibility of differences in Paf receptors on different subpopulations of macrophages. Inhibition of Paf-induced PGI_2 generation by BN 52021, WEB 2086 and L-652,731 appears to be a result of blockade of specific receptors since the same concentrations of these antagonists failed to affect A23187-induced PGI_2 generation. However, WEB 2086 and L-652,731 did reduce the resting generation of PGI_2 which could indicate that under these conditions there is a basal generation of Paf.

There is evidence that Paf receptor antagonists are bound by plasma proteins to a significant extent (Nunez *et al.*, 1986). Therefore, the possible effects of BSA in the platelet and PMNL experiments must be considered. The reduced apparent affinities of the Paf antagonists for macrophages incubated in the absence of BSA suggests that binding to BSA may have reduced the differences in pA_2 values between macrophages and platelets.

The selectivity of the antagonists for PMNL responses to Paf has not been studied. Such experiments are complicated by the fact that other stimuli for PMNL aggregation such as A23187 and C5a (Camussi *et al.*, 1981; Mueller *et al.*, 1983) also stimulate synthesis of Paf. Moreover, using cross-desensitization to Paf (O'Flaherty, 1985) or Paf antagonism (Bureau *et al.*, 1987) it has been found that A23187-induced activation of rabbit peritoneal PMNLs and N-formyl methionyleucyl phenylalanine and leukotriene B_4 induced activation of rat pleural PMNLs may be partly mediated by Paf. Therefore, we have made no attempt in this study to establish the selectivity of the Paf antagonists on the PMNL.

On guinea-pig washed platelets, WEB 2086 had a similar pA_2 value to that on rabbit platelets, arguing against the possibility of species differences explaining the relatively low affinities of the Paf antagonists for the macrophage receptor. The agreement between the pA_2 values for kadsurenone on human PMNLs (Shen *et al.*, 1985) and those on pig PMNLs (Lambrecht & Parnham, 1986) provides further evidence against species differences in Paf receptors on these cells or others. However, some effects of Paf are species-dependent. For example, rat platelets are insensitive to Paf (Terashita *et al.*, 1983) and leukotrienes are involved to a major extent in the cardiac and pulmonary actions of Paf in the rat (Piper & Stewart, 1986b; Voelkel *et al.*, 1986), to a lesser extent in the guinea-pig (Piper & Stewart, 1987) and not at all in the rabbit lung (Seale *et al.*, 1986). It would be of interest to identify whether Paf responses linked to the generation of arachidonic acid metabolites were mediated via receptors distinct from those evoking a direct effect, as appears to be the case for the responses investigated in this study.

It is apparent from a number of studies that endogenously synthesized Paf remains largely cell-associated (Camussi *et al.*, 1983; Jouvin-Marche *et al.*, 1984; Prescott *et al.*, 1984; Roubin *et al.*, 1986), stimulating speculation that intracellular Paf may have a function within the cell of origin. Henson (1987) has suggested that Paf may act independently of receptors since the high concentrations achieved

could have direct effects on membranes (Harris *et al.*, 1985). It also seems possible that intracellular Paf could act via specific receptors located within the cell, providing a potential explanation for decreased antagonist affinities in the macrophage.

The importance of PMNLs and macrophages in vascular injury, inflammation and atherosclerosis is well recognized (Harlan, 1987; Seljelid, 1987). Thus, the existence of Paf receptor subtypes on effector cells of the immune system provides the potential for the development of selective antagonists. Furthermore, the present findings predict that some actions of Paf *in vivo* should be relatively resistant to inhibition by WEB 2086, L-652,731 and BN 52021. It is therefore interesting that the anti-bronchoconstrictor and the anti-hypotensive actions of BN 52021 are dissociated in guinea-pigs (Desquand *et al.*, 1986). In addition, inhibition of Paf-induced rat paw oedema appears to be less susceptible to inhibition by L-652, 731 (Hwang *et al.*, 1986) than a number of other actions such as hypotension and leukopenia (Wu *et al.*, 1986). Some *in vivo* actions of Paf that are relatively resistant to the above antagonists may involve macrophages.

A.G.S. was supported by the C R Roper Bequest to the Faculty of Medicine, University of Melbourne and financial support was also provided by the National Health and Medical Research Council of Australia. We thank Dr J. Salmon, Wellcome Research Laboratories (U.K.) for a gift of 6-oxo-$PGF_{1\alpha}$ antiserum, the Wellcome Foundation Ltd for prostacyclin, Dr P. Braquet, IHB Laboratories (France) for BN 52021, Dr J. Chabala, Merck, Sharp & Dohme (U.S.A.) for L-652,731 and Dr N. Latt, Boehringer Ingelheim (Australia) for WEB 2086. We also thank Mr G. Vairo for assistance with the non-specific esterase staining of macrophages.

References

ADNOT, S., LEFORT, J., LAGENTE, V., BRAQUET, P. & VARGAFTIG, B.B. (1986). Interference of BN 52021, a Paf acether antagonist, with endotoxin-induced hypotension in the guinea-pig. *Pharmacol. Res. Commun.*, **18**, 197–200.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.*, **14**, 48–58.

BENVENISTE, J. (1985). Paf-Acether (Platelet-activating factor). In *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Vol. 13, ed. Neri Serneri, C.G. pp. 11–18. New York: Raven.

BRAQUET, P., SPINNEWYN, B., BRAQUET, M., BOURGAIN, R.H., TAYLOR, J.E., ETIENNE, A. & DRIEU, K. (1985). BN 52021 and related compounds: A new series of highly specific Paf-acether receptor antagonists isolated from *Ginkgo Biloba L. Blood & Vessel*, **16**, 558–572.

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

BUREAU, M., JOSEPH, D. & VARGAFTIG, B.B. (1987). Desensitization and antagonism of rat polymorphonuclear leukocytes stimulated with Paf acether. *Prostaglandins*, **33**, 37–50.

CAMUSSI, G., AGLIETTA, M., CODA, R., BUSSOLINO, F., PIACIBELLO, W. & TETTA, C. (1981). Release of platelet-activating factor and histamine. II. The cellular origin of human Paf: monocytes, polymorphonuclear neutrophils and basophils. *Immunopharmacol.*, **42**, 191–199.

CASALS-STENZEL, J. (1987a). Protective effect of WEB 2086, a novel antagonist of platelet activating factor, in endotoxin shock. *Eur. J. Pharmacol.*, **185**, 117–122.

CASALS-STENZEL, J. (1987b) Triazolodiazepines are potent antagonists of platelet activating factor in vitro and in vivo. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **335**, 351–355.

CASALS-STENZEL, J., MUACEVIC, G. & WEBER, K-H. (1987). Pharmacological actions of WEB 2086, a new specific antagonist of platelet activating factor. *J. Pharmacol. Exp. Ther.*, **241**, 974–981.

CUNNINGHAM, F.M., SHIPLEY, M.E. & SMITH, M.J.H. (1980). Aggregation of rat polymorphonuclear leukocytes *in vitro*. *J. Pharm. Pharmacol.*, **32**, 377–380.

DESQUAND, S., TOUVAY, C., RANDON, J., LAGENTE, V., MARIDONNEAU-PARINI, I., ETIENNE, A., LEFORT, J., BRAQUET, P. & VARGAFTIG, B.B. (1986). Interference of BN 52021 (Ginkgolide B) with the broncho-pulmonary effects of Paf-acether in the guinea-pig. *Eur. J. Pharmacol.*, **127**, 83–95.

DIEM, K. (ed.) (1962). *Documenta Geigy, Scientific Tables.* 6th. edn. pp. 171–178. St. Leonards: Geigy Pharmaceuticals.

HARLAN, J.M. (1987). Neutrophil-mediated vascular injury. *Acta. Med. Scand.*, (suppl). **715**, 123–129.

HARRIS, R.A., CLAY, K., MURPHY, R. & HENSON, P.M. (1985). Effects of Paf and related lipids on membrane physical properties. *Fed. Proc.*, **44**, 858.

HASLETT, C., GUTHRIE, L.A., KOPANIAK, M.M., JOHNSTON, R.B. & HENSON, P.M. (1985). Modulation of multiple neutrophil functions by preparative methods or trace concentrations of bacterial lipopolysacharide. *Am. J. Pathol.*, **119**, 101–110.

HELLEWELL, P.G. & WILLIAMS, T.J. (1986). A specific antagonist of platelet-activating factor supresses oedema formation in an Arthus reaction but not oedema induced by leukocyte chemoattractants in rabbit skin. *J. Immunol.*, **137**, 302–307.

HENSON, P.M. (1987). Extracellular and intracellular activities of platelet-activating factor. In *New Horizons in Platelet-Activating Factor Research.* ed. Winslow, C.M. & Lee, M.L. pp. 3–10. Chichester: John Wiley & Sons Ltd.

HWANG, S-B., LAM, M-H., BIFTU, T., BEATTIE, T.R. & SHEN, T-Y. (1985). Trans-2,5-bis-(3,4,5-trimethoxyphenyl) tetrahydrofuran an orally active specific and competitive receptor antagonist of platelet activating factor. *J. Biol.*

Chem., **260**, 15,639–15,645.

HWANG, S-B., LAM, M-H., LI, C-L. & SHEN, T-Y. (1986). Release of platelet-activating factor and its involvement in the first phase of carrageen in induced rat foot oedema. *Eur. J. Pharmacol.*, **120**, 33–41.

HWANG, S-B., LEE, C.S.B., CHEAH, M.J. & SHEN, T-Y. (1983). Specific receptor sites for 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine (platelet-activating factor) on rabbit and guinea-pig smooth muscle membranes. *Biochem.*, **22**, 4756–4763.

LAMBRECHT, G. & PARNHAM, M.J. (1986). Kadsurenone distinguishes between different platelet-activating factor receptor subtypes on macrophages and polymorphonuclear leucocytes. *Br. J. Pharmacol.*, **87**, 287–289.

JOUVIN-MARCHE, E., NINIO, E., BEAURAIN, G., TENCE, M., NIAUDET, P. & BENVENISTE, L. (1984). Biosynthesis of Paf-acether (platelet-activating factor). VII. Precursors of Paf-acether and acetyltransferase activity in human leukocytes. *J. Immunol.*, **133**, 892–898.

MUELLER, H.W., O'FLAHERTY, J.T. & WYKLE, R.L. (1983). Biosynthesis of platelet activating factor in rabbit polymorphonuclear neutrophils. *J. Biol. Chem.*, **258**, 6213–6218.

NUNEZ, D., CHIGNARD, M., KORTH, R., LE COUEDIC, J-P., NOREL, X., SPINNEWYN, B., BRAQUET, P. & BENVENISTE, J. (1986). Specific inhibition of Paf-acether-induced platelet activation by BN 52021 and comparison with the Paf-acether inhibitors kadsurenone and CV 3988. *Eur. J. Pharmacol.*, **123**, 197–205.

O'FLAHERTY, J.T. (1985). Neutrophil degranulation: Evidence pertaining to its mediation by the combined effects of leukotriene B_4, platelet-activating factor and 5-HETE. *J. Cell Physiol.*, **122**, 229–239.

PIPER, P.J. & STEWART, A.G. (1986a). Evidence of a role for platelet-activating factor in antigen-induced coronary vasoconstriction in guinea-pig perfused hearts. *Br. J. Pharmacol. Proc. Suppl.*, **88**, 238P.

PIPER, P.J. & STEWART, A.G. (1986b). Coronary vasoconstriction in the rat, isolated perfused heart induced by platelet-activating factor is mediated by leukotriene C_4. *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.

PRESCOTT, S.M., ZIMMERMAN, G.A. & McINTYRE, T.M. (1984). Human endothelial cells in culture produce platelet-activating factor (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) when stimulated with thrombin. *Proc. Natn. Acad. Sci. U.S.A.*, **81**, 3534–3538.

ROUBIN, R., DULIOUST, A., HAYE-LEGRAND, I., NINIO, E. & BENVENISTE, J. (1986). Biosynthesis of Paf-acether. VIII. Impairment of Paf-acether production in activated macrophages does not depend upon acetyltransferase activity. *J. Immunol.*, **136**, 1796–1802.

SALMON, J.A. (1978). A radio-immunoassay for 6-keto-prostaglandin $F_{1\alpha}$. *Prostaglandins*, **15**, 383–397.

SEALE, J.P., HELLEWELL, P.G. & WILLIAMS, T.J. (1986). Effects of platelet activating factor (PAF) in the pulmonary circulation of rabbit isolated perfused lungs. *Aust. N.Z. J. Med.*, **16**, 629.

SELJELID, R. (1987). Effector functions of macrophages. *Acta Med. Scand.*, (suppl.) **715**, 131–138.

SHEN, T.Y., HWANG, S-B., CHANG, M.N., DOEBBER, T.W., LAM, M-H.T., WU, M.S., WANG, X., HAM, G.Q. & LI, R.Z. (1985). Characterization of a platelet-activating factor receptor antagonist isolated from Haifenteng (*Piper futokadsura*): Specific inhibition of *in vitro* and *in vivo* platelet-activating factor-induced effects. *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 672–676.

TERASHITA, Z-E., IMURA, Y., NISHIKAWA, K. & SUMIDA, S. (1985). Is platelet activating factor (PAF) a mediator of endotoxin shock? *Eur. J. Pharmacol.*, **109**, 257–261.

TERASHITA, Z-I., TSUSHIMA, S., YOSHIOKA, Y., NOMURA, H., INADA, Y. & NISHIKAWA, K. (1983). CV-3988 – a specific antagonist of platelet activating factor (PAF). *Life Sci.*, **32**, 1975–1982.

TOUVAY, C., ETIENNE, A. & BRAQUET, P. (1985). Inhibition of antigen-induced lung anaphylaxis in the guinea-pig by BN 52021 a new specific Paf-acether receptor antagonist isolated from Gingko Biloba. *Agents Actions*, **17**, 371–372.

VARGAS, J.R., RADANOUKI, M. & MONCADA, S. (1982). The use of prostacyclin in the separation from plasma and washing of human platelets. *Prostaglandins*, **23**, 929–945.

VILAIN, B., LAGENTE, V., TOUVAY, C., DESQUAND, G., RANDON, S., LEFORT, J., BRAQUET, P. & VARGAFTIG, B.B. (1986). Pharmacological control of the in vivo passive anaphylactic shock by the Paf-acether antagonist compound BN 52021. *Pharmacol. Res. Commun.*, **18**, 119–126.

VOELKEL, N.F., CHANG, S-W., PFEFFER, K.D., WORTHEN, S.G., McMURTRY, I.F. & HENSON, P.M. (1986). Paf antagonists: different effects on platelets, neutrophils, guinea-pig ileum and Paf-induced vasodilatation in isolated rat lung. *Prostaglandins*, **32**, 359–372.

WU, M.S., BIFTU, T. & DOEBBER, T.W. (1986). Inhibition of the platelet activating factor (PAF)-induced in vivo responses in rats by Trans-2,5-(3,4,5-trimethoxyphenyl) tetrahydrofuran (L-652,731), a PAF receptor antagonist. *J. Pharmacol. Exp. Ther.*, **239**, 841–845.

(Received November 11, 1987
Revised February 29, 1988
Accepted March 15, 1988)