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Antagonism of vasoconstriction induced by platelet-activating factor in guinea-pig perfused hearts by selective platelet-activating factor receptor antagonists

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1 Platelet-activating factor (Paf) is a potent coronary vasoconstrictor in rat, guinea-pig, dog and pig. The present study investigated the mechanism and duration of action of Paf in guinea-pig isolated, Krebs-perfused hearts.

2 Dose-related and sustained decreases in cardiac contractility and increases in coronary perfusion pressure were elicited by bolus doses of Paf (0.3–100 pmol).

3 Platelet-activating factor (30 pmol) induced increases in the production of immunoreactive thromboxane B_2 (TXB_2), leukotriene B_4 (LTB_4) and LTC_4, but not 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$). In addition, the release of leukotriene-like material following Paf was observed using on-line superfusion bioassay.

4 The coronary vasoconstrictor actions of Paf were partially antagonized by the leukotriene receptor antagonist, FPL 55712 (1.9 μM), or by indomethacin (2.8 μM). The combined use of these compounds did not result in further significant inhibition.

5 The Paf receptor antagonists, BN 52021 (30 μM) and L 652, 731 (10 μM), antagonized both the increase in coronary perfusion pressure and the decrease in cardiac contractility induced by Paf (10–100 pmol) in a surmountable and relatively selective manner.

6 The effects of a bolus dose of 100 pmol Paf were sustained in excess of 18 min. Exogenous Paf underwent little metabolism on passing through the coronary circulation with only 2% being converted to lyso-Paf and approximately 4% being retained by the heart after 18 min of perfusion.

7 These results suggest that the coronary vasoconstrictor actions of Paf are partially dependent on the release of vasoactive arachidonic acid metabolites. The extraordinary potency and the long-lasting action of Paf indicate a potential role for this pro-inflammatory mediator in disorders of the coronary circulation.

Introduction

Coronary vascular tone is increased by several products released by activated platelets, including 5-hydroxytryptamine and thromboxane A_2 (TXA_2), which have received considerable attention as mediators of coronary vasospasm. However, recent studies indicate that platelet-activating factor (Paf) is an extremely potent coronary vasoconstrictor (Benveniste *et al.*, 1983; Piper & Stewart, 1986a). Platelets may be activated by contact with the subendothelial structures or by stimulation with a number of vasoactive substances (Harker, 1986). Furthermore, the release of Paf from activated platelets has been reported (Chignard *et al.*, 1979; Chap *et al.*, 1981). The potential role of Paf in coronary artery disease is supported by the recent finding of intravascular Paf release in patients with coronary artery disease undergoing atrial pacing (Montrucchio *et al.*, 1986).

In a previous study, we showed that Paf elicited vasoconstriction in rat isolated, perfused hearts (Piper & Stewart, 1986a) by an indirect mechanism involving the release of the coronary vasoconstrictors leukotriene C_4 (LTC_4, Letts & Piper, 1983) and, to a lesser extent, TXA_2. Platelet-activating factor has been shown to release cysteinyl-containing leukotrienes from rat perfused lungs (Voelkel *et al.*, 1982), rat

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chopped lung (Beaubien *et al.*, 1984) and cat chopped, pulmonary and coronary blood vessels (Lefer *et al.*, 1984b). In addition, studies *in vivo* provide indirect evidence that Paf-induced coronary vasoconstriction is mediated, at least in part, by lipoxygenase products in the dog (Bessin *et al.*, 1983; Kenzora *et al.*, 1984; Sybertz *et al.*, 1985) and the pig (Feuerstein *et al.*, 1984). However, there is disagreement on the mechanism of action of Paf in the guinea-pig isolated perfused heart: Levi *et al.* (1984) have suggested that Paf acts independently of the release of vasoactive arachidonic acid (AA) metabolites on the basis of a lack of effect of either indomethacin or FPL 55712; on the other hand, it has recently been shown that FPL 55712 has inhibitory effects on Paf-induced coronary vasoconstriction and that LTC_4-like immunoreactivity is released from Paf-challenged hearts (Benveniste, personal communication).

The purpose of the present study was to determine the extent of the dependence of Paf-induced coronary vasoconstriction on the release of AA metabolites in guinea-pig perfused hearts. In addition, the ability of the selective Paf receptor antagonists, BN 52021 (Nunez *et al.*, 1986) and L-652, 731 (Hwang *et al.*, 1986) to antagonize the cardiac effects of Paf have been examined.

A preliminary account of some of these findings has been presented to the British Pharmacological Society (Piper & Stewart, 1986b).

Methods

Isolated, perfused hearts

Male Dunkin-Hartley guinea-pigs (350–550 g) were killed by a blow to the head and exsanguinated, 15 min after receiving 3000 u heparin kg^{-1}, i.p. The heart was rapidly excised and perfused using Krebs solution, as previously described for rat hearts (Piper & Stewart, 1986a). Heart rate, cardiac contractility and coronary perfusion pressure stabilized following a 30 min equilibration period at which time an infusion of vehicle (30% dimethyl sulphoxide, DMSO, 0.1 ml min^{-1}), 30 μM BN 52021 or 10 μM L-652, 731, was commenced. After a further 10 min period, vasoactive substances were administered as bolus injections (10–100 μl) into the perfusion fluid 2 cm proximal to the aorta. Measurements of cardiac functions were made before vehicle/Paf antagonist administration, again immediately before Paf (0.3–100 pmol) administration and then at 2, 5 and 10 min after Paf.

Bioassay

Male Dunkin-Hartley guinea-pigs (0.5–1.0 kg) were killed by a blow to the head and exsanguinated. Three strips of longitudinal smooth muscle from the ileum (GPISM) were prepared and superfused as previously described (Piper & Stewart, 1986a). Strips of GPISM were continuously superfused with cardiac effluent to detect the release of leukotriene-like substances.

Radioimmunoassay

In some experiments, the cardiac effluents were collected on ice for periods of 10 min before and 10 min after Paf, to determine the production and release of AA metabolites. The cardiac effluents were passed through C_{18} Sep-Paks and the AA metabolites were eluted with methanol, evaporated to dryness and stored under N_2 at −20°C until radioimmunoassay (RIA) of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and TXB_2 or further purification of leukotrienes on reverse phase-h.p.l.c. (Piper & Stewart, 1986a). The percentage recoveries, assessed by the addition of tritium-labelled prostanoids to 40 ml of Krebs solution, for both 6-keto-$PGF_{1\alpha}$ (99 ± 3, $n = 3$) and TXB_2 (82 ± 2, $n = 3$) were high and showed little variability. Thus, the values of 6-keto-$PGF_{1\alpha}$ and TXB_2 presented in the present study have not been corrected for recovery.

The percentage cross-reactions for 6-keto-$PGF_{1\alpha}$ and TXB_2 have been previously demonstrated (Watts *et al.*, 1982). The limits of detection of assays for 6-keto-$PGF_{1\alpha}$ and TXB_2 were in the range of 0.1 to 0.5 pmol which corresponds to 0.05 to 0.25 pmol min^{-1}. The percentage cross-reactions of the LTC_4 antiserum at 50% binding were LTC_4-sulphone, 68.9; LTD_4, 28.9; LTE_4-sulphone, 7.1; LTE_4, 0.7. The following substances showed less than 0.02% cross-reaction: LTB_4, 20-OH-LTB_4, 20-COOH-LTB_4, PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2, 6-keto-$PGF_{1\alpha}$ and arachidonic acid. The percentage cross-reactions of the LTB_4 antiserum at 50% binding were less than 0.1% for the following substances: 20-OH-LTB_4, 20-COOH-LTB_4, LTC_4, LTD_4, LTE_4, LTC_4-sulphone, LTE_4-sulphone, PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2 and AA. The percentage recoveries for LTB_4 (94 ± 5, $n = 32$) and LTC_4 (61 ± 6, $n = 32$) were more variable than those of the cyclo-oxygenase products and have been used to correct the levels of these lipoxygenase products. The limits of detection of the assays for LTB_4 and LTC_4 were in the range of 0.03 to 0.1 pmol, which corresponds to 0.03–0.1 pmol min^{-1}. The inter-assay coefficients of variation over a 12 month period were: LTB_4 (17%, $n = 6$); LTC_4 (8%, $n = 6$); 6-keto-$PGF_{1\alpha}$ (12%, $n = 6$); TXB_2 (17%, $n = 6$). The intra-assay coefficients of variation quoted below are for the assays carried out in the present study and were: LTB_4 (15%); LTC_4 (3%); 6-keto-$PGF_{1\alpha}$ (15%); TXB_2 (16%).

Metabolism of [^{3}H]-Paf

The metabolism of [^{3}H]-Paf was examined in hearts

perfused with Krebs containing bovine serum albumin (BSA, 0.25% w/v). Approximately 10^5 d.p.m. [^{3}H]-Paf together with 100 pmol of unlabelled Paf were administered as a bolus injection in a volume of 100 μl. Cardiac parameters were measured, as previously described, throughout an 18 min period during which the cardiac effluent was collected on ice in 9 aliquots. Platelet-activating factor was isolated from acidified Krebs (pH~3.5) by passage through C_{18} Sep-Pak cartridges (Waters Associates), previously washed with methanol (5 ml) followed by distilled H_2O (5 ml). The samples were then passed through the Sep-Pak cartridges at a flow rate of 5 ml min^{-1}. Following sample application, the Sep-Pak cartridges were washed with distilled H_2O (5 ml) and Paf was then eluted with a mixture of chloroform:methanol (80:20; 5 ml). The recovery of [^{3}H]-Paf was 95 ± 5% (n = 20).

An aliquot of the eluate (1.0 ml) was evaporated to dryness under reduced pressure before being counted for tritium (Packard Tri-Carb 4640, liquid scintillation spectrometer). The remaining eluate was evaporated to dryness and stored under N_2 at −20°C for subsequent thin-layer chromatography (t.l.c.). The method of Parente & Flower (1985) was used to separate Paf from lyso-Paf on t.l.c. Briefly, samples were redissolved in the mobile phase (chloroform: methanol:H_2O 65:35:6) and applied to silica t.l.c. plates (Whatman LK 5D) together with 50 μg of authentic Paf, lyso-Paf, phosphatidylcholine and phosphatidylinositol. After drying the plates, phospholipids were stained with iodine vapour and R_F values were calculated. The plates were then scraped at 1 cm intervals into glass tubes containing 0.5 ml of saline (0.9% w/v NaCl; 0.25% w/v BSA) to elute tritium-labelled Paf and metabolites from the silica. Following the addition of instagel (Packard) the samples were counted (Packard Tri-Carb 4640).

In addition, to determine whether any of the injected Paf was retained by the hearts, at the end of the perfusion period hearts were placed in a solubilizing liquid (Lumasolv, Lumac System A G). Following complete digestion of the tissue, the radioactive content of the solubilized tissue was determined.

Statistical analyses

The data were analysed by Student's two-tailed t test. An analysis of variance was initially performed when several groups were being compared. Differences were subsequently identified by Student's t test using Bonferroni's correction. When the variances of the groups being tested differed significantly ($P < 0.05$, F-test), a modified t test using a separate variances estimate was carried out. When appropriate, a paired t test was used to analyse the data, i.e., when paired observations were made in the same heart. A P value less than 0.05 was considered to be significant. Data are presented as the means ± s.e.mean of n observations.

Materials

All chemicals used were of analytical grade. The solvents for h.p.l.c. were of h.p.l.c. grade. Compounds used and the source of their supply were as follows: BN 52021 (3-(1, 1-dimethylethyl) hexahydro-1, 4, 7b-trihydroxy-8-methyl-9H-1, 7-α-(epoxymethano)-1H, 6αH-cyclopenta [C] furo (2, 3b) [3′, 2′:3, 4] cyclopenta (1, 2-d] furan-5, 9, 12 (4H)-trione, IHB-IPSEN), Institute for therapeutic research, Le-Plessis-Robinson, France; bovine serum albumin, essentially fatty acid free, L-α-phosphatidylcholine, L-α-phosphatidylinositol, Sigma; FPL 55712 (sodium 7-(3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxypropyl-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate), Fisons Pharmaceuticals; heparin, Evans; indomethacin and L-652, 731 (trans-2, 5-Bis-(3, 4, 5-trimethoxyphenyl) tetrahydrofuran, Merck, Sharp & Dohme; LTB_4, LTC_4, LTD_4 and LTE_4, Merck Frosst, Canada; hexadecyl-Paf and hexadecyl-lyso-Paf, Bachem Inc., Essex; U44069 ((15S) - hydroxy - 9α, 11α - (epoxymethano) prosta - 5Z, 13E - dienoic acid) Upjohn; 1-O-alkyl-1′, 2′-[^{3}H]-Paf 59.5 Ci mmol^{-1}, 1-O-alkyl-1′, 2′-[^{3}H]-lyso-Paf 45 Ci mmol^{-1}, 14, 15-[^{3}H]-LTB_4 32 Ci mmol^{-1}, 14, 15-[^{3}H]-LTC_4 40 Ci mmol^{-1} and 6-5, 8, 9, 11, 12, 14, 15-[^{3}H]-keto-PGF_1 100 Ci mmol^{-1}, New England Nuclear; 5, 6, 8, 9, 11, 12, 14, 15-[^{3}H]-TXB_2 180 Ci mmol^{-1}, Amersham. The antisera for LTB_4 and LTC_4 were raised by Mr R.O. Thomas and Mr. J. Zakrzewski in this department. The antisera for 6-keto-$PGF_{1\alpha}$ and TXB_2 were gifts from Dr L. Myatt and Dr J.B. Smith, respectively.

Results

Cardiac actions of platelet-activating factor

Intracoronary administration of Paf (0.3–100 pmol) resulted in dose-related increases in coronary perfusion pressure (Table 1). Higher doses of Paf (3–100 pmol) decreased cardiac contractility. Heart rate was unaffected by doses of Paf of up to 30 pmol, whereas 100 pmol induced dysrhythmia in 6 of 14 preparations and a small degree of tachycardia in the remaining preparations (Figure 1). The increases in coronary perfusion pressure elicited by 1–100 pmol were maintained for a period in excess of 10 min. In contrast, only the higher doses of Paf (30 and 100 pmol) elicited a sustained decrease in cardiac contractility (Table 1).

Superfusion bioassay of leukotriene-like substances.

Paf (30 pmol) evoked the release of a substance(s)

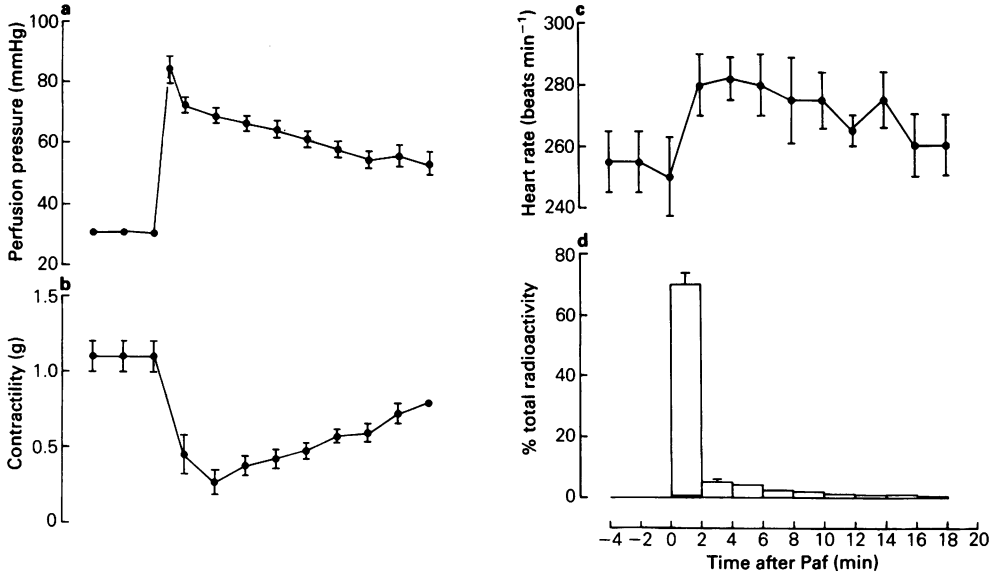

Figure 1 The duration of action of a bolus dose of Paf (100 pmol + 10^5 d.p.m. [^{3}H]-Paf) and its profile of metabolism as assessed by thin layer chromatography (t.l.c.) of partially purified cardiac effluents. The parameters were measured at 2 min intervals from 4 min before Paf administration to 18 min after Paf, and are presented as the mean with vertical lines indicating s.e.mean, of 6 observations; 2 min fractions were collected for analysis of the recovery and metabolism of [^{3}H]-Paf. (a) Coronary perfusion pressure in mmHg. (b) Cardiac contractility in g. (c) Heart rate in beat min^{-1}. (d) Efflux of tritium as a percentage of the total injected; solid portion of the histogram indicates the proportion of tritium co-eluting with lyso-Paf on t.l.c. whereas the open portion indicates the proportion co-eluting with Paf on t.l.c. $^*P < 0.05$, compared to the value of the parameter 2 min before Paf administration, Student's paired t test.

Table 1 The time-course and dose-dependence of platelet-activating factor (Paf)-induced (a) increases in coronary perfusion pressure and (b) decreases in cardiac contractility

a						
Paf	n	*Pre-Paf*[1]	*Increase in coronary perfusion pressure* (mmHg)			
(pmol)		(mmHg)	*Peak*	*2 min*	*5 min*	*10 min*
0.3	3	38.0 ± 1.5	3.5 ± 0.3	1.2 ± 1.1	1.0 ± 0.6	1.0 ± 0.6
1.0	3	44.7 ± 2.7	21.3 ± 1.2	12.2 ± 0.6	3.7 ± 0.9	3.7 ± 0.3
3.0	4	47.8 ± 1.4	23.3 ± 3.3	16.0 ± 2.2	9.3 ± 1.1	6.3 ± 1.7
10.0	4	35.4 ± 1.4	20.8 ± 2.7	17.4 ± 2.7	16.9 ± 3.5	17.5 ± 3.7
30.0	8	37.6 ± 3.4	35.6 ± 3.9	27.3 ± 3.6	20.6 ± 2.4	20.1 ± 2.6
100.0	14	38.3 ± 2.0	40.0 ± 3.6	31.1 ± 3.1	26.8 ± 2.8	22.8 ± 2.6
b		*Pre-Paf*[1]		*Decrease in cardiac contractility* (g)		
		(g)		*2 min*	*5 min*	*10 min*
0.3	3	1.00 ± 0.06		0 ± 0.06	0 ± 0.06	0.10 ± 0.12
1.0	3	1.07 ± 0.09		−0.23 ± 0.12	−0.17 ± 0.09	−0.10 ± 0.06
3.0	4	1.22 ± 0.06		−0.33 ± 0.06	−0.09 ± 0.07	−0.11 ± 0.15
10.0	4	1.09 ± 0.18		−0.22 ± 0.15	−0.08 ± 0.15	−0.10 ± 0.23
30.0	8	1.02 ± 0.09		−0.59 ± 0.10	−0.59 ± 0.10	−0.57 ± 0.13
100.0	14	0.89 ± 0.12		−0.58 ± 0.14	−0.71 ± 0.09	−0.63 ± 0.10

[1]Absolute values of (a) coronary perfusion pressure and (b) cardiac contractility before Paf administration.

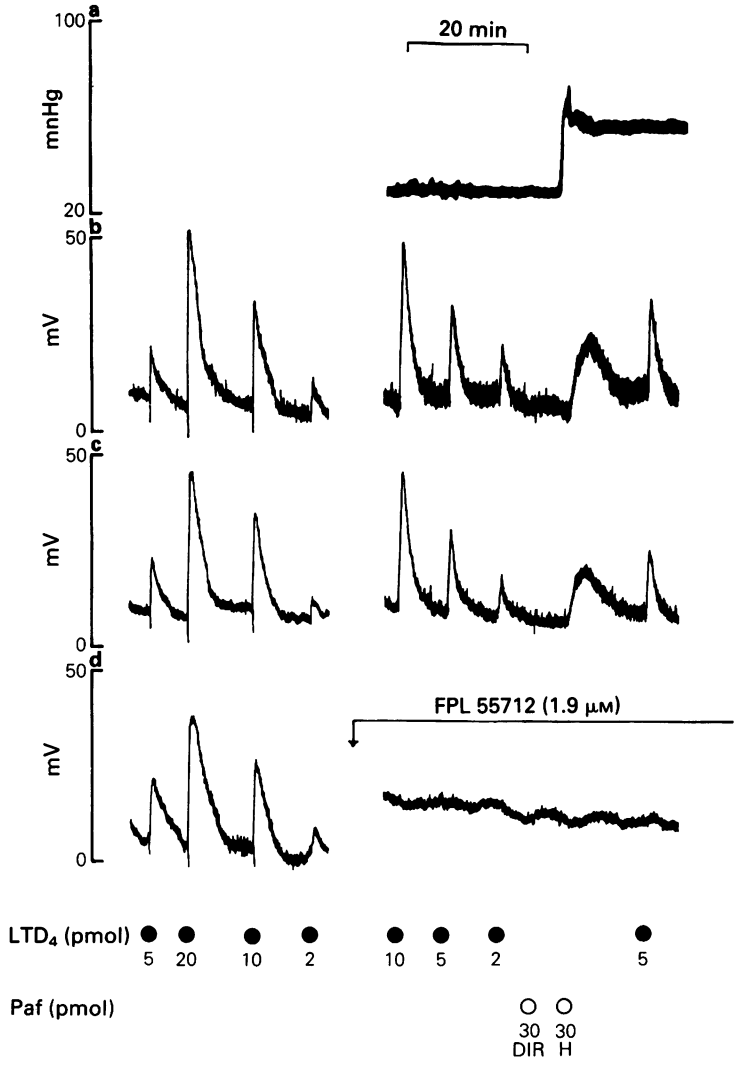

Figure 2 The effects of a bolus dose of platelet-activating factor (Paf, 30 pmol) on coronary perfusion pressure (a) and on superfused guinea-pig longitudinal smooth muscle from the ileum (GPISM; b–d). Leukotriene D_4 (LTD_4, 2–20 pmol) was administered directly over the GPISM tissues. The last tissue was continuously exposed to FPL 55712 (1.9 μM) for 2 h before the Paf experiment. Paf administered directly (DIR) over the assay tissues did not affect the tone which was increased in the upper 2 assay tissues by Paf administered through the heart (H). This tracing is representative of results obtained in 3 other experiments.

having leukotriene-like activity on the superfused GPISM (Figure 2). The contractile activity was evident after a delay of approximately 60 s, coinciding with the peak of the vasoconstrictor response to Paf. The contraction of the assay tissues subsided 10–15 min after Paf administration. A subsequent injection of Paf resulted in no further increase in coronary perfusion pressure, nor was there any further release of leukotriene-like material into the cardiac effluent. The amount of leukotriene-like material released by Paf (30 pmol) was 2.6 ± 0.2 pmol leukotriene D_4 equivalents (mean ± s.e.mean of 4 observations).

Table 2 The inhibitory effects of FPL 55712 (1.9 μM) and indomethacin (2.8 μM) on (a) increases in coronary perfusion pressure and (b) decreases in cardiac contractility elicited by Paf (30 pmol)

a		*Pre-Paf*[1]	*Increase in coronary perfusion pressure* (mmHg)			
Treatment	n	(mmHg)	*Peak*	*2 min*	*5 min*	*10 min*
DMSO	8	37.6 ± 3.4	35.6 ± 3.9	27.3 ± 3.6	20.6 ± 2.4	20.1 ± 2.6
Indomethacin	5	46.6 ± 1.1	27.6 ± 3.6	12.2 ± 2.8*	12.0 ± 1.7*	9.8 ± 1.4*
FPL 55712	8	46.6 ± 3.3	23.0 ± 4.1	15.1 ± 3.0*	10.4 ± 2.8*	8.1 ± 2.4*
b		*Pre-Paf*[1] (g)		*Decrease in cardiac contractility* (g) *2 min*	*5 min*	*10 min*
DMSO	8	1.02 ± 0.09		−0.59 ± 0.10	−0.59 ± 0.13	−0.57 ± 0.13
Indomethacin	5	1.42 ± 0.16		−1.10 ± 0.11*	−1.00 ± 0.13*	−1.04 ± 0.14*
FPL 55712	8	0.97 ± 0.10		−0.41 ± 0.20	−0.35 ± 0.10	−0.30 ± 0.09

[1]Absolute values of coronary perfusion pressure and cardiac contractility before Paf administration.
*$P < 0.05$, compared to DMSO at the corresponding time.

Effects of indomethacin and FPL 55712

Indomethacin pretreatment (2.8 μM) attenuated the increase in coronary perfusion pressure elicited by 30 pmol Paf at 2–10 min after challenge (Table 2). In contrast, the decrease in contactility appeared to be exacerbated by indomethacin. However, when the post-Paf contractility was expressed as a percentage of that before Paf administration in control hearts (77 ± 18%), indomethacin did not appear to enhance the Paf-induced decrease in cardiac contractility (65 ± 28%).

The leukotriene receptor antagonist, FPL 55712 (1.9 μM), inhibited the increases in coronary perfusion pressure induced by Paf (1–30 pmol) but did not significantly alter ($P < 0.05$, Student's t test) the decrease in cardiac contractility (Tables 2 and 3). The peak responses to Paf appeared to be less susceptible to the antagonistic actions of FPL 55712 than those occurring 5 to 10 min after Paf administration.

The combination of indomethacin (2.8 μM) and FPL 55712 (1.9 μM) pretreatments did not result in a more marked inhibition than FPL 55712 alone.

Release of arachidonic acid metabolites by platelet-activating factor

Platelet-activating factor (30 pmol) selectively

Table 3 The inhibitory effects of FPL 55712 (1.9 μM)-pretreatment on Paf (1–30 pmol)-induced (a) increases in coronary perfusion pressure and (b) decreases in cardiac contractility

a			*Increase in coronary perfusion pressure* (mmHg)			
Paf (pmol)	n	*Pre-Paf*[1] (mmHg)	*Peak*	*2 min*	*5 min*	*10 min*
1.0	3	38.0 ± 1.0	7.7 ± 0.9*	2.8 ± 0.9*	0.3 ± 0.2*	0.3 ± 0.3*
3.0	4	40.6 ± 2.7	13.6 ± 1.0	8.5 ± 1.3*	5.1 ± 0.5*	3.6 ± 1.1*
10.0	4	44.1 ± 2.7	21.5 ± 4.9	15.1 ± 0.9	8.0 ± 2.3*	7.4 ± 0.7*
30.0	8	46.6 ± 3.3	23.0 ± 4.1*	15.1 ± 3.0*	10.4 ± 2.8*	8.1 ± 2.4*
b		*Pre-Paf*[1] (g)		*Decrease in cardiac contractility* (g) *2 min*	*5 min*	*10 min*
1.0	3	0.87 ± 0.07		−0.07 ± 0.12	−0.07 ± 0.12	0.07 ± 0.20
3.0	4	1.08 ± 0.11		+0.04 ± 0.07	+0.03 ± 0.05	0 ± 0.18
10.0	4	1.08 ± 0.15		−0.41 ± 0.20	−0.35 ± 0.10	−0.30 ± 0.09
30.0	8	0.97 ± 0.10	−0.44 ± 0.08	−0.48 ± 0.09	−0.49 ± 0.11	

[1]Absolute value of parameter before Paf administration.
*$P < 0.05$, compared to the response at the corresponding time after Paf administration in controls (see Table 1 for Paf-induced changes in cardiac contractility and coronary perfusion pressure in control hearts).

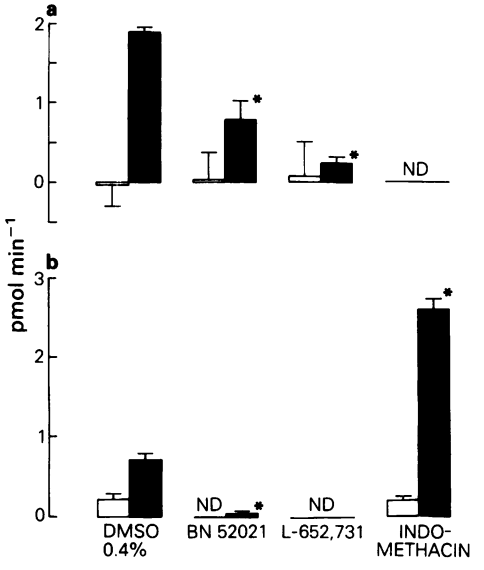

Figure 3 Platelet-activating factor (30 pmol)-induced increase in the production and release of immunoreactive (ir) arachidonic acid metabolites from hearts treated with vehicle control (0.4% DMSO), BN 52021 (30 μM), L-652, 731 (10 μM), and indomethacin (2.8 μM). Ordinate scales: (a) increase in release of cyclo-oxygenase products (open columns: ir-6-keto-prostaglandin $F_{1\alpha}$, solid columns: ir-thromboxane B_2) in pmol min^{-1} ($n = 4$); (b) increase in release of lipoxygenase products open columns: ir-leukotriene C_4, solid columns: ir-leukotriene B_4 in pmol min^{-1} ($n = 4$).
*$P < 0.05$, compared to DMSO-treated hearts (Student's unpaired t test).

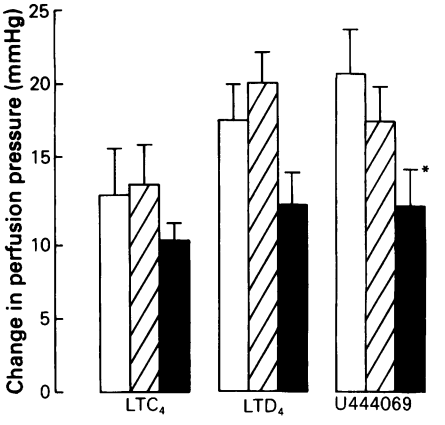

Figure 4 The effects of BN 52021 (30 μM, hatched columns) and L 652, 731 (10 μM, solid columns) on leukotriene C_4 (LTC_4 30 pmol), LTD_4 (100 pmol) and U44069 (100 pmol)-induced increased in coronary perfusion pressure. Open columns: effect of 0.4% DMSO on increases in perfusion pressure. *$P < 0.05$, compared to responses in vehicle-treated hearts (0.4% v/v DMSO).

increased the production of TXB_2; there was no corresponding increase in the generation of 6-keto-$PGF_{1\alpha}$ (Figure 3). The increase in TXB_2 levels in the post-Paf cardiac effluents was prevented by indomethacin which also reduced the basal release of both TXB_2 and 6-keto-$PGF_{1\alpha}$ (0.13 ± 0.08 and 1.98 ± 0.46 pmol min^{-1}, respectively) to below the assay detection limited (~ 0.05 pmol min^{-1}).

Paf evoked the release of both LTB_4 and LTC_4 (Figure 3), neither of which was detected in significant amounts (LTC_4 < 0.03 pmol min^{-1}; LTB_4 0.07 ± 0.02 pmol min^{-1}) before Paf administration. Neither LTD_4 nor LTE_4 was detected in cardiac effluents following Paf. Pretreatment of hearts with indomethacin resulted in a significant increase in the release of LTB_4 but not that of LTC_4.

Inhibitory actions of BN 52021 and L-652, 731

The selectivity of the Paf receptor antagonists was assessed by examining their effects on the increases in coronary perfusion pressure elicited by leukotrienes C_4 (LTC_4, 30 pmol) and D_4 (LTD_4, 100 pmol) and the TXA_2-mimetic, U44069 (100 pmol). These agonist doses were chosen to give a peak increase in coronary perfusion pressure similar in magnitude to that of 10 pmol Paf. However, it may be of importance that the durations of the coronary vasoconstrictor effects of LTC_4 (~ 4 min), LTD_4 (~ 2 min) and U44069 (~ 30 s) were considerably less than that of Paf (greater than 10 min). Neither BN 52021 nor L-652, 731 had any significant effect on the coronary vasoconstrictor actions of the leukotrienes (Figure 4). On the other hand, L-652,731 reduced the response to U44069 by 40%.

The increases in coronary perfusion pressure and the decreases in cardiac contractility induced by Paf (10–100 pmol) were antagonized in an apparently surmountable manner by either BN52021 (30 μM) or L-652, 731 (10 μM) pretreatment (Table 4 and Figure 5). Antagonism of the increase in coronary perfusion pressure in response to 100 pmol Paf was evident as a more rapid recovery rather than a reduction in the peak response (Figure 6). In addition to inhibiting the cardiac actions of Paf, both receptor antagonists reduced the increase in production of vasoactive AA metabolites (Figure 3) without significantly altering ($P > 0.05$, Student's t test) the basal release of cyclo-oxygenase products (6-keto-$PGF_{1\alpha}$ in pmol min^{-1}: 1.98 ± 0.46, 1.60 ± 0.24 and 1.61 ± 0.15 for control, BN 52021 and L-652, 731, respectively; TXB_2

Table 4 The effects of Paf receptor antagonists on coronary perfusion pressure, cardiac contractility and heart rate

Pretreatment	n	*Coronary perfusion pressure* (mmHg)	*Cardiac contractility* (g)	*Heart rate* (beats min^{-1})
DMSO 0.4%	29	41.4 ± 1.0	1.02 ± 0.06	237 ± 17
BN52021 30 μM	13	39.6 ± 0.9	0.73 ± 0.06*	238 ± 5
L-652,731 10 μM	13	37.3 ± 0.8*	0.83 ± 0.06	226 ± 5

*$P < 0.05$, compared to control values, i.e. 0.4% DMSO.

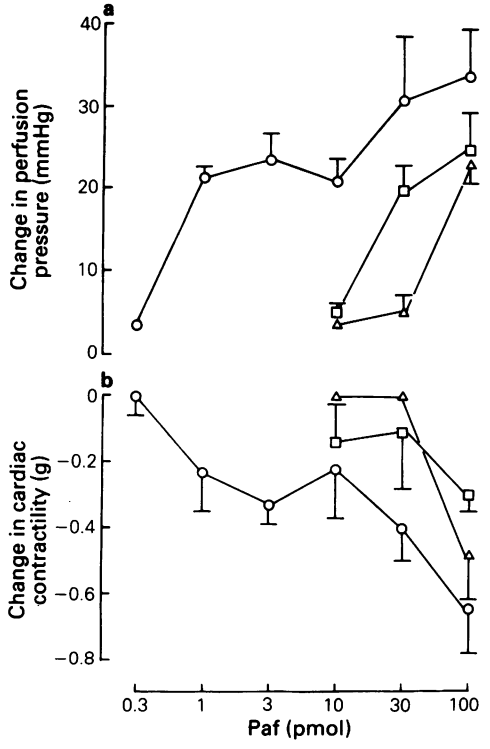

Figure 5 The effects of platelet-activating factor (Paf 0.3–100 pmol) on (a) coronary perfusion pressure and (b) cardiac contractility and the antagonistic effects of BN 52021 (30 μM, □) and L-652,731 (10 μM, △). The responses were measured at their peak which corresponds to 20–40 s after Paf administration for increases in coronary perfusion pressure and 2–4 min for decreases in cardiac contractility. (○) Response to Paf after 0.4% DMSO. Each point represents the mean of at least 3 experiments and vertical lines show s.e.mean.

in pmol min^{-1}: 0.13 ± 0.08, 0.16 ± 0.05 and 0.33 ± 0.10 for control, BN 52021 and L-652,731, respectively).

Duration of action of Paf

Ten minutes after the administration of Paf (100 pmol) in the presence of BN 52021 or L-652,731, the increase in coronary perfusion pressure was inhibited compared to vehicle controls. Cessation of antagonist infusion at this time resulted in a rapid increase in perfusion pressure (Figure 6), whereas removal of the vehicle had no significant effect ($P > 0.05$, paired t test). When infusions were re-started at 15 min, there was a rapid decrease in coronary perfusion pressure in hearts receiving antagonist but not those receiving vehicle infusions. At 20 min, cessation of antagonist infusion had a similar effect to that observed 10 min after Paf.

The duration of action of Paf and its metabolism were investigated in hearts perfused with BSA (0.25% w/v)-containing Krebs solution. The increase in perfusion pressure reached a maximum at 34 ± 5 s ($n = 6$) after Paf (100 pmol + 10^5 d.p.m. [^{3}H]-Paf). The maximum decrease in cardiac contractility was observed at 4 min (Figure 1). Neither coronary perfusion pressure nor cardiac contractility returned to control values during the 18 min period following Paf administration whereas the small increase in heart rate had subsided after 16 min. The elution profile of tritium shows that the conversion of exogenous Paf to lyso-Paf did not proceed to a significant extent; during the 18 min collection period, 87% of the administered tritium was recovered, of which only 2% co-eluted with lyso-Paf (R_F 0.25), the remainder co-eluted with Paf (R_F 0.40). The hearts retained 3.8 ± 0.3% ($n = 6$) of the administered tritium. Radioactivity was not detected on regions of the t.l.c. plate other than those associated with Paf or lyso-Paf. Thus, the remaining 9% of tritium may be accounted for by losses occurring within the apparatus or during the extraction procedure.

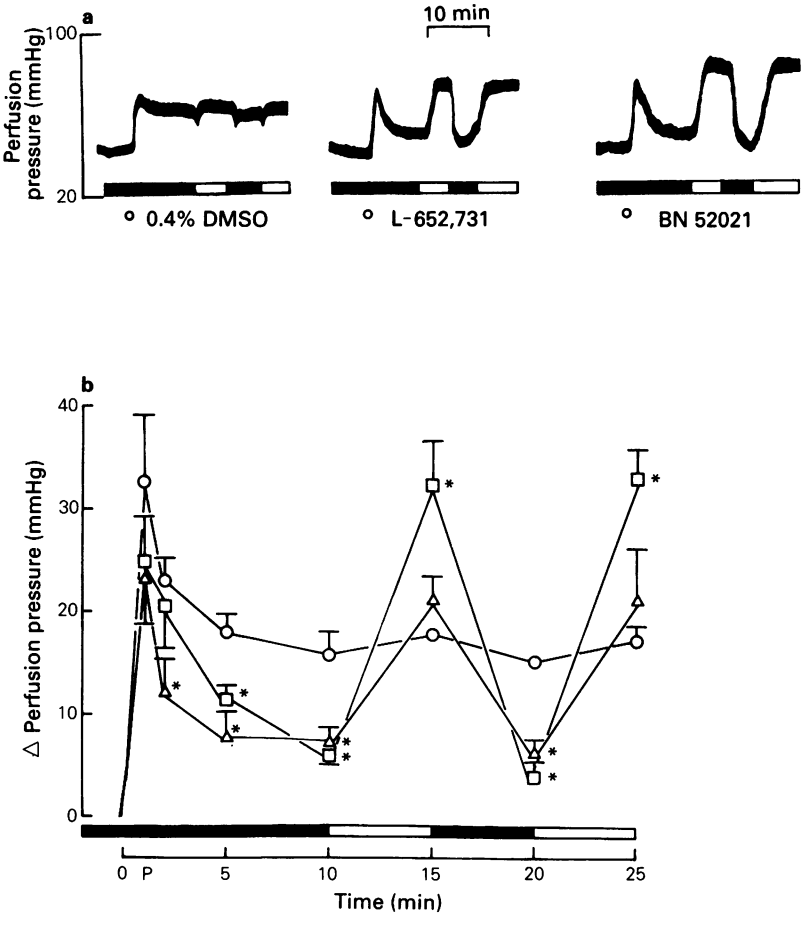

Figure 6 Effects of Paf (100 pmol, O) on coronary perfusion pressure and its antagonism by BN 52021 (30 μM) and L-652,731 (10 μM). (a) Representative tracings from 3 separate experiments in hearts treated with the vehicle (0.4% v/v DMSO) or the Paf receptor antagonists BN 52021 or L 652,731. The open bars indicate the period during which vehicle/antagonist infusions were stopped. (b) Mean data for the increases in coronary perfusion pressure in the representative experiments shown in (a). Filled bars indicate infusion of vehicle/antagonist whereas open bars indicate the period in which infusions were stopped. Effects of (O) 0.4% DMSO, (□) BN 5202 and (△) L-652731. *$P < 0.05$ compared to increase in vehicle-treated hearts ($n = 4$).

Discussion

Paf administration evoked a long-lasting cardiac dysfunction comprising coronary vasoconstriction and impaired contractility with dysrhythmias at the highest dose. It has been suggested that these effects of Paf are independent of the release of vasoactive AA metabolites (Levi *et al.*, 1984). The findings of the present study indicate that Paf-induced coronary vasoconstriction in the guinea-pig isolated heart is the result of at least two mechanisms: one dependent on the release of the vasoconstrictors TXA_2 and LTC_4; the other, an undefined action of Paf which may represent a direct effect on the coronary vasculature. The complexity of the cardiac actions of Paf *in vivo* is further underscored by a potent platelet-activating action in the guinea-pig and a number of other species (Benveniste, 1985) with the exception of the rat (Terashita *et al.*, 1983).

Intracoronary administration of Paf resulted in the release of leukotriene-like material which was subsequently identified as LTC_4 using a combination of

r.p.-h.p.l.c. and detection by RIA. Neither LTD_4 nor LTE_4 was detected in cardiac effluents following Paf-challenge, suggesting that the released LTC_4 did not undergo significant metabolism. The amount of LTC_4 released by guinea-pig hearts was an order of magnitude less than that released by rat hearts (Piper & Stewart, 1986a), as assessed by either on-line superfusion bioassay or by RIA. Even though the rat heart is considerably less sensitive to the cardiac actions of Paf, the potency of leukotrienes in the guinea-pig is similar to that in the rat (Letts & Piper, 1983; Lefer & Roth, 1985; present study cf. Piper & Stewart, 1986a). It may be concluded from these comparisons that the amount of LTC_4 released from guinea-pig hearts was insufficient to account for all of the coronary vasoconstriction induced by Paf. This conclusion is strengthened by the failure of the leukotriene receptor antagonist, FPL 55712, to inhibit completely the increases in coronary perfusion pressure, particularly when higher doses of Paf were administered.

Since LTB_4 does not exert any measurable acute effects in guinea-pig isolated heart (Letts & Piper, 1983) it seems unlikely that the release of LTB_4 by Paf is of any consequence in this respect. It is noteworthy that the amounts of LTB_4 release by Paf were considerably greater than those of LTC_4, and indomethacin further increased LTB_4 release without significantly altering that of LTC_4. These observations may be explained by the existence of different sources for LTC_4 and LTB_4. Paf has been shown to activate human polymorphonuclear leukocytes (PMNLs) aggregation with a concomitant generation of LTB_4 (Gorman *et al.*, 1983). Neutrophils stimulated by the ionophore, A23187, release large amounts of LTB_4 with only a relatively small release of LTC_4 (Sun *et al.*, 1985). In addition, recent studies using *in vivo* models of myocardial infarction in the rabbit demonstrate that hearts, taken from animals previously subjected to coronary artery ligation and reperfusion, release increased amounts of prostanoids and leukotrienes in response to formyl-methionyl-leucyl-phenylalanine (Barst & Mullane, 1985; Evers *et al.*, 1985), an activator of PMNLs (Schiffman *et al.*, 1975). Thus, it is suggested that neutrophils or other immunocompetent cell types may represent a potential target for Paf in the guinea-pig perfused heart. In addition, the release of LTB_4 by Paf would be consistent with a pro-inflammatory role *in vivo* in cardiac tissue for this potent phospholipid mediator.

The combined use of indomethacin and FPL 55712 failed to inhibit completely the coronary vasoconstrictor actions of Paf, suggesting that the actions of Paf are, at least in part, independent of the release of vasoactive AA metabolites. The nature of this latter action is unknown. However, there is considerable evidence that Paf, at concentrations relevant to inflammation, does not exert direct effects on vascular and other types of smooth muscle (Cervoni *et al.*, 1983; Lefer *et al.*, 1984a). Thus, a further secondary mediator of coronary vasoconstriction may be released by Paf.

In contrast to earlier observations in the rat heart (Piper & Stewart, 1986a), Paf selectively increased TXB_2 production without a corresponding increase in 6-keto-$PGF_{1\alpha}$. This finding is in apparent conflict with the observation that exogenous LTC_4 and LTD_4 released 6-keto-$PGF_{1\alpha}$ but not TXB_2 from perfused guinea-pig hearts (Terashita *et al.*, 1981) and suggests that Paf-induced release of LTC_4 does not, in turn, release PGI_2. Similarly, in antigen-challenged hearts which release large amounts of LTC_4, FPL 55712 treatment failed to reduce the release of cyclo-oxygenase products (Aehringhaus *et al.*, 1983). In rat hearts, Paf-induced release of cyclo-oxygenase products was not altered by FPL 55712 treatment (Piper & Stewart, 1986a) and responses to leukotrienes were not inhibited by indomethacin in this species (Letts & Piper, 1983). However, in guinea-pig hearts in which the coronary vasoconstrictor actions of leukotrienes are partially sensitive to indomethacin (Letts & Piper, 1982), the independence of Paf-induced TXA_2 generation from an intermediary role of LTC_4 has not been established. Indomethacin inhibited the coronary vasoconstrictor actions of Paf to an extent similar to that of FPL 55712. One interpretation of these observations is that indomethacin inhibited the Paf response by reducing the formation of the potent coronary vasoconstrictor TXA_2 (Anhut *et al.*, 1978; Allan & Levi, 1981; Mullane *et al.*, 1982) and that the final stimulus for TXA_2 generation was LTC_4 rather than Paf. An alternative mediator of the indomethacin-sensitive component of the coronary vasoconstriction is PGD_2 (Anhut *et el.*, 1978; Allan & Levi, 1980). Nevertheless, it has recently been demonstrated that the coronary vasoconstrictor actions of PGD_2 may be mediated by TXA_2 (Hattori & Levi, 1986). It may be concluded that endogenously released LTC_4 elicits a different profile of release of AA metabolites favouring those with vasoconstrictor activity whereas exogenous LTC_4 releases PGI_2 (Terashita *et al.*, 1981) a potent vasodilator in the coronary vasculature (Schror *et al.*, 1978).

Both BN 52021 (Nunez *et al.*, 1986) and L-652, 731 (Hwang *et al.*, 1985) have been described as specific and selective Paf-receptor antagonists on washed human and rabbit platelets respectively. Furthermore, these receptor antagonists have been used to implicate Paf in the pathogenesis of a variety of inflammatory models including endotoxin shock (Terashita *et al.*, 1985), systemic anaphylaxis (Touvay *et al.*, 1985), immune complex-induced hypotension (Sanchez-Crespo 1985; Doebber *et al.*, 1986), carrageenin-induced oedema (Hwang *et al.*, 1986) and the Arthus reaction in rabbit skin (Hellewell & Williams, 1986). In addi-

tion, we have recently found that BN 52021 and L-652, 731 attenuated antigen-induced coronary vasoconstriction in guinea-pig perfused hearts (Piper & Stewart, 1986b). The present study shows that these receptor antagonists inhibit the cardiac response to Paf in an apparently competitive manner. The inability to obtain repeated responses to Paf in the isolated heart makes an analysis of pA_2 values impractical. Nevertheless, the relative potencies of these antagonists in the heart is similar to that obtained on washed rabbit platelets (Hwang *et al.*, 1985). BN 52021 had no effect on leukotriene or TXA_2-mimetic-induced vasoconstriction whereas L-652,731 inhibited the latter by 40%, suggesting a lack of specificity. However, L-652,731 showed a degree of selectivity with respect to the TXA_2-mimetic since the response to an equiactive dose of Paf was inhibited by more than 90%. Neither antagonist altered the basal release of either TXB_2 or 6-keto-$PGF_{1\alpha}$, indicating a lack of inhibitory effects on either cyclo-oxygenase or phospholipase A_2. Thus, both BN 52021 and L-652, 731 appear to be useful tools in determining a role for Paf in cardiac dysfunction.

A striking feature of the actions of Paf on isolated hearts is its protracted nature. This feature is not shared by other putative mediators of coronary vasospasm such as LTC_4, LTD_4, TXA_2 or 5-HT. Indeed, only antigen-challenge exhibits a similar protracted action. Furthermore, the duration of the response does not appear to be attributable to a long-lasting production of leukotrienes, since the contraction of the bioassay tissues subsided after 15 min whereas the perfusion pressure remained elevated. In addition, neither FPL 55712, indomethacin nor the combination accelerated the recovery. Thus, the propagative actions of Paf may not be associated with the release of vasoactive AA metabolites. However, a close association to the Paf receptor is suggested by the ability of the Paf antagonists, to reduce rapidly the vasoconstriction up to 30 min after a single bolus dose of Paf. In experiments following the time-course of the response beyond the usual 10 min observation period, cessation of antagonist infusion resulted in a rapid increase in coronary perfusion pressure which could be readily reversed by further antagonist infusion. This remarkable cycle of events has been paralleled in experiments on the thrombotic action of Paf in superfused mesenteric bed *in situ* in which Paf has a protracted action which may be reversed by BN 52021 up to 3 h after superfusion of Paf (Bourgain *et al.*, 1985). These authors suggested that the long-lasting effect of Paf may be due to autogeneration. Platelets were suggested as being a source of the endogenous Paf since none of the original superfused dose of radiolabelled Paf could be detected at times when BN 52021 still exerted an inhibitory action on thrombus formation. Similarly, in the present study a bolus dose of 100 pmol resulted in a retenion of only 4% after 18 min of perfusion with 80% of the dose appearing in the cardiac effluent in the first 4 min. Nevertheless, it remains possible that this relatively small amount of Paf, if concentrated near its site of action, could sustain a vasoconstrictor response. A study of Paf metabolism in Krebs-BSA perfused lungs showed little conversion of exogenous Paf to its inactive precursor/metabolite, lyso-Paf (Fitzgerald *et al.*, 1986), an observation similar to that in the Krebs-BSA perfused heart. However, Paf is rapidly inactivated after intravenous injection, due to large amounts of plasma acetyl hydrolase (Pinckard *et al.*, 1979).

A further possible explanation for the protracted action of Paf in the perfused heart is a failure to activate modulatory mechanisms. Prostacyclin is a potent vasodilator in the perfused guinea-pig heart (Schror *et al.*, 1978) and endothelium-derived relaxant factor (EDRF) has been shown to relax isolated coronary arteries (Cocks & Angus, 1983). However, intracoronary administration of Paf does not appear to increase 6-keto-$PGF_{1\alpha}$ generation by guinea-pig hearts. High concentrations (0.1–1.0 μM) of Paf fail to increase 6-keto-$PGF_{1\alpha}$ generation by porcine cultured aortic endothelial cells (Gryglewski *et al.*, 1986) or by human cultured umbilical vein endothelial cells (Fan, Lewis, Piper & Stewart, unpublished observation). Furthermore, Paf failed to release EDRF from porcine cultured aortic endothelial cells (Gryglewski *et al.*, 1986) or from the rabbit thoracic aorta. Thus, the relatively unopposed nature of Paf-induced vasoconstriction may contribute to its duration of action. Interestingly, intracoronary administration of Paf in anaesthetized dogs releases a platelet-derived coronary artery vasodilator substance (Jackson *et al.*, 1986).

In conclusion, Paf causes a severe and long-lasting dysfunction in the guinea-pig perfused heart. A similar action *in vivo* may contribute to the precipitous decrease in systemic arterial blood pressure which accompanies modest intravenous doses of Paf (Bessin *et al.*, 1983; Lefer *et al.*, 1984; Feuerstein *et al.*, 1984; Sanchez-Crespo *et al.*, 1985; Terashita *et al.*, 1985; Doebber *et al.*, 1986) which do not appear to be explained solely by reductions in total peripheral vascular resistance (Kenzora *et al.*, 1984). The extent to which the cardiac actions of Paf contribute to the various forms of cardiovascular shock remains to be elucidated.

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