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Involvement of nitric oxide and eicosanoids in platelet-activating factor-induced haemodynamic and haematological effects in dogs

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1 Platelet-activating factor (PAF) is a phospholipid mediator with potent cardiovascular and haematological actions. But its mechanisms of action *in vivo* have not been fully elucidated, probably due to difficulties arising from previous findings that the effects of PAF are largely mediated by the release of a variety of other autacoids. In the present study, the roles of nitric oxide and eicosanoids in the effects of PAF (0.01–0.25 $\mu g\ kg^{-1}$ i.v.) on systemic and pulmonary vasculatures and circulating blood cell count were pharmacologically evaluated in anaesthetized dogs.

2 Higher doses of PAF (>0.1 $\mu g\ kg^{-1}$) produced a biphasic systemic hypotension. The first hypotension seen 30 s after the injection was accompanied by a decrease in systemic vascular resistance, thrombocytopenia and leukopenia, while the second hypotension seen 1–2 min after PAF was accompanied by a marked rise in pulmonary vascular resistance and decreases in aortic blood flow and cardiac contractility. Lower doses of PAF (0.01–0.05 $\mu g\ kg^{-1}$) caused only the first responses in a dose-dependent manner.

3 Pretreatment with indomethacin inhibited the second responses to PAF without affecting the first responses. The thromboxane A_2/prostaglandin H_2 (TP)-receptor antagonist vapiprost blocked the PAF-induced rise in pulmonary vascular resistance. AA-861, a 5-lipoxygenase inhibitor, attenuated the PAF-induced cardiac depression. The nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester inhibited the PAF-induced early decrease in systemic vascular resistance.

4 All observed changes in haemodynamics and blood cell count after PAF were almost abolished by TCV-309, a PAF-receptor antagonist.

5 Reproducible hypotension and thrombocytopenia produced by a lower dose of PAF (0.05 $\mu g\ kg^{-1}$) were respectively attenuated and potentiated by pretreatment with N^G-nitro-L-arginine, another nitric oxide synthase inhibitor. Administration of L-arginine reversed the effects of the nitric oxide synthase inhibitor.

6 These results indicate that PAF-receptor-mediated production of not only eicosanoids but also nitric oxide may contribute to the cardiovascular and haematological responses to PAF in the dog.

Keywords: Platelet-activating factor; nitric oxide; thromboxane A_2; cyclo-oxygenase metabolites; 5-lipoxygenase metabolites; pulmonary hypertension; systemic hypotension; thrombocytopenia; leukopenia

Introduction

Platelet-activating factor (PAF) is an endogenous phospholipid mediator with potent biological activities, and has been implicated in a variety of physiopathological conditions (Braquet *et al.*, 1987). Intravascular administration of PAF in different species of animals elicits not only haemodynamic effects such as systemic hypotension (Blank *et al.*, 1979; McManus *et al.*, 1980; 1981; Lai *et al.*, 1983; Kenzora *et al.*, 1984) and pulmonary hypertension (Bessin *et al.*, 1983; Toyofuku *et al.*, 1988) but also haematological alterations such as transient leukopenia and thrombocytopenia (McManus *et al.*, 1980; 1981), and increased vascular permeability (McManus *et al.*, 1981; Bessin *et al.*, 1983; Braquet *et al.*, 1984; Handley *et al.*, 1984) with the respective patterns of time-course. The mechanisms or the pathways involved in the circulatory effects of PAF particularly in the whole body are complex probably because the PAF receptor has been recognized to activate multiple signaling pathways including activation of phospholipid (via phospholipase A_2, C or D), protein kinase C and Ca^{2+} mobilization (Shukla, 1992), and therefore are not completely understood. In fact, the vascular effects of PAF have been shown to be largely mediated by the release of other vasoactive substances such as prostaglandins

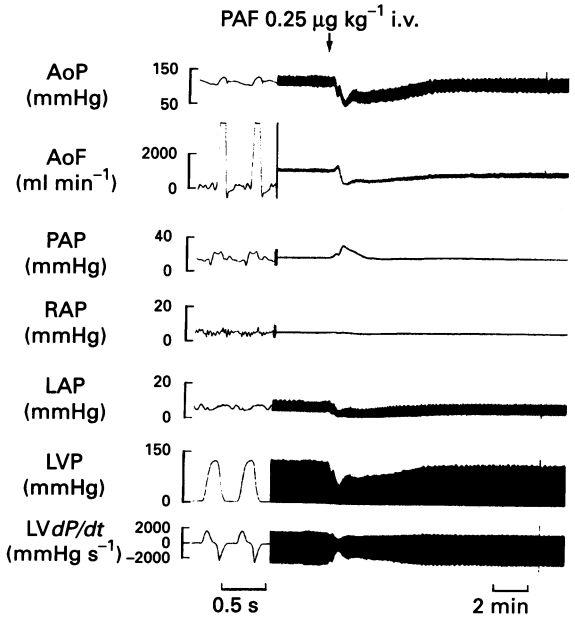

Figure 1 A representative example of haemodynamic responses to platelet-activating factor (PAF, 0.25 $\mu g\ kg^{-1}$ i.v.). AoP = aortic pressure, AoF = aortic blood flow, PAP = pulmonary arterial pressure, RAP = right atrial pressure, LAP = left atrial pressure, LVP = left ventriculr pressure, LV*dP/dt* = first derivative of LVP.

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(Heffner *et al.*, 1983; Piper & Stewart, 1986; Hu & Man, 1991), leukotrienes (Voelkel *et al.*, 1982; Sybertz *et al.*, 1985; Piper & Stewart, 1986) and PAF itself (Tessner *et al.*, 1989). In addition, several lines of evidence indicate that production of endothelium-derived relaxing factor (EDRF), which is thought to be nitric oxide (NO; Palmer *et al.*, 1987), may contribute to the vasodilator actions of PAF in *in vitro* preparations (Kamitani *et al.*, 1984; Kamata *et al.*, 1989; Chiba *et al.*, 1990; Moritoki *et al.*, 1992; Juncos *et al.*, 1993). Nevertheless, the role of NO in PAF-induced hypotensive actions in *in vivo* experimental models remains controversial (Filep & Földes-Filep, 1993; Takekoshi *et al.*, 1993).

Thus, the present study was carried out to examine whether endogenous NO as well as eicosanoids participate in the effects of PAF on the circulatory system *in vivo*. For this purpose, we evaluated the time course of changes in systemic and pulmonary haemodynamic parameters and circulating leukocyte and platelet counts, following the intravenous injection of PAF, in the presence or absence of specific inhibitors or receptor blockers of eicosanoids and NO in anaesthetized dogs.

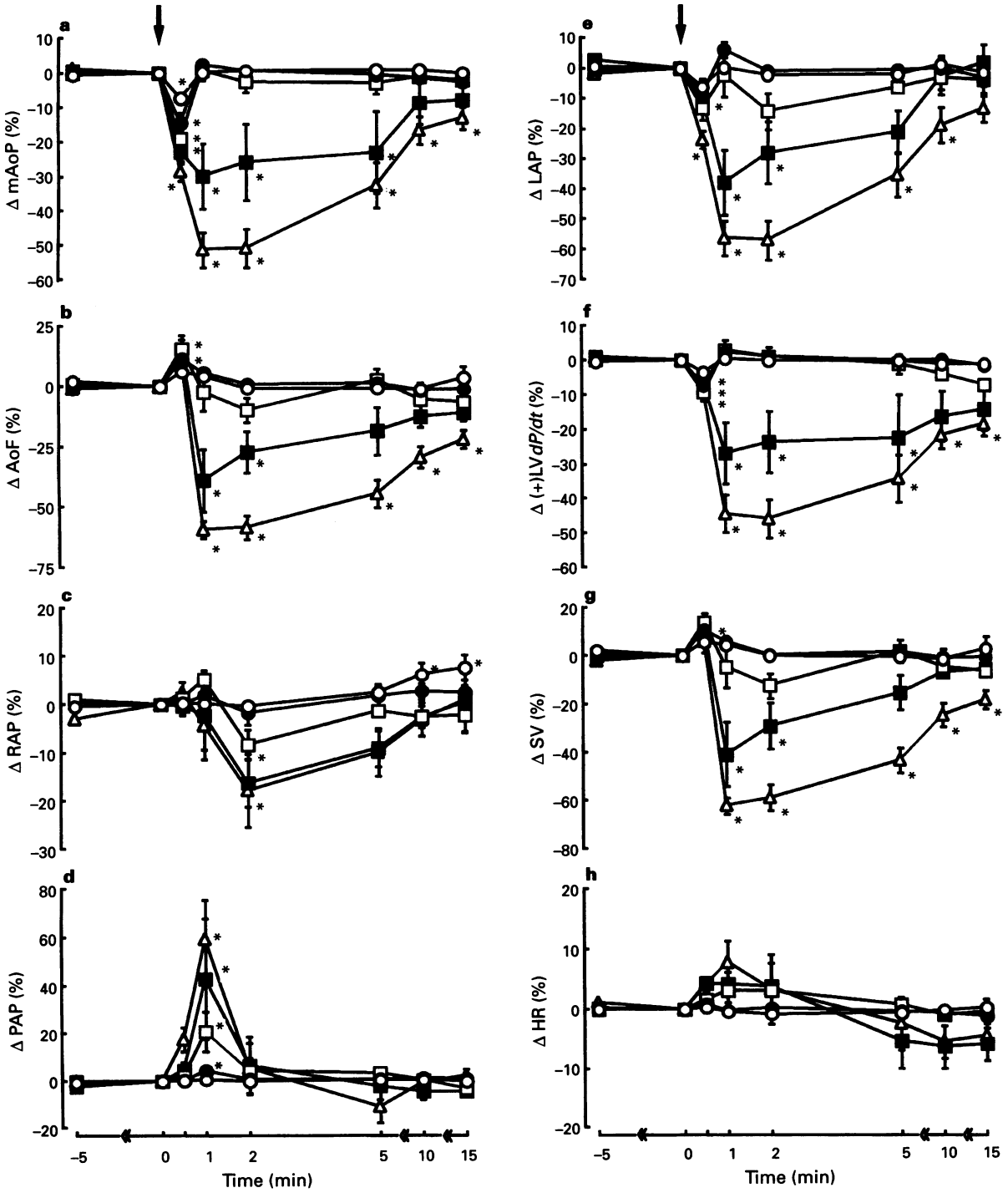

Figure 2 Effects of PAF on haemodynamic variables. (○) PAF 0.01 $\mu g\,kg^{-1}$ i.v. $n=5$, (●) 0.02 $\mu g\,kg^{-1}$ i.v. $n=5$, (□) 0.05 $\mu g\,kg^{-1}$ i.v. $n=6$, (■) 0.10 $\mu g\,kg^{-1}$ i.v. $n=6$, (△) 0.25 $\mu g\,kg^{-1}$ i.v. $n=12$. (a) Mean aortic pressure; (b) aortic blood flow; (c) right atrial pressure; (d) pulmonary arterial pressure; (e) left atrial pressure; (f) peak positive LV*dP/dt*; (g) stroke volume; (h) heart rate. Data represent means ± s.e. $*P<0.05$ vs preadministration value (0 min).

Methods

Experimental preparation

Mongrel dogs of either sex weighing between 9 and 17 kg were anaesthetized with pentobarbitone sodium (25 mg kg^{-1}, i.v.) and were artificially ventilated with room air supplemented with oxygen. A constant level of anaesthesia was maintained with a continuous infusion of pentobarbitone sodium (4–5 mg kg^{-1} h^{-1}, i.v.). A catheter was inserted through the left carotid artery into the aortic root and connected to a pressure transducer (TP-200T, Nihon Kohden) for measurement of aortic pressure (AoP). A left thoracotomy was performed through the fourth intercostal space to expose the heart. The pericardium was opened, and the heart was suspended in a pericardial cradle. The root of the ascending aorta was dissected free from the surrounding tissues, and an electromagnetic flow probe (SP7515, Spectramed) of appropriate size was placed around it and connected to an electromagnetic flowmeter (SP2204, Gould) for measurement of aortic blood flow (AoF). Two catheter-tip manometers (PC-350, Millar) were introduced through the left atrial appendage into the left ventricular cavity and the left atrium to measure left ventricular pressure (LVP) and left atrial pressure (LAP), respectively. A wedge pressure catheter (5Fr.) was inserted through the right femoral vein into the pulmonary artery. A Berman angiographic catheter (5Fr. or 6Fr.) was introduced through the left jugular vein into the right atrium. These two catheters were connected to pressure transducers (TP-200T) for measurement of pulmonary artery pressure (PAP) and right atrial pressure (RAP), respectively. Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated from the following equations; SVR (mmHg min l^{-1}) = (mean AoP − mean RAP)/mean AoF; PVR (mmHg min l^{-1}) = (mean PAP − mean LAP)/mean AoF. Heart rate was counted continuously with a cardiotachometer (AT-600G, Nihon Kohden) triggered by the pressure pulse. The first derivative of LVP (LV$dP/dt$) was derived from differentiating the LVP signal with an electronic differentiator (ED-601G, Nihon Kohden).

A polyethylene tube filled with heparinized saline was inserted into the abdominal aorta via the right femoral artery for periodic sampling of arterial blood. Red blood cell (RBC) count, white blood cell (WBC) count, platelet (PLT) count and haematocrit (Hct) in the arterial blood sample were measured by an automatic blood cell counter (MEK-6158, Nihon Kohden).

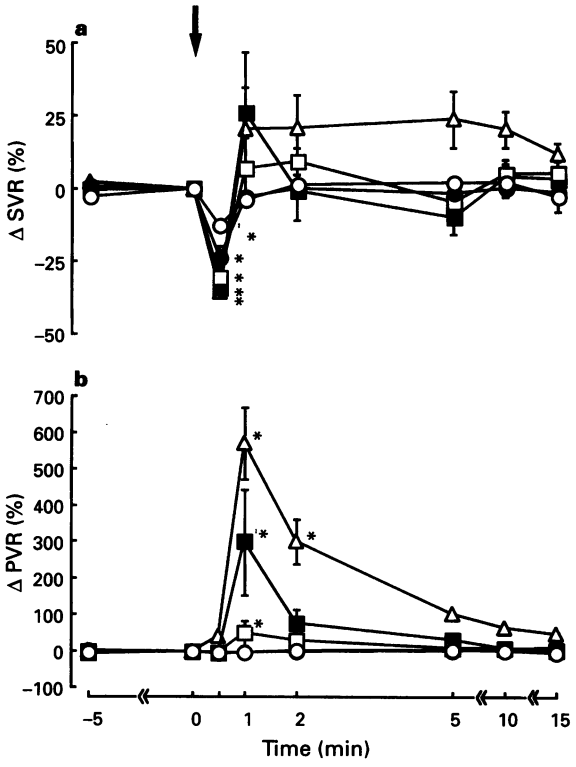

Figure 3 Effects of PAF on (a) systemic vascular resistance (SVR) and (b) pulmonary vascular resistance (PVR). Symbols are the same as those in Figure 2.

Experimental protocol

Experiments were started after a stabilization period of at least 30 min. Measurement of control haemodynamics and blood sampling were performed and then PAF (0.01, 0.02, 0.05, 0.1 or 0.25 μg kg^{-1}) was administered. Administration of vehicle of PAF (0.1 ml kg^{-1}) caused no change in any of the parameters measured. To examine the effect of pretreatment with various inhibitors or antagonists on PAF-induced cardiovascular and haematological effects, each of indomethacin (2 mg kg^{-1}, $n=6$), vapiprost (100 μg kg^{-1}, $n=7$), AA-861 (5 mg kg^{-1}, $n=7$), L-NAME (5 mg kg^{-1}, $n=6$) and TCV-309 (300 μg kg^{-1}, $n=5$) was respectively given 30, 5, 10, 35 and 5 min before an injection of PAF (0.25 μg kg^{-1}), and the re-

Table 1 Basal values for haemodynamic and haematological variables

	PAF (μg kg^{-1} i.v.)				
	0.01	*0.02*	*0.05*	*0.10*	*0.25*
Number of dogs	5	5	6	6	12
Variable					
mAoP (mmHg)	124±9	112±10	115±10	104±10	112±5
AoF (ml min^{-1})	1138±135	1032±82	1080±101	1277±155	1035±59
RAP (mmHg)	4.5±0.5	5.2±0.5	5.0±0.5	4.2±0.4	3.6±0.3
PAP (mmHg)	19.0±2.7	20.1±3.0	20.0±2.5	20.6±1.0	18.2±1.0
LAP (mmHg)	7.7±0.9	7.5±0.9	7.5±0.7	5.5±0.5	6.6±0.6
(+) LV$dP/dt$ (mmHg s^{-1})	2414±332	2360±329	2336±286	2268±161	2034±102
Heart rate (beats min^{-1})	160±18	164±19	157±18	160±9	146±8
WBC count (10^9 l^{-1})	10.2±2.6	12.9±2.9	12.6±2.5	8.5±2.3	10.2±2.5
Platelet count (10^9 l^{-1})	179±41	190±41	196±35	176±17	185±19
RBC count (10^{12} l^{-1})	7.0±0.3	6.8±0.5	6.9±0.4	6.4±0.3	6.3±0.3
Haematocrit (ratio)	0.43±0.03	0.42±0.03	0.42±0.03	0.40±0.02	0.40±0.02

mAoP = mean aortic pressure; AoF = aortic blood flow; RAP = right atrial pressure; PAP = pulmonary arterial pressure; LAP = left atrial pressure; (+) LV$dP/dt$ = first derivative of peak positive left ventricular pressure; WBC = white blood cell count; RBC = red blood cell count. Data represent means ± s.e.

sults were compared with those obtained from the dogs ($n=12$) receiving PAF (0.25 μg kg^{-1}) alone. The dose of AA-861 employed in this study was selected by considering the previous report demonstrating that AA-861 apparently inhibited the antigen-induced increases in plasma levels of leukotriene C_4 and D_4 in passively sensitized guinea-pigs (Sakuma *et al.*, 1991).

In 7 dogs, PAF at a dose of 0.05 μg kg^{-1} was repeatedly given at an interval of 60 min but saline (1 ml kg^{-1}) was administered 30 min before the second injection of PAF. In a further 6 dogs, PAF (0.05 μg kg^{-1}) was repeatedly given at an interval of 60 min, but L-NNA (20 mg kg^{-1}) instead of saline was administered 30 min before the second injection of PAF. Subsequently 30 min after the second injection of PAF, L-arginine (200 mg kg^{-1}) was given in a bolus followed by a continuous infusion of 10 mg kg^{-1} min^{-1} for 45 min, and the third injection of PAF (0.05 μg kg^{-1}) was performed 15 min after the L-arginine administration. The reason why a lower dose of PAF (0.05 μg kg^{-1}) was used in the experiments with repeated administration of PAF to the same animal was that development of tachyphylaxis in the response of PVR to the highest dose of PAF (0.25 μg kg^{-1}) was observed (unpublished data).

Drugs

PAF (1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine) was purchased from Bachem (Bubendorf, Switzerland). Lyso PAF (1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine), indomethacin (a cyclo-oxygenase inhibitor), N^G-nitro-L-arginine methyl ester (an NO synthase inhibitor; L-NAME) and N^G-nitro-L-arginine (an NO synthase inhibitor; L-NNA) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). AA-861 (2-(12-hydroxydodeca-5,10-dinyl)-3,5,6-trimethyl-1,4-benzoquinone) and L-arginine were obtained from Wako Pure Chemical Industries (Osaka, Japan). AA-861, a 5-lipoxygenase inhibitor (Yoshimoto *et al.*, 1982), was dissolved in polyethylene glycol 400/0.9% saline (2:1 by volume). Vapiprost ([1**R**-[1a(Z),2β,3β,5α]]-(+)-7-[5-[[1,1'-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)-cyclopentyl]-4-heptenoic acid hydrochloride), which is a specific TP-receptor antagonist (Lumley *et al.*, 1989), was kindly donated by Nippon Glaxo (Tokyo, Japan). The vehicle for vapiprost was made of β-cyclodextrin (0.166%), mannitol (5.0%), $NaH_2PO_4.2H_2O$ (0.093%), $NaHPO_4$ (0.011%) and distilled water. Intravenous administration of vapiprost (100 μg kg^{-1}) itself or the vehicle (0.2 ml kg^{-1}) produced virtually no haemodynamic or haematological effect. The dose of vapiprost employed in this study has previously been demonstrated to inhibit selectively and significantly vasoconstrictor responses to U46619 (0.5 μg kg^{-1}), a stable thromboxane A_2 analogue, in anaesthetized open-chest dogs (Noguchi *et al.*, 1992). TCV-309 (3-bromo-5-[*N*-phenyl-*N*-[2-[[2-(1,2,3,4-tetrahydro-2-isoquinolyl-carbonyloxy)ethyl]carbamoyl]ethyl]carbamoyl]-1-propylpyridinium nitrate), which is a selective PAF-receptor antagonist (Terashita *et al.*, 1992), was supplied from Takeda Chemical Industries (Osaka, Japan). PAF and lyso PAF were dried down from ethanol stock solution and resuspended in saline (0.9% NaCl) containing 2.5 mg ml^{-1} bovine serum albumin immediately before use. Indomethacin was dissolved in saline containing 0.2% sodium carbonate just before use. L-NAME, L-NNA, L-arginine and TCV-309 were dissolved and diluted with saline. All drugs used in this study were given intravenously in a bolus.

Data analysis

The time sequence data were analysed by use of two-way analysis of variance followed by Dunnett's test for multiple comparisons. The results of the treatment groups were analysed by one-way analysis of variance with Dunnett's test. All values are presented as means ± s.e. Statistical significance was set at $P<0.05$.

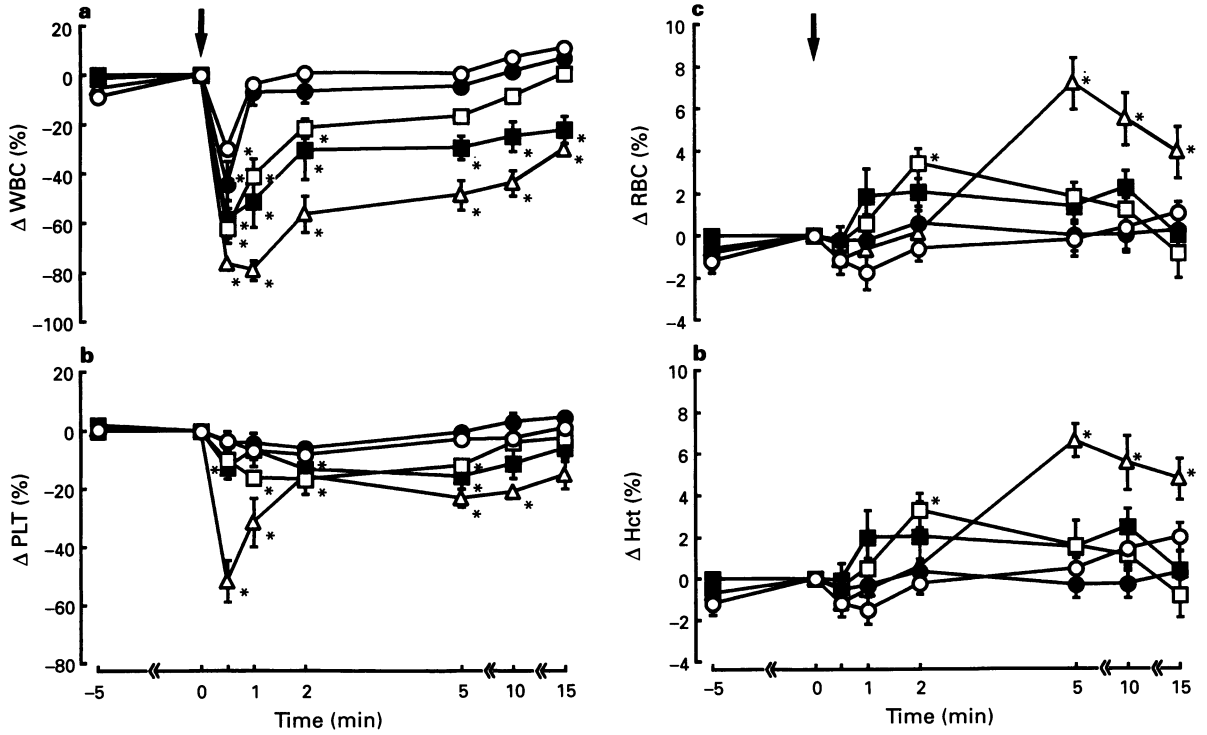

Figure 4 Effects of PAF on (a) white blood cell count (WBC), (b) platelet count (PLT), (c) red blood cell count (RBC) and (d) haematocrit (Hct) in arterial blood. Symbols are the same as those in Figure 2.

Results

Effects of PAF on systemic and pulmonary haemodynamic variables

A representative example of haemodynamic responses to PAF at a dose of 0.25 $\mu g\ kg^{-1}$ is presented in Figure 1. Administration of PAF produced an immediate and small fall in AoP around 30 s after the injection followed by a maximum fall at 1 min. The first phase of hypotension was accompanied by a small increase in AoF, while the second phase of hypotension was accompanied by marked reductions in AoF, LAP and LV*dP/dt*, and a prominent rise in PAP. RAP slightly decreased around 2 min after the injection. These changes occurred in a dose-related manner, as summarized in Figure 2, although the lower doses of PAF (0.01–0.05 $\mu g\ kg^{-1}$) failed to cause the second phase of hypotension as in the case of the higher doses (0.1 and 0.25 $\mu g\ kg^{-1}$). Significant and dose-dependent decreases in SVR were seen 30 s after administration of PAF at all doses, as shown in Figure 3. In contrast, PVR increased maximally 1 min after PAF injection especially at the higher doses. All of the significant changes mentioned above returned toward the baseline, presented in Table 1, 30 min after the injection.

Effects of PAF on haematological variables

Figure 4 shows the effects of PAF on arterial WBC, PLT and RBC counts, and Hct. WBC count rapidly decreased to a nadir within 1 min following PAF administration in a dose-related manner, and then recovered to the pre-administration value after 30 min. PLT count also markedly decreased as early as 30 s after the PAF injection, particularly at the highest dose. RBC count and Hct significantly increased from 5 to 15 min with a similar pattern when 0.25 $\mu g\ kg^{-1}$ PAF was given.

Effects of pretreatment with indomethacin, vapiprost, AA-861, L-NAME and TCV-309 on PAF-induced haemodynamic and haematological responses

Administration of indomethacin increased PVR (12.1±2.4 to 15.0±3.5 mmHg min l^{-1}, $P<0.05$), PAP (17.7±0.6 to 20.0±0.6 mmHg, $P<0.01$) and LAP (7.0±1.0 to 8.0±1.2 mmHg, $P<0.05$). Administration of AA-861 itself did not affect the baseline haemodynamic and haematological values except (+)LV*dP/dt* (2067±223 to 1842±188 mmHg s^{-1}, $P<0.01$). Significant changes seen 30 min after administration of L-NAME alone were increases in AoP (94±6 to 118±7 mmHg, $P<0.01$), PAP (17.9±1.8 to 21.3±3.0 mmHg, $P<0.05$), LAP (5.6±0.7 to 7.9±0.9 mmHg, $P<0.05$), RAP (4.8±0.3 to 5.6±0.4 mmHg, $P<0.05$), SVR (74±9 to 158±24 mmHg min l^{-1}, $P<0.01$) and PVR (10.1±1.4 to 17.3±3.4 mmHg min l^{-1}, $P<0.05$), and decreases in heart rate (136±11 to 125±14 beats min^{-1}, $P<0.05$), AoF(1268±153 to 821±152 ml min^{-1}, $P<0.01$) and (+)LV*dP/dt* (1963±170 to 1679±151 mmHg s^{-1}, $P<0.01$).

Effects of various inhibitors on the response of haemodynamic and haematological variables to an injection of PAF 0.25 $\mu g\ kg^{-1}$ i.v. are summarized in Figures 5 and 6, and Table 2. The first phase of systemic hypotension and the small increase in AoF 30 s after injection of PAF was not affected by any of the inhibitors except TCV-309 (Figure 5), while the following systemic hypotension, which appeared 1 min after injection, was significantly attenuated by indomethacin and AA-861 in addition to TCV-309 (Figure 6). Treatment with indomethacin, vapiprost, AA-861 and TCV-309 significantly modified the PAF-induced prominent reduction in AoF 1 min after injection (Figure 6). The decrease in SVR seen 30 s after the PAF injection was significantly inhibited in L-NAME-treated dogs (Figure 5). On the other hand, the increase in PVR induced by PAF was markedly inhibited with either indomethacin and vapiprost (Figure 6). Both indomethacin- and AA-861-treated dogs showed an attenuated PAF-induced reduction in (+)LV*dP/dt* as compared to that of control dogs (Figure 6) but there was no difference in the response of LAP between the treated and control groups. As summarized in Table 2, pretreatment with either indomethacin, vapiprost, AA-861 or L-NAME did not significantly influence the leukopenia, thrombocytopenia and increases in RBC count and Hct produced by PAF administration. Lyso PAF at the same dose as that of the parent phospholipid (0.25 $\mu g\ kg^{-1}$ i.v.) failed to cause any appreciable change in the measured parameters (data not shown), and the selective PAF-receptor antagonist TCV-309 almost completely abolished the haemodynamic and haematological responses to PAF (Figures

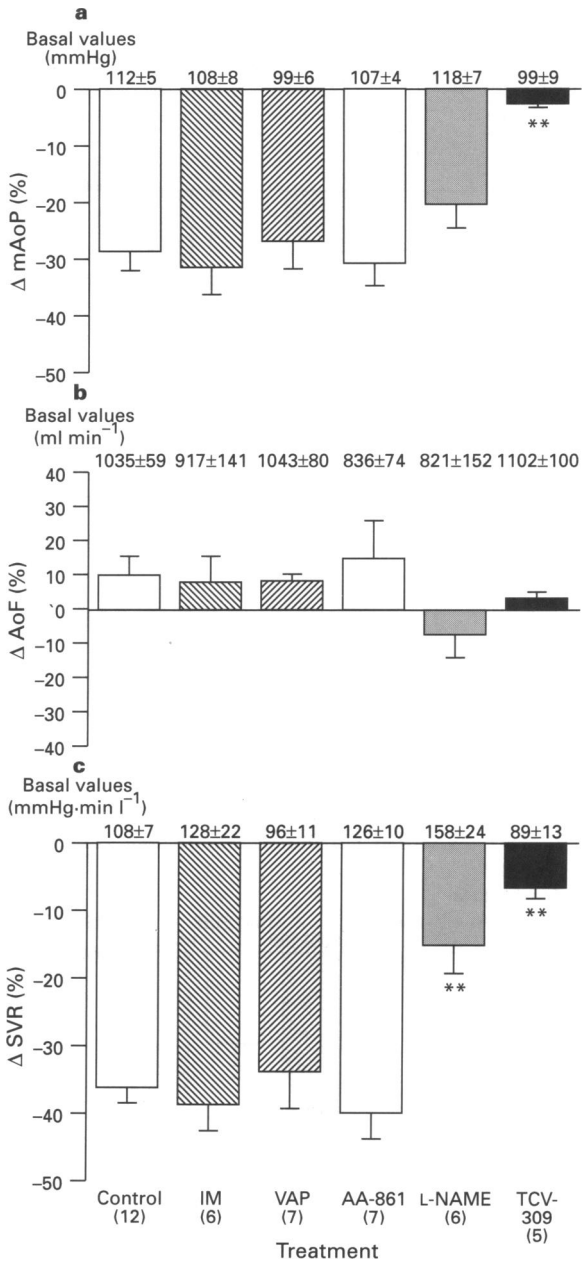

Figure 5 Effects of various inhibitors on the responses of (a) mean aortic pressure (mAoP), (b) aortic blood flow (AoF) and (c) systemic vascular resistance (SVR) to an injection of PAF 0.25 $\mu g\ kg^{-1}$ i.v. Data were obtained 30 s after administration of PAF. IM = indomethacin (2 $mg\ kg^{-1}$ i.v.), VAP = vapiprost (100 $\mu g\ kg^{-1}$ i.v.), AA-861 (5 $mg\ kg^{-1}$ i.v.), L-NAME = N^G-nitro-L-arginine methyl ester (5 $mg\ kg^{-1}$ i.v.) and TCV-309 (300 $\mu g\ kg^{-1}$ i.v.). Data represent means±s.e. $^{**}P<0.01$ vs control response.

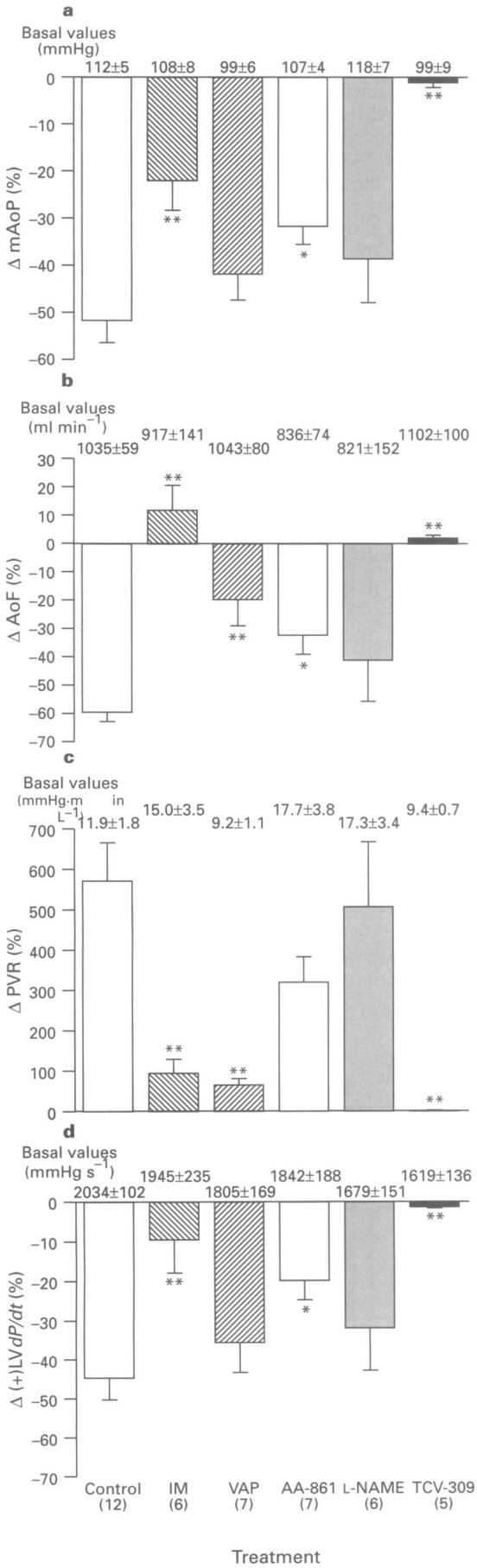

Figure 6 Effects of various inhibitors on the responses of (a) mean aortic pressure (mAoP), (b) aortic blood flow (AoF), (c) pulmonary vascular resistance (PVR) and (d) peak positive LV*dP*/*dt* ((+)LV*dP*/*dt*) to an injection of PAF 0.25 μg kg^{-1} i.v. Data were obtained 1 min after administration of PAF and represent means±s.e. *$P<0.05$, **$P<0.01$ vs control response.

5 and 6, and Table 2), indicating that all the changes observed following PAF injection probably occur through the PAF-receptor rather than a non-specific mechanism.

Effects of an NO synthase inhibitor and replenishment with L-arginine on the PAF responses

Effects of pretreatment with L-NNA, another NO synthase inhibitor, and subsequent treatment with L-arginine, a substrate for NO synthase, were examined further to clarify a role of NO in the PAF-induced haemodynamic and haematological effects. In the L-NNA-arginine both group, both AoP and SVR, but not WBC and PLT counts, significantly increased from the baseline values of 105±9 mmHg and 89±12 mmHg min l^{-1} respectively to 123±6 mmHg and 156±20 mmHg min l^{-1} 30 min after L-NNA administration (just before the second injection of PAF), and these variables recovered to 104±8 mmHg and 114±16 mmHg min l^{-1} during an infusion of L-arginine (just before the third injection of PAF). As shown in Figure 7a, repeated administration of this dose of PAF consistently produced changes in the measured variables including PVR. On the other hand, treatment with L-NNA significantly inhibited the immediate reductions in AoP and SVR seen 30 s following the injection of PAF, and significantly potentiated the thrombocytopenia (Figure 7b) when compared with the corresponding value with the first PAF injection. Subsequent administration of L-arginine restored the inhibited or enhanced responses to PAF produced by L-NNA treatment (Figure 7b).

Discussion

In the present study, we pharmacologically analysed the acute responses of the cardiovascular system to exogenous PAF in dogs, and demonstrated that mechanisms involved in the PAF-induced systemic hypotension may be different when a low dose (less than 0.05 μg kg^{-1}) rather than a higher dose (more than 0.1 μg kg^{-1}) of PAF is injected. The lower doses of PAF only caused a rapid but slight hypotension associated with a significant decrease in SVR, indicating that systemic vasodilatation occurred. Whereas the higher doses caused marked hypotension in addition to the preceding moderate hypotension, which was accompanied by a large decrease in AoF and a considerable increase in PVR. Indomethacin treatment potently attenuated both the large reductions in mAoP and AoF and the large increase in PVR produced by the highesr dose of PAF without affecting the first phase of changes in mAoP and AoF. These results indicate the involvement of cyclo-oxygenase metabolites in the second phase of hypotension as previously demonstrated (Yamanaka *et al.*, 1992). The TP-receptor antagonist vapiprost as well as indomethacin markedly inhibited PAF-induced increase in PVR and reduction in AoF, which is compatible with previous data in various species that consistently demonstrated that thromboxane A_2 (TXA_2) is responsible for pulmonary hypertension produced by PAF (Heffner *et al.*, 1983; Toyofuku *et al.*, 1988; Laurindo *et al.*, 1989; Yamanaka *et al.*, 1992; Argiolas *et al.*, 1995). These findings suggest that pulmonary vasoconstriction due to TXA_2 generation may explain, in part, the systemic hypotension through reduction of cardiac output. Moreover, simultaneous generation of vasodilator prostaglandins also conceivably contributes to the hypotension to some extent

Table 2 Effects of various inhibitors on the maximal responses of white blood cell count, platelet count, red blood cell count and haematocrit in arterial blood to an injection of PAF (0.25 $\mu g\ kg^{-1}$, i.v.)

	White blood cell count		*Platelet count*		*Red blood cell count*		*Haematocrit*	
Inhibitors	*Baseline* ($10^9\ l^{-1}$)	*PAF response* (*Δ*%)	*Baseline* ($10^9\ l^{-1}$)	*PAF response* (*Δ*%)	*Baseline* ($10^{12}\ l^{-1}$)	*PAF response* (*Δ*%)	*Baseline* (*Ratios*)	*PAF response* (*Δ*%)
Control ($n=12$)	10.2±2.5	−76.4±3.0	[a]185±19	−51.6±7.3	6.3±0.3	7.2±1.2	0.40±0.02	6.6±0.8
IM, ($n=6$) ($2\ mg\ kg^{-1}$)	12.6±1.8	−85.0±3.1	165±19	−72.4±6.7	6.1±0.5	5.6±1.0	0.39±0.03	5.9±1.0
VAP, ($n=7$) ($100\ \mu g\ kg^{-1}$)	15.2±3.1	−85.5±2.1	188±37	−45.5±6.4	6.7±0.5	8.9±2.2	0.40±0.02	8.4±2.0
AA-861, ($n=7$) ($5\ mg\ kg^{-1}$)	12.7±2.5	−83.3±3.0	169±33	−44.3±8.5	7.0±0.4	6.0±1.2	0.43±0.02	6.1±1.2
L-NAME, ($n=6$) ($5\ mg\ kg^{-1}$)	14.3±2.2	−69.0±5.3	133±14	−59.7±6.7	5.8±0.7	5.5±2.2	0.36±0.04	5.0±2.2
TCV-309, ($n=5$) ($300\ \mu g\ kg^{-1}$)	16.9±2.4	−7.2±3.6**	188±31	−0.2±0.6**	6.7±0.4	0.4±0.8*	0.42±0.02	0.3±0.9*

IM = indomethacin, VAP = vapiprost, L-NAME = N^G-nitro-L-arginine methyl ester. Maximal reductions in WBC count and platelet count were seen 30 s after the PAF injection, and maximal increases in RBC count and haematocrit were seen at 5 min. Data represent means±s.e. *$P<0.05$, **$P<0.01$ vs control response. [a]Number of dogs for platelet count in control group is 10.

because selective blockade of the TP-receptor by vapipost failed to attenuate PAF-induced decrease in AoP, although sympathetic vasoconstriction due to baroreflex would oppose the vasodilator effects. In addition, a part of the decrease in AoF induced by PAF may be attributable to a decrease in cardiac contractility mediated by leukotrienes since the depressant response of (+)LV*dPdt* to PAF was significantly inhibited by AA-861, which reduces the generation of 5-lipoxygenase metabolites, such as leukotriene C_4 (LTC_4) and LTD_4, that have been shown to exert a negative inotropic action in dogs (Fiedler *et al.*, 1987). Also, PAF itself had direct negative inotropic effects (Tamargo *et al.*, 1985; Robertson *et al.*, 1988). Thus, it can be postulated that the hypotensive response to higher doses of PAF was caused mainly by a reduction in cardiac output resulting from profound TXA_2-mediated pulmonary vasoconstriction and at least partly from a cardiodepressive action presumably due to leukotrienes and PAF, and additionally by systemic vasodilatation via the formation of vasodilator prostaglandins, other mediators and/or PAF itself. In support of this hypothesis, increased plasma levels of TXB_2 and 6-keto $PGF_{1\alpha}$ (Toyofuku *et al.*, 1988; Yamanaka *et al.*, 1992), and leukotrienes (Piper & Stewart, 1986) in response to exogenous PAF have been observed.

Several *in vitro* experiments (Kamitani *et al.*, 1984; Kamata *et al.*, 1989; Chiba *et al.*, 1990; Moritoki *et al.*, 1992; Juncos *et al.*, 1993) have suggested the involvement of EDRF or NO in the vasodilator effects of PAF. In this study, we observed that, firstly, decreases in SVR seen immediately following the injection of PAF 0.25 $\mu g\ kg^{-1}$ were significantly inhibited by pretreatment with the NO synthase inhibitor L-NAME and also with the PAF antagonist TCV-309; secondly, decreases in mAoP and SVR induced by the lower dose of PAF (0.05 $\mu g\ kg^{-1}$) were significantly attenuated by another NO synthase inhibitor L-NNA; thirdly, these reduced effects of PAF produced by L-NNA were restored with administration of L-arginine, a substrate for NO synthase. Therefore, the immediate hypotensive response to i.v. administration of PAF, particularly seen at lower doses, can be considered to be mediated, at least partly, through NO generation via stimulation of PAF-receptors in dogs. On the other hand, Filep & Földes-Filep (1993) have mentioned that the hypotensive action of PAF (0.1 and 1.0 $\mu g\ kg^{-1}$, i.v.) seems to be independent of NO formation in the conscious rat. However, this discrepant view may possibly be explained by the higher doses of PAF used in their study compared to the present study (0.05 $\mu g\ kg^{-1}$) and/or species differences in the actions of PAF (Namm *et al.*, 1982). Also the effects of NO are only transient and small.

In contrast to the cardiohaemodynamic effects, dose-dependent and reversible haematological effects of PAF including thrombocytopenia, leukopenia and haemoconcentration were not significantly affected by indomethacin, vapiprost, AA-861 or L-NAME, although all of these effects were almost abolished by TCV-309. This indicates that the PAF-induced haematological changes are independent of cyclo-oxygenase, 5-lipoxygenase and NO synthase products. Toyofuku *et al.* (1988) have demonstrated that an infusion of PAF results in transient and severe decreases in circulating leukocytes and platelets, both of which were unchanged by pretreatment with a thromboxane synthetase inhibitor, and therefore they have suggested that the synthesis of TXA_2 is unrelated to leukopenia and thrombocytopenia. This is well in accordance with the present study in which a selective TP-receptor antagonist was used. Hence these haematological actions of PAF have been presumed to be due to direct effects on platelets, leukocytes and vascular endothelial cells (Braquet *et al.*, 1987). However, it is likely that NO, a potent platelet adhesion/aggregation inhibitor (Moncada *et al.*, 1991), may participate in moderating the thrombocytopenia induced by PAF particularly at lower doses, since pretreatment with the NO synthase inhibitor L-NNA significantly enhanced the PAF-induced decrease in circulating platelet count, an effect that was reversed by supplementation with L-arginine, a substrate for NO synthase. A similar tendency to potentiate the platelet response to 0.25 $\mu g\ kg^{-1}$ PAF was seen in the L-NAME-treated dogs, although the changes were not statistically significant when compared with the control response. Thus, these results imply that NO might play a role in lessening the effects of PAF on platelets.

Constitutive NO synthase, unlike the inducible form of the enzyme, is activated with rapid onset and short duration through an increase in $[Ca^{2+}]_i$ by various activators such as acetylcholine (ACh) and PAF, and is widely distributed in endothelial cells, neutrophils, platelets and etc. (Nathan, 1992). According to the *in vitro* experiments of James-Kracke *et al.* (1994), a rapidly increased $[Ca^{2+}]_i$ produced by PAF at 10^{-12} M without any activation of protein kinase C or phospholipase C probably occurs normally when low concentrations of PAF cause platelets to undergo reversible shape change, whereas phospholipase C or other signal transduction systems seems to be activated additionally when PAF 10^{-9} M or more is applied. If this is the case *in vivo*, a low concentration of PAF should initially produce Ca^{2+} mobilization of the target cells leading to the appearance of transient and modest actions of PAF, for instance NO-mediated hypotension, which may precede activation of other pathways.

In conclusion, the data suggest that generation of NO significantly contributes to both the systemic vasodilatation and

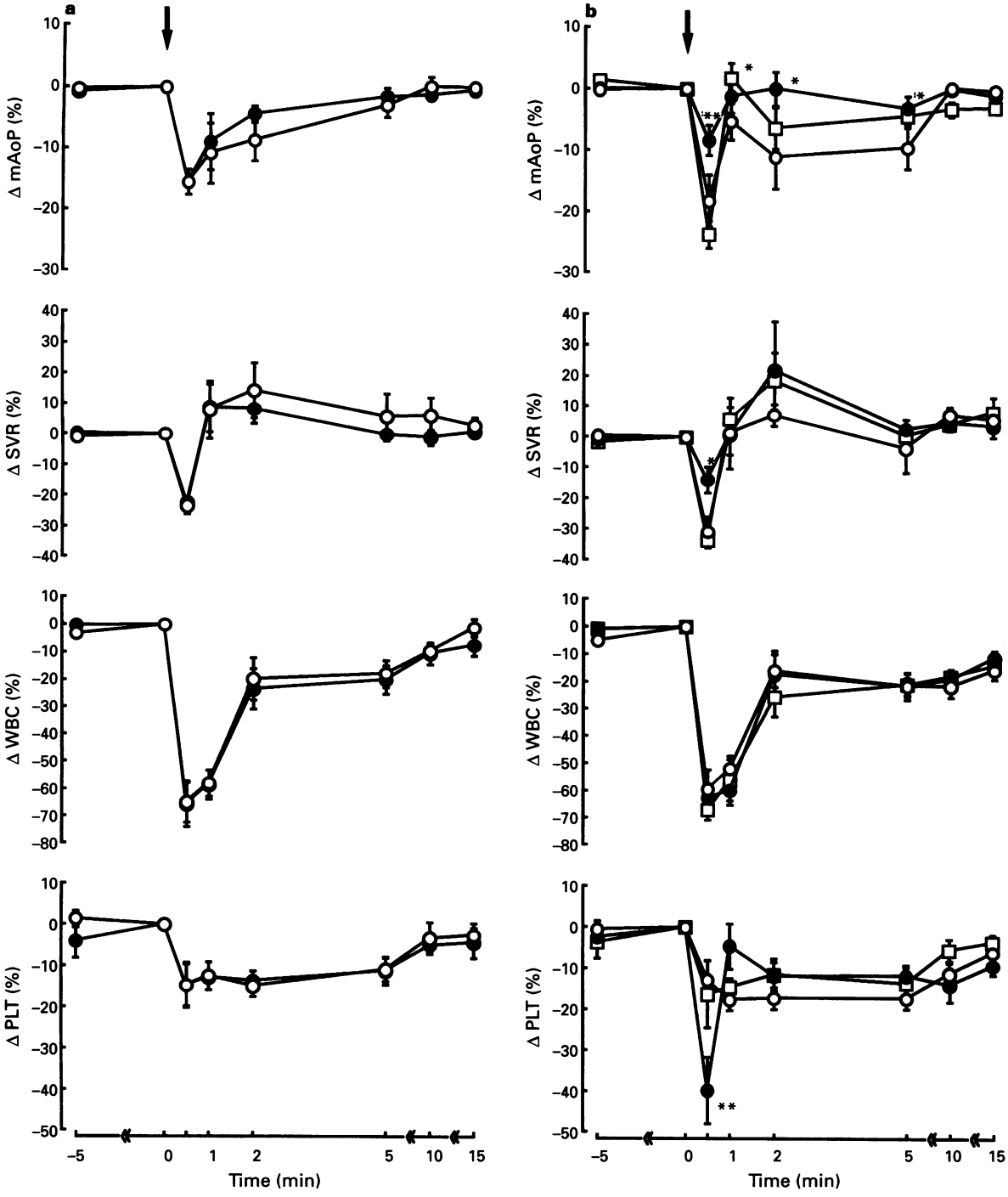

Figure 7 (a) Effects of repeated administration of PAF (0.05 $\mu g\,kg^{-1}$ i.v., $n=7$) on mean aortic pressure (mAoP), systemic vascular resistance (SVR), white blood cell count (WBC) and platelet count (PLT). PAF was repeatedly given at an interval of 60 min. Saline was administered 30 min before the second injection of PAF. Baseline values for mAoP, SVR, WBC and PLT were 102±9 mmHg, 99±18 mmHg $min\,l^{-1}$, 10.7±2.4 $10^9\,l^{-1}$ and 151±13 $10^9\,l^{-1}$, respectively. (○) and (●) Represent % changes from the pre-administration value following the first and second injections of PAF, respectively. (b) Effects of N^G-nitro-L-arginine (20 $mg\,kg^{-1}$ i.v.) and L-arginine (200 $mg\,kg^{-1}$ + 10 $mg\,kg^{-1}\,min^{-1}$ i.v.) on PAF (0.05 $\mu g\,kg^{-1}$ i.v.)-induced responses of mAoP, SVR, WBC and PLT in 6 dogs. Baseline values for mAoP, SVR, WBC and PLT were 105±9 mmHg, 89±12 mmHg $min\,l^{-1}$, 9.7±1.6 $10^9\,l^{-1}$ and 213±49 $10^9\,l^{-1}$, respectively. (○) Represent control responses to PAF. (●) Represent responses to PAF 30 min after treatment with N^G-nitro-L-arginine. (□) Represent responses to PAF during the L-arginine infusion that was given after the second injection of PAF subsequent to N^G-nitro-L-arginine treatment. Data represent means±s.e. $^*P<0.05$; $^{**}P<0.01$ vs PAF alone.

moderating thrombocytopenia seen immediately (within 30 s) after i.v. injection of PAF at lower doses. Thus, NO, in addition to eicosanoids, seems to be an important mediator for the PAF-induced haemodynamic and haematological effects in the whole animal preparation.

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