PAF-induced bronchial hyperresponsiveness in the rabbit: contribution of platelets and airway smooth muscle

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1 Aerosol administration of platelet activating factor (PAF) to normal rabbits induced an enhanced airway responsiveness to inhaled histamine, 6 and 24 h after exposure. Following exposure to bovine serum albumin (BSA) as the carrier molecule for PAF, there was an increase in airway responsiveness to histamine 6 h after challenge, although by 24 h this was not significantly different from the responsiveness of airways to histamine before BSA.

2 PAF-induced bronchial hyperresponsiveness at 24 h was associated with a substantial increase in the number of neutrophils and mononuclear cells and a small, but significant increase in the number of eosinophils in the lungs as assessed by bronchoalveolar lavage. BSA exposure failed to alter the total number of cells in the lungs, although there was a significant increase in the number of neutrophils in the bronchoalveolar lavage fluid.

3 Selective platelet depletion with a guinea-pig anti-rabbit platelet serum inhibited PAF-induced bronchial hyperresponsiveness. In addition, there was an attenuation of PAF-induced airway inflammation in animals rendered thrombocytopenic.

4 The contractile potency to histamine, methacholine and carbachol was similar in intrapulmonary bronchi taken from rabbits exposed to an aerosol of BSA or PAF. Furthermore, the relaxant potency to the non-selective β -adrenoceptor agonist isoprenaline, was unaltered in PAF-treated rabbits. In contrast, there was a 2.58 fold reduction in the relaxant potency to theophylline in rabbits exposed to PAF compared with rabbits exposed to BSA.

5 These results suggest that in the rabbit, PAF-induced bronchial hyperresponsiveness at 24 h is associated with airways inflammation and is dependent upon platelet activation, but is not related to changes in airway smooth muscle function.

Introduction

Platelet activating factor (PAF) has been implicated in the pathogenesis of bronchial asthma as PAF has a number of biological properties of relevance to this disease (Page, 1988). PAF is of particular interest as it is able to induce a long-lasting and non-specific bronchial hyperresponsiveness both in experimental animals (Mazzoni *et al.*, 1985; Chung *et al.*, 1986; Barnes *et al.*, 1987; Robertson *et al.*, 1988) and man (Cuss *et al.*, 1986). However, the precise mechanisms underlying this heightened airway responsiveness are unknown. It has been suggested that bronchial hyperresponsiveness is secondary to airway inflammation as a number of stimuli capable of inducing bronchial hyperresponsiveness also induce an infiltration of inflammatory cells, principally neutrophils and eosinophils into the airways (Chung *et al.*, 1985; Murphy *et al.*, 1986). Moreover, depletion of circulating leucocytes has been demonstrated to inhibit the development of bronchial hyperresponsiveness in experimental animals (O'Byrne *et al.*, 1984; Marsh *et al.*, 1985), but this has not been observed by other investigators (Murlas & Roum, 1985; Thompson *et al.*, 1986; Cibulas *et al.*, 1988). Other investigators have also suggested that alterations in airway smooth muscle function (Antonissen *et al.*, 1979), bronchial wall oedema (Persson, 1986) or geometric factors (James *et al.*, 1989) may contribute to bronchial hyperresponsiveness.

It is now recognized that some of the biological activities of PAF are secondary to platelet activation (Vargaftig *et al.*, 1980; Mazzoni *et al.*, 1985; Lellouch-Tubiana *et al.*, 1988) and that the platelet may act as an inflammatory cell in its own right (Page, 1989).

The present study has therefore attempted to investigate whether PAF-induced bronchial hyperresponsiveness is secondary to changes in airway smooth muscle function or platelet activation.

Methods

In vivo experiments

New Zealand White rabbits (3–3.5 kg) were anaesthetized with diazepam (5 mg ml⁻¹, 5 mg kg⁻¹ i.p.) and fentanyl citrate (0.315 mg ml⁻¹, 0.4 ml kg⁻¹ i.m.) and placed in a supine position. Rabbits were intubated with a 3.0 mm endotracheal tube which was connected to a pneumotachograph (Bore 00) and the flow determined by a Validyne pressure transducer. Pleural pressure was estimated by placing an oesophageal balloon in the lower third of the oesophagus to obtain the maximum expiratory pressure. Thoracic pressure was measured by a sidehole catheter on the distal end of the tracheal tube. Transpulmonary pressure, the difference between thoracic and pleural pressure was measured with a differential pressure transducer (Validyne). The flow was integrated to obtain a continuous recording of tidal volume. Measurements of total lung resistance (R_L) and dynamic compliance (C_{dyn}) were calculated by a pulmonary mechanical analyser (Buxco, Model 6, Sharon, CT, U.S.A.) as described previously (Giles *et al.*, 1974). Anaesthesia was maintained throughout the course of the experiment by administration of 0.2–0.3 ml fentanyl citrate intramuscularly every 30–40 min, according to the protocol described by Flecknell (1987).

Assessment of airway responsiveness to histamine

Airway responsiveness to histamine was determined by exposing rabbits to increasing concentrations of histamine (1.25–80 mg ml⁻¹) administered to the lungs directly via an endotracheal tube. Aerosols were generated by a Devilbiss ultrasonic nebuliser which has previously been demonstrated to generate particles of which 80% are less than 0.5 μ m in diameter.

After measurement of baseline lung function, animals were exposed to an aerosol of sterile saline for 2 min immediately

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followed by lung function measurements. Increasing concentrations of histamine were administered, with each dose being given for 2 min, until there was at least a doubling in R_L or a 30% fall in C_{dyn} . The provocation dose (PD) of histamine which produced a 100% increase in R_L (PD_{100}) and 30% fall in C_{dyn} (PD_{30}) was determined for each animal and used as an index of airway responsiveness.

On day 2 animals were re-anaesthetized and a PAF challenge performed. After exposure to an aerosol of 0.25% bovine serum albumin (BSA) for 2 min, rabbits were exposed to PAF ($80 \mu\text{g ml}^{-1}$) administered over a 1 h period. Lung function was then monitored for a 1 h period after exposure to PAF. Control animals received an aerosol of BSA followed by exposure to an aerosol of BSA containing the same concentration of ethanol as that received by the PAF-treated animals (approx. 1%).

On day 3, increasing concentrations of histamine were administered to the anaesthetized rabbits as on day 1 and the PD_{100} for histamine determined.

Bronchoalveolar lavage

On completion of the concentration-effect curve to histamine on day 1 and day 3, a bronchoalveolar lavage (BAL) was performed. Rabbits were lavaged by passing a cannula into the airways via the endotracheal tube until it was wedged. Three millilitres of 10% normal rabbit serum in 0.9% sterile saline was injected and immediately aspirated from the lungs. Total cell counts were determined under light microscopy by an improved Neubauer haemocytometer. Cytospins were prepared, fixed and stained with Lendrum's stain. A total of 200 cells were counted differentially and classified as neutrophils, eosinophils or mononuclear cells based on standard morphological criteria. The return volume of BAL was $1.97 \pm 0.13 \text{ ml}$ ($n = 15$) and had a coefficient of variation of 26%.

Preparation of anti-platelet serum

Guinea-pig anti-rabbit platelet serum (APS) was a kind gift of Dr K.D. Butler (Ciba-Geigy Pharmaceuticals, Horsham, U.K.), prepared according to the protocol described by Butler & Smith (1982).

Administration of anti-serum

Animals were treated with 0.5 ml kg^{-1} of APS approximately 16 h before PAF or BSA challenge. Normal guinea-pig serum (NGPS) was administered in an identical manner to serve as an appropriate control. In 2 rabbits, administration of APS induced only a 70% depletion of platelets. In such circumstances, 0.2–0.4 ml of APS was administered slowly over a 1 h period via the marginal ear vein until the platelet count was reduced by at least 90%. In 5 rabbits, APS was administered 16 h before a second histamine dose-response curve to determine if thrombocytopenia *per se* modified airway responsiveness to histamine.

Enumeration of platelet and white blood cell counts

Before and 15 min after exposure to PAF, 3 ml of blood was taken from the central ear artery into 0.3 ml 3.8% sodium tricitrate. Total white blood cell counts were obtained under light microscopy following dilution with Turks reagent. Two millilitres of blood was then spun at 200 *g* for 3 min and the platelet-rich plasma removed. The blood platelet count was enumerated under phase contrast light microscopy following dilution with brilliant cresyl blue and 3.8% sodium citrate. In addition, blood samples were taken from the ear vein before and 16 h after administration of either APS or NGPS and total blood counts performed as described above. Blood smears were also obtained, stained with Lendrum's stain and a total of 200 cells counted differentially.

In vitro experiments

New Zealand White rabbits (3–3.5 kg) treated with NGPS and exposed to an aerosol of either 0.25% BSA or PAF ($80 \mu\text{g ml}^{-1}$) for 1 h and then challenged 24 h later with histamine, followed by BAL, were killed by exsanguination whilst under anaesthesia. The lungs were quickly removed and placed in cold, oxygenated Krebs-Henseleit solution. Rabbit intrapulmonary bronchi (1–3 mm i.d.) were dissected free of parenchymal tissue and visible blood vessels. Bronchial rings were suspended under 1 g tension in organ baths containing Krebs-Henseleit solution aerated with 95% O_2 , 5% CO_2 and maintained at 37°C. Changes in isometric tension were measured with a Grass force-displacement transducer (FTO3C) and recorded on a 6 channel ectromed. All bronchial preparations were allowed to equilibrate for 45 min and decreases in resting tension which may have occurred during this period were compensated for by readjustment of tension to 1 g. The bathing solution was changed every 15 min before the addition of pharmacological agonists.

Following equilibration in Krebs-Henseleit solution, each bronchial preparation was exposed to a single concentration of carbachol ($1 \mu\text{M}$). When the contractile response had plateaued the preparations were once again washed and allowed to equilibrate for a further 45 min. Cumulative concentrations of either carbachol (0.1–100 μM), histamine (1–100 μM), methacholine (0.1–100 μM), isoprenaline (0.01–10 μM) or theophylline (0.01–5 mM) were added to bronchial preparations chosen randomly. In the case of isoprenaline and theophylline, bronchial preparations were contracted with a concentration of carbachol which produced 25% of the maximal response (EC_{25}) and cumulative concentrations of isoprenaline or theophylline superimposed on this contractile response. The pharmacological testing of all agonists in bronchi from 0.25% BSA and PAF-treated animals was performed blind. In a separate experiment, PAF (0.001–1 μM ; dissolved in Krebs containing 0.25% BSA) was added to epithelium-intact bronchi from naïve rabbits, suspended in silicone-treated organ baths. Only one concentration of PAF was tested in each preparation to avoid the effects of tachyphylaxis.

Analysis of results

Results from *in vivo* studies are expressed as mean $\pm$ s.e.mean. *In vivo* histamine potency derived from measurements of resistance (PD_{100}) and dynamic compliance (PD_{30}) are expressed as geometric mean $\pm$ s.e.mean. Some of the data are expressed as the derived PD_{100} (antilog PD_{100}) together with upper and lower values for s.e.mean.

For the *in vitro* data, agonist potency ($pD_2 = \log_{10} EC_{50}$) and the maximal tension generated (E_{max}) were obtained. On each experimental day a mean pD_2 and E_{max} value respectively were obtained for each agonist. On completion of the study, the mean values were grouped according to the treatment and a grand mean $\pm$ s.e.mean was obtained for both pD_2 and E_{max} .

The probability (*P*) of differences between mean values was determined by Student's two tailed, paired or non-paired *t* test and was considered significant if $P < 0.05$. Analysis of variance (ANOVA) was used to analyse baseline R_L and C_{dyn} data. Analysis of covariance (ANCOVA) was used to analyse the BAL data and Kruskal-Wallis one-way analysis of variance was used to analyse the eosinophil data.

Drugs

Carbamylcholine hydrochloride, methacholine hydrochloride, histamine dihydrochloride, histamine diphosphate, ($\pm$)-isoprenaline hydrochloride, theophylline, bovine serum albumin Grade V (endotoxin free) and normal rabbit serum were all obtained from Sigma (Poole, Dorset); PAF was purchased from Novabiochem (Nottingham, U.K.); diazepam (Roche, U.K.); fentanyl citrate (Janssen Pharmaceutical Ltd, U.K.).

Anti-platelet serum was a kind gift of Dr K.D. Butler, Ciba-Geigy Pharmaceuticals (Horsham, U.K.). Composition of the Krebs-Henseleit solution (mm): NaCl 117.6, NaHCO_3 25, D-glucose 11.1, KCl 5.4, MgSO_4 0.57, KH_2PO_4 1.03 and CaCl_2 2.5. Isoprenaline was dissolved in 0.9% NaCl solution containing $20 \mu\text{g ml}^{-1}$ ascorbic acid.

Results

In vivo studies

PAF ($80 \mu\text{g ml}^{-1}$) induced acute bronchoconstriction in control animals, R_L increased by $146.2 \pm 40.7\%$ ($n = 6$) and C_{dyn} decreased by $30.6 \pm 4.7\%$ ($n = 6$) (Figure 1). PAF-induced changes in pulmonary function diminished over the next hour, although the values for R_L and C_{dyn} did not return completely to baseline (Figure 1). Exposure to 0.25% BSA induced a small but significant bronchoconstriction ($R_L +10.7 \pm 2.3\%$, $C_{\text{dyn}} -7.2 \pm 3.2\%$, $P < 0.05$). Platelet depletion reduced the magnitude of PAF-induced bronchoconstriction compared with rabbits treated with NGPS ($R_L +29.3 \pm 5.5\%$, $C_{\text{dyn}} -11.8 \pm 6.6\%$, $n = 5$, $P < 0.05$, Figure 2).

Effect of PAF on airway responsiveness to histamine

Resistance The concentration of histamine required to increase R_L by 100% (PD_{100}) was 47.1 mg ml^{-1} (42.0–52.8; $n = 10$). A significant increase in airway responsiveness to histamine was observed 6 h (12.0 mg ml^{-1} (9.5–15.1), $P < 0.05$; $n = 4$) and 24 h (19.0 mg ml^{-1} (14.4–25.1), $P < 0.05$; $n = 5$) but not 72 h (52.5 mg ml^{-1} (44.2–62.4), $P > 0.05$; $n = 4$) following exposure to an aerosol of PAF.

Airway responsiveness to histamine was not significantly different 6 h (33.1 mg ml^{-1} (25.9–42.4), $P > 0.05$; $n = 2$), 24 h (44.7 mg ml^{-1} (38.5–51.8), $P > 0.05$; $n = 5$) and 72 h (61.4 mg ml^{-1} (45.5–82.8), $P > 0.05$; $n = 4$) following exposure to an aerosol of BSA. The mean results shown in Figure 3a were obtained after logarithmic transformation of the data.

Compliance The concentration of histamine required to decrease C_{dyn} by 30% (PD_{30}) was 60.7 mg ml^{-1} (55.3–66.0; $n = 10$). A significant increase in airway responsiveness to histamine was observed 6 h (20.4 mg ml^{-1} (12.0–34.7), $P < 0.05$; $n = 5$) and 24 h (23.4 mg ml^{-1} (18.2–30.2), $P < 0.05$; $n = 5$) but not 72 h (74.1 mg ml^{-1} (61.6–100.0), $P > 0.05$; $n = 4$) following exposure to an aerosol of PAF.

Figure 1 Changes in total lung resistance (R_L) and dynamic compliance (C_{dyn}) recorded over a 1 h period following a 1 h aerosol exposure to 0.25% bovine serum albumin (○) or PAF $80 \mu\text{g ml}^{-1}$ (●) in rabbits. Each point is the mean from 6 animals and vertical lines show s.e.mean.

Figure 2 Changes in total lung resistance (R_L) and dynamic compliance (C_{dyn}) in rabbits treated with normal guinea-pig serum (NGPS) (columns a and b, $n = 5$) and in rabbits rendered thrombocytopenic (cross-hatched columns c, $n = 5$) following a 1 h aerosol exposure to 0.25% bovine serum albumin (a, open columns) or $80 \mu\text{g ml}^{-1}$ PAF (columns b and c). Results expressed as mean and bars show s.e.mean. * PAF-induced bronchoconstriction significantly reduced in platelet-depleted rabbits compared with NGPS-treated rabbits ($P < 0.05$, non-paired t test).

Airway responsiveness to histamine was significantly increased 6 h after exposure to BSA (30.9 mg ml^{-1} (25.6–37.2), $P < 0.05$; $n = 4$), but to a lesser extent than that observed to PAF. Airway responsiveness to histamine was not significantly different 24 h (60.2 mg ml^{-1} (54.0–67.3), $P > 0.05$;

Figure 3 Airway responsiveness to histamine (a) log PD_{100} resistance and (b) log PD_{30} compliance, 6, 24 and 72 h after aerosol exposure to 0.25% bovine serum albumin (BSA) (open columns) or $80 \mu\text{g ml}^{-1}$ PAF (shaded columns). Results are expressed as geometric mean from 4–5 observations and bars show s.e.mean. * $P < 0.05$ (paired t test) compared with airways responsiveness to histamine before exposure to BSA or PAF (pretreatment (Pre) value).

$n = 5$) and 72 h (61.6 mg ml^{-1} (57.8–65.8), $P > 0.05$; $n = 4$) following exposure to an aerosol of BSA. The mean results shown in Figure 3b were obtained after logarithmic transformation of the data.

Mean pretreatment baseline R_L and C_{dyn} values were $15.8 \pm 1.5 \text{ cmH}_2\text{O l}^{-1} \text{ s}^{-1}$ ($n = 10$) and $3.2 \pm 0.4 \text{ ml cmH}_2\text{O}^{-1}$ ($n = 10$), respectively. Furthermore, there was no significant difference in baseline R_L and C_{dyn} at 6, 24 and 72 h ($P > 0.05$, ANOVA).

Effect of PAF on circulating platelets and leucocytes

Aerosol administration of PAF failed to alter the number of circulating platelets (pretreatment: $3.39 \pm 0.35 \times 10^5 \text{ cells } \mu\text{l}^{-1}$; post-treatment: $3.50 \pm 0.53 \times 10^5 \text{ cells } \mu\text{l}^{-1}$; $n = 3$) or leucocytes (pretreatment: $1.28 \pm 0.25 \times 10^4 \text{ cells ml}^{-1}$; post-treatment: $1.08 \pm 0.50 \times 10^4 \text{ cells ml}^{-1}$; $n = 3$). No differential cell count was performed on the latter cell type.

Effect of administration of anti-platelet or normal serum on circulating platelet and white blood cell count

Administration of either anti-platelet serum (APS) or normal guinea-pig serum (NGPS) failed to alter the total number of circulating leucocytes compared with untreated animals (Table 1). A significant increase in the number of neutrophils and a decrease in the number of mononuclear cells following administration of APS compared with control animals was observed. However, administration of the NGPS induced a similar neutrophilia and reduction in the number of mononuclear cells which was not significantly different from that induced by APS (Table 1).

Effect of platelet depletion on histamine-induced bronchoconstriction

The R_L PD_{100} following histamine administration was 50.1 mg ml^{-1} (42.7–58.9; $n = 5$). Administration of APS failed to modify histamine-induced bronchoconstriction (PD_{100} 44.7 mg ml^{-1} (33.9–58.9), $n = 5$; $P > 0.05$).

Effect of platelet depletion on PAF-induced bronchial hyperresponsiveness

In another series of experiments, an increase in airways responsiveness to histamine was observed 24 h following exposure to PAF in naïve (pretreatment PD_{100} : 51.3 mg ml^{-1} (43.2–60.8); 24 h post-treatment: PD_{100} 30.2 mg ml^{-1} (24.0–38.0), $n = 9$, $P < 0.05$, Figure 4a) and NGPS-treated rabbits (pretreatment: 58.9 mg ml^{-1} (47.0–73.8); (post-treatment: 28.8 mg ml^{-1} (21.9–38.0), $n = 6$, $P < 0.05$, Figure 4a). NGPS treatment alone failed to alter the PAF-induced increase in airway responsiveness to histamine ($P < 0.05$). PAF increased airway responsiveness to histamine by 2 fold or greater in 5

Figure 4 Individual histamine PD_{100} values before and 24 h after aerosol exposure to (a) PAF $80 \mu\text{g ml}^{-1}$ in naïve rabbits ($\circ$) or rabbits treated with normal guinea-pig serum ($\bullet$), (b) 0.25% bovine serum albumin or (c) PAF $80 \mu\text{g ml}^{-1}$ in rabbits treated with guinea-pig anti-rabbit platelet serum.

rabbits, by 1.4 fold in 7 rabbits and minimally in 3 rabbits (Figure 4a). In contrast, airway responsiveness to histamine was not increased 24 h following exposure to BSA in NGPS-treated rabbits (pretreatment: 45.3 mg ml^{-1} (32.2–63.7); post-treatment: 43.2 mg ml^{-1} (32.1–58.2), $n = 5$, $P > 0.05$, Figure 4b). In animals rendered thrombocytopenic, PAF failed to increase airway responsiveness to histamine (pretreatment: 56.0 mg ml^{-1} (43.6–72.4); post-treatment: 54.2 mg ml^{-1} (38.4–76.6), $n = 5$, $P > 0.05$, Figure 4c). The individual data for the 3 groups of rabbits are shown in Figure 4.

Bronchoalveolar lavage

Before PAF exposure, mononuclear cells comprised greater than 95% of the resident cell population with a small (generally less than 5%) number of neutrophils. The mean pretreatment cell counts from the 3 groups were not significantly different ($P > 0.05$, ANOVA) and consequently the pretreatment cell counts from each group were pooled, as presented in Table 2. PAF induced a significant increase in the total number of leucocytes in the airways as assessed by BAL, 24 h after PAF exposure in animals treated with NGPS (ANCOVA $F(2,11) = 6.2$, $P < 0.01$; Table 2). Although there was an increase in the numbers of neutrophils and monocytes in BAL following PAF this was not significant ($P > 0.05$, ANCOVA). In contrast, a small but significant increase in the numbers of BAL eosinophils following PAF was observed ($P < 0.05$). Exposure to an aerosol of BSA in rabbits treated with NGPS induced a small but insignificant increase in the

Table 1 Effect of normal guinea-pig serum (NGPS) and anti-platelet serum (APS) on circulatory cell numbers in the rabbit

	Pretreatment	Post NGPS	Post APS
		(cells μl^{-1})	
Platelets	381545 ± 56056	436800 ± 79867	$29286 \pm 4082^*$
		($\times 10^3 \text{ cells } \mu\text{l}^{-1}$)	
White blood cells	13.2 ± 1.00	11.4 ± 1.30	12.2 ± 1.70
Neutrophils	4.80 ± 0.46	$6.43 \pm 1.23^*$	$5.89 \pm 0.65^{*\dagger}$
Eosinophils	0.25 ± 0.03	0.30 ± 0.05	0.29 ± 0.04
Mononuclear cells	6.60 ± 1.00	$3.64 \pm 0.37^*$	$4.91 \pm 1.00^{*\dagger}$
Basophils	1.00 ± 1.10	1.03 ± 0.22	1.07 ± 0.16

Results are expressed as mean $\pm$ s.e.mean. $n = 5$ –6 rabbits.

* Significant difference compared with pretreatment value ($P < 0.05$, paired t test).

$\dagger$ No significant difference compared with post NGPS value ($P > 0.05$, non-paired t test).

Table 2 Effect of PAF on pulmonary cell accumulation in rabbits treated with normal guinea-pig serum (NGPS) or anti-platelet serum (APS)

	Total	Neutrophils	Eosinophils	Monocytes
	($\times 10^5$ cells ml^{-1})			
Pretreatment	3.38 ± 0.44	0.16 ± 0.05	0	2.79 ± 0.39
BSA	4.78 ± 0.83	1.78 ± 0.28	0.006 ± 0.006	2.96 ± 0.84
PAF/NGPS	$15.88 \pm 4.26^*$	5.57 ± 2.14	$0.568 \pm 0.207^*$	9.69 ± 5.0
PAF/APS	$3.57 \pm 1.18^\dagger$	2.11 ± 0.59	$0.014 \pm 0.006^\dagger$	2.04 ± 0.81

Results are expressed as mean $\pm$ s.e.mean. $n = 5$ animals in each group. The prechallenge lavage data are the mean of 15 rabbits.

* Significant increase in total cell numbers ($P < 0.01$, non-paired t test) and eosinophils ($P < 0.05$, Mann-Whitney test) compared with BSA group.

No significant difference in total cell numbers or eosinophils compared with BSA group. ($\dagger P > 0.05$).

total cell infiltrate (Table 2). However, there was a significant increase in the number of neutrophils in the airways after BSA ($P < 0.05$, paired t test). BSA treatment induced no significant changes in the number of eosinophils or mononuclear cells. Depletion of circulating platelets reduced the PAF-induced leucocyte infiltration ($P < 0.05$) and this was associated with a reduction in the number of eosinophils (Table 2).

In vitro studies

Contractile agonists Carbachol, methacholine and histamine produced concentration-dependent contractions in bronchial

Table 3 Contractile and relaxant potency (pD_2) of various agonists in bronchial preparations from rabbits exposed 24 h previously to 0.25% BSA or PAF ($80 \mu\text{g ml}^{-1}$)

	BSA	PAF
	pD_2	
Contractile agonists		
Carbachol	-6.356 ± 0.064 5(17)	-6.211 ± 0.047 5(17)
Methacholine	-5.768 ± 0.071 5(18)	-5.773 ± 0.150 5(17)
Histamine	-5.100 ± 0.156 5(23)	-4.974 ± 0.159 5(21)
Relaxant agonists		
Isoprenaline	-6.871 ± 0.226 5(11)	-6.690 ± 0.130 4(7)
Theophylline	-4.101 ± 0.105 5(12)	$-3.691 \pm 0.068^*$ 5(14)

Results are expressed as mean $\pm$ s.e.mean. $\text{pD}_2 = \log_{10} \text{EC}_{50}$.

n (number below each result) = number of animals and n (number in parentheses) indicates the total number of bronchial preparations. * Relaxant potency for theophylline significantly reduced in rabbits exposed to PAF compared with BSA ($P < 0.01$, non-paired t test).

Table 4 Maximal tension generated (E_{max}) by various contractile agonists in rabbits treated 24 h previously with 0.25% BSA or PAF ($80 \mu\text{g ml}^{-1}$)

	BSA	PAF
	E_{max} (mg mg^{-1} wet weight)	
Carbachol	200 ± 50	136 ± 27
Methacholine	202 ± 48	167 ± 41
Histamine	168 ± 46	131 ± 27

Results are expressed as mean $\pm$ s.e.mean. $n =$ similar to those given in Table 2.

preparations from rabbits exposed to BSA or PAF. However, there was no significant difference in either the contractile potency (pD_2) or maximal tension generated (mg mg^{-1} wet weight) for carbachol, methacholine and histamine in bronchi from rabbits exposed to BSA or PAF ($P > 0.05$; Tables 3 and 4). PAF ($0.001\text{--}1 \mu\text{M}$) itself failed to contract isolated bronchi from naïve rabbits ($n = 3$ rabbits providing 18 bronchial preparations).

Relaxant agonists Isoprenaline and theophylline produced concentration-dependent relaxation of carbachol-induced tone in bronchial preparations from both 0.25% BSA-treated and PAF-treated rabbits. The maximal relaxant response was $70 \pm 11\%$ ($n = 5$) and $78 \pm 22\%$ ($n = 4$) in rabbits exposed to BSA and PAF, respectively. Similarly, for theophylline the maximal relaxant response was $154 \pm 19\%$ ($n = 5$) and $147 \pm 12\%$ ($n = 5$) in rabbits exposed to BSA and PAF, respectively. The relaxant potency of isoprenaline was not significantly different in bronchial preparations from rabbits exposed to BSA compared with PAF ($P > 0.05$, Table 3). In contrast the relaxant potency to theophylline was reduced by 2.58 fold in bronchial preparations from rabbits exposed to PAF compared with those from rabbits exposed to BSA (Table 3).

Discussion

It is becoming increasingly apparent that PAF may play a significant role in the pathogenesis of asthma, as it mimics many of the features which are characteristic of asthma (Barnes *et al.*, 1988; Page, 1988). We have therefore investigated the ability of PAF to induce airway hyperresponsiveness and inflammation in the rabbit and assessed the relative contribution of the platelet and airway smooth muscle function to these responses.

Exposure of rabbits to an aerosol of PAF induced acute bronchoconstriction. PAF has previously been shown to induce a platelet-dependent bronchoconstriction in this and other species following intravenous administration (Vargaftig *et al.*, 1980; Halonen *et al.*, 1985). Similarly in our experiments, aerosol administration of PAF induced a platelet-dependent bronchoconstriction. Furthermore, isolated bronchial preparations failed to contract to PAF under conditions where they readily contracted to other spasmogens. Our *in vitro* results are consistent with the inability of PAF to contract canine trachea (Popovich *et al.*, 1988), guinea-pig trachea (Cerrina *et al.*, 1983) and human bronchus (Schellenberg *et al.*, 1983) unless platelets are present (Schellenberg *et al.*, 1983; Popovich *et al.*, 1988). However, our results are in contrast to those of Lefort *et al.* (1984), who demonstrated that whilst bronchoconstriction induced by systemically administered PAF was platelet-dependent in the guinea-pig, an aerosol of PAF induced a platelet-independent bronchoconstriction.

No change in the number of circulating platelets or leucocytes was observed following aerosol exposure to PAF. This is

consistent with studies performed in the guinea-pig (Lefort *et al.*, 1984). However, a change in a minority cell population may still have occurred which we did not detect as we did not perform differential leucocyte counts on peripheral blood samples. In man, a transient neutropenia without thrombocytopenia was observed following inhalation of PAF (Roberts *et al.*, 1988). The lack of an effect on circulating platelets in man and rabbit suggests that platelet aggregation *per se* is not necessary for PAF-induced effects in the lung, consistent with observations made in other species (Robertson & Page, 1987).

Following exposure to PAF, airway responsiveness to histamine was increased at 6–24 h later. Such effects are consistent with those obtained following systemic administration or inhalation of PAF in the dog (Chung *et al.*, 1986), guinea-pig (Mazzoni *et al.*, 1985; Barnes *et al.*, 1987; Robertson & Page, 1987; Coyle *et al.*, 1988a; Robertson *et al.*, 1988), rabbit (Nieminen *et al.*, 1988) and in man (Cuss *et al.*, 1986). In our study the ability of PAF to induce airway hyperresponsiveness to histamine was relatively small (approx 1.5–4 fold) and did not occur in all animals. This observation is of interest for it has recently been found that PAF will not induce bronchial hyperresponsiveness in all subjects (Jenkins *et al.*, 1989) and in the rabbit at least, is susceptible to changes in body temperature (Nieminen *et al.*, 1989).

The underlying mechanisms whereby PAF can induce an increase in airway responsiveness is unclear. However, platelet activation appears to be important, as platelet depletion with a selective anti-serum inhibited PAF-induced airway hyperresponsiveness. Our results confirm previous observations in the guinea-pig that PAF induces a platelet-dependent increase in airway responsiveness (Mazzoni *et al.*, 1985).

Studies performed in both experimental animals and man suggest airway inflammation is important in heightened airway responsiveness (Chung, 1987). In the present study, PAF induced a 4 fold increase in the number of leucocytes in the airways as assessed by BAL and our results are consistent with previous *in vivo* findings that PAF can elicit airway inflammation (Camussi *et al.*, 1983; Lellouch-Tubiana *et al.*, 1988; Coyle *et al.*, 1988a). The differential cell counts revealed a trend towards an increase in the number of neutrophils and mononuclear cells which, due to the variability in the magnitude of the change between animals, did not reach statistical significance. However, there was a significant increase in the number of eosinophils in BAL fluid obtained from rabbits 24 h after exposure to PAF, which was small when compared with the increase in the numbers of neutrophils and mononuclear cells. Exposure to BSA also produced an increase in the number of neutrophils, probably as a result of non-specific inflammation.

PAF has been demonstrated to be a potent activation stimulus for neutrophils (Kurihara *et al.*, 1989), mononuclear cells (Yasaka *et al.*, 1982) and eosinophils (Wardlaw *et al.*, 1986; Tamura *et al.*, 1987). Furthermore, specific binding sites for PAF have recently been identified on the surface of these cell types (Kurihara *et al.*, 1989). PAF has also been demonstrated to induce neutrophil and mononuclear cell accumulation *in vivo* following intracutaneous administration in healthy individuals (Archer *et al.*, 1985), whereas local administration of PAF to the skin of allergic subjects induces predominantly an eosinophil-rich infiltrate (Henocq & Vargaftig, 1986). Aerosol administration of PAF in man induces a transient neutropenia which probably reflects sequestration of neutrophils in the pulmonary vasculature (Roberts *et al.*, 1988). PAF exposure also induced an increase in the number of mononuclear cells in the BAL 24 h after challenge and these observations are consistent with those observed following intratracheal administration of PAF in the rabbit (Camussi *et al.*, 1983).

In these experiments, the ability of PAF to induce leucocyte infiltration into the airways was dependent on platelet activation, since the total number of inflammatory cells found in lavage fluid after exposure to PAF was significantly reduced in thrombocytopenic animals. The differential cell counts only revealed significance for the effect of APS on PAF-induced

eosinophil infiltration, although there was a tendency for the number of neutrophils and mononuclear cells to fall as well. The precise mechanisms underlying platelet-dependent cellular infiltration are unknown, but our results are consistent with a platelet-dependent eosinophilia observed in the rabbit following exposure to allergen (Coyle *et al.*, 1988b) and in guinea-pig following exposure to PAF or allergen (Lellouch-Tubiana *et al.*, 1988). However, recent studies in the guinea-pig have not found any significant effect of platelet depletion on eosinophil infiltration in the lung induced by an aerosol of PAF (Sanjar *et al.*, 1990). Nevertheless, it is recognized that platelets can release a variety of chemotactic factors for neutrophils (e.g. platelet factor 4 (PF4), platelet derived growth factor (PDGF) and certain lipoxigenase metabolites of arachidonic acid), eosinophils (PF4) and mononuclear cells (e.g. PF4, transforming growth factor-beta (TGF- β)) (Page, 1989). Our results are of interest because platelet activation has recently been suggested to occur chiefly in allergic asthmatics, and increased platelet turnover (Gresele *et al.*, 1987), reduced platelet survival time (Taytard *et al.*, 1986) and prolonged bleeding times (Szczeklik *et al.*, 1986) have all been found to be features of allergic asthma. However, such changes have not been observed consistently within the allergic population (Hemmendinger *et al.*, 1989). Nevertheless, platelet aggregates have recently been found in the lung vasculature (Beasley *et al.*, 1989), platelets have been observed on damaged airway epithelium in symptomatic asthmatics (Jeffery *et al.*, 1989) and in BAL fluid obtained from allergic asthmatics (Metzger *et al.*, 1986) following allergen challenge. Furthermore, the exercise-induced rise in plasma levels of PF4 and β -thromboglobulin were attenuated in asthmatics following treatment with the PAF-antagonist BN 52036 (Wilkins *et al.*, 1990).

We have also investigated whether altered smooth muscle function could account for the bronchial hyperresponsiveness observed *in vitro*. The contractile potency to histamine, carbachol and methacholine was not enhanced in rabbits exposed to PAF compared with rabbits exposed to BSA. Similarly, the maximum tension developed in response to these spasmogens was similar in bronchi taken from both sources. These results are consistent with those found in guinea-pigs exposed to PAF (Robertson *et al.*, 1988) and suggest that alterations in airway smooth muscle function *per se* are not responsible for PAF-induced bronchial hyperresponsiveness. Similarly, no alteration in airway smooth muscle function has been observed in bronchi taken from asthmatic lung (Cerrina *et al.*, 1986; Goldie *et al.*, 1986) except in isolated cases (Schellenberg & Foster, 1984; De Jongste *et al.*, 1987).

We also assessed whether PAF-induced bronchial hyperresponsiveness was associated with a loss in airway β -adrenoceptor function. There was no alteration in the relaxant potency to isoprenaline in bronchi from rabbits exposed to PAF compared with BSA-treated rabbits, consistent with previous findings in other species (Barnes *et al.*, 1987). However, other investigators have demonstrated that incubation of lung membranes with PAF did result in a reduction in β -adrenoceptor density (Agrawal & Townley, 1987), an effect presumably due to the high concentrations of PAF utilised *in vitro*. Furthermore, our observations in the rabbit are consistent with those obtained in mild asthma where no alteration in β -adrenoceptor function has been observed (Svedymyr *et al.*, 1977). However, in severe asthma, β -adrenoceptor dysfunction has been demonstrated (Cerrina *et al.*, 1986; Goldie *et al.*, 1986). In contrast, the potency of theophylline was reduced in bronchi taken from rabbits previously exposed to PAF. The reasons for this remain unclear but may involve a cyclic AMP-independent mechanism, since β -adrenoceptor function was unaltered.

PAF has recently been shown to be a potent substrate at inducing vascular permeability in the airways (Evans *et al.*, 1987) and airway oedema has been suggested to contribute to airway hyperresponsiveness (Persson, 1986). However, recent investigations in the guinea-pig have suggested no causal relationship between airway vascular permeability and bron-

chial hyperresponsiveness (Roberts & Barnes, 1989). Chronic administration of PAF has also been shown to induce changes in smooth muscle thickening (Touvey *et al.*, 1990) and airway connective tissue (Schellenberg, 1987) which may occur via a different mechanism from that described in the present study. The contribution of platelets and airways inflammation to those long-term anatomical changes remains unknown.

In summary, we have demonstrated that PAF induces both bronchial hyperresponsiveness and airway inflammation

which are dependent on some aspect of platelet activation. However, this increase in airway responsiveness is not a consequence of changes in airway smooth muscle function *per se*.

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References

- AGRAWAL, D.K. & TOWNLEY, R.G. (1987). Effects of platelet activating factor on beta-adrenoceptors in human lung. *Biochem. Biophys. Res. Commun.*, **143**, 1–6.
- ANTONNISSEN, L.A., MITCHELL, R.W., KROEGER, W., KEPRON, K.S. & STEPHEN, N.L. (1979). Mechanical alterations of airway smooth muscle in canine asthmatic model. *J. Appl. Physiol.*, **46**, 681–687.
- ARCHER, C.B., PAGE, C.P., MORLEY, J. & MACDONALD, D.M. (1985). Accumulation of inflammatory cells in response to intracutaneous platelet activating factor (Paf-acether) in man. *Br. J. Dermatol.*, **112**, 285–290.
- BARNES, P.J., GRANDORDY, B.M., PAGE, C.P., RHODEN, K.J. & ROBERTSON, D.N. (1987). The effect of platelet activating factor on pulmonary β -adrenoceptors. *Br. J. Pharmacol.*, **90**, 709–715.
- BARNES, P.J., CHUNG, K.F. & PAGE, C.P. (1988). Inflammatory mediators and asthma. *Pharmacol. Rev.*, **40**, 49–84.
- BEASLEY, R., ROCHE, W.R., ROBERTS, J.A. & HOLGATE, S.T. (1989). Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am. Rev. Respir. Dis.*, **139**, 806–817.
- BUTLER, K.D. & SMITH, J.R. (1982). Mechanisms of forssman-induced bronchospasm and their inhibition. *Br. J. Pharmacol.*, **73**, 25–32.
- CAMUSSI, G., PAWLOWSKI, I., TETTA, C., ROFFINELLO, C., ALBERTSON, M., BRENTJENS, J. & ANDRES, G. (1983). Acute lung inflammation induced in the rabbit by local instillation of 1-O-octadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine or of native platelet-activating factor. *Am. J. Pathol.*, **112**, 78–88.
- CERRINA, J., RAFFESTIN, B., LABAT, C., BOULLET, C., BAYOL, A., GATEAU, O. & BRINK, C. (1983). Effects of PAF-acether on isolated muscle preparations from the rat, guinea-pig and human lung. In *Platelet-Activating Factor and Structurally Related Ether-Lipids*. ed. Benveniste, J. & Arnoux, B. pp. 205–212. Amsterdam: Elsevier.
- CERRINA, J., LAUDRIE, M.L.R., LABAT, C., RAFFESTIN, B., BAYOL, A. & BRINK, C. (1986). Comparison of human bronchial muscle responses to histamine in vivo with histamine and isoproterenol agonists in vitro. *Am. Rev. Respir. Dis.*, **134**, 57–61.
- CHUNG, K.F., BECKER, A.B., LAZARUS, S.C., FRICK, O.L., NADEL, J.A. & GOLD, W.M. (1985). Antigen-induced airway responsiveness and pulmonary inflammation in allergic dogs. *J. Appl. Physiol.*, **58**, 1347–1353.
- CHUNG, K.F., AIZAWA, H., LEIKAUF, G.D., UEKI, I.F., EVANS, T.W. & NADEL, J.A. (1986). Airway hyperresponsiveness induced by platelet-activating factor: Role of thromboxane generation. *J. Pharmacol. Exp. Ther.*, **236**, 580–584.
- CHUNG, K.F. (1987). The role of inflammation in hyperreactivity of the airways in asthma. *Thorax*, **41**, 657–662.
- CIBULAS, W., BROOKS, S.M., MURLAS, C.G., MILLER, M.L. & McKAY, R.T. (1988). Toluene diisocyanate-induced airway hyperreactivity in guinea-pigs depleted of granulocytes. *J. Appl. Physiol.*, **64**, 1773–1778.
- COYLE, A.J., URWIN, S.C., PAGE, C.P., TOUVAY, C., VILLAIN, B. & BRAQUET, P. (1988a). The effect of the selective PAF antagonist BN 52021 on PAF- and antigen-induced bronchial hyperreactivity and eosinophil accumulation. *Eur. J. Pharmacol.*, **148**, 51–58.
- COYLE, A.J., BROWN, L.A., PAGE, C.P. & METZGER, W.J. (1988b). The role of platelets in late phase asthma, bronchial hyperreactivity and eosinophil recruitment in allergic rabbits. *Am. Rev. Respir. Dis.*, **137**, A135.
- CUSS, F.M., DIXON, C.M.S. & BARNES, P.J. (1986). Effect of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. *Lancet*, **ii**, 189–192.
- DE JONGSTE, J.C., MONS, H., BONTA, I.L. & KERREBIJN, K.F. (1987). In vitro response of airways from an asthmatic patient. *Eur. J. Respir. Dis.*, **71**, 23–29.
- EVANS, T.W., CHUNG, K.F., ROGERS, D.F. & BARNES, P.J. (1987). Effect of platelet activating factor on airway vascular permeability: possible mechanisms. *J. Appl. Physiol.*, **63**, 479–484.
- FLECKNALL, P.A. (1987). *Laboratory Animal Anaesthesia*. London: Academic Press.
- GILES, R.E., FINKEL, M.P. & MAZUROWSKI, J. (1974). Use of an analogue on line computer for the evaluation of pulmonary resistance and dynamic compliance in the anaesthetised dog. *Arch. Int. Pharmacodyn.*, **194**, 213–222.
- GOLDIE, R.G., SPINA, D., HENRY, P.J., LULICH, K.M. & PATERSON, J.W. (1986). In vitro responsiveness of human asthmatic bronchus to carbachol histamine, β -adrenoceptor agonists and theophylline. *Br. J. Clin. Pharmacol.*, **22**, 669–676.
- GRESELE, P., RIBALDI, E., GRASSELLI, S., TODISCO, T. & NENCI, G.G. (1987). Evidence for platelet activation in allergic asthma. In *Platelets, PAF and Asthma. Agents & Actions*, Vol. 21, ed. Schmitz-Schumann, M., Menz, G. & Page, C.P. pp. 119–128. Basle, Birkhauser-Verlag.
- HALONEN, M., LOHMAN, I.C., DUNN, A.M., McMANUS, L.M. & PALMER, J.D. (1985). Participation of platelets in the physiologic alterations of the AGEPC response and of IgE anaphylaxis in the rabbit. *Am. Rev. Respir. Dis.*, **131**, 11–17.
- HEMMENDINGER, S., PAULI, G., TENABENE, A., PUJOL, J.L., BESSOT, J.C., EBER, M. & CAZENAVE, J.-P. (1989). Platelet function: Aggregation by PAF or sequestration in lung is not modified during immediate or late allergen-induced bronchospasm in man. *J. Allergy Clin. Immunol.*, **83**, 990–996.
- HENOCQ, E. & VARGAFTIG, B.B. (1986). Accumulation of eosinophils in response to intracutaneous PAF-acether and allergens in man. *Lancet*, **ii**, 1378–1379.
- JAMES, A.L., PARE, P.D. & HOGG, J.C. (1989). The mechanics of airway narrowing in asthma. *Am. Rev. Respir. Dis.*, **139**, 242–246.
- JEFFERY, P.K., WARDLAW, A.J., NELSON, F.C., COLLINS, J.V. & KAY, A.B. (1989). Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am. Rev. Respir. Dis.*, **140**, 1745–1753.
- JENKINS, J.R., LAI, C.K.W. & HOLGATE, S.T. (1989). Effect of increasing doses of platelet-activating factor (PAF) on normal human airways. *J. Allergy Clin. Immunol.*, **83**, 443 (Abstract).
- KURIHARA, K., WARDLAW, A.J., MOQBEL, R. & KAY, A.B. (1989). Inhibition of platelet activating factor (PAF) induced chemotaxis and binding to human eosinophils and neutrophils by the specific ginkgolide derived PAF antagonist BN 52021. *J. Allergy Clin. Immunol.*, **83**, 83–90.
- LEFORT, J., ROTILIO, D. & VARGAFTIG, B.B. (1984). The platelet-independent release of thromboxane A_2 by Paf-acether from guinea-pig lungs involves mechanisms distinct from those for leukotriene. *Br. J. Pharmacol.*, **82**, 565–575.
- LELLOUCH-TUBIANA, A., LEFORT, J., SIMON, M.-T., PFISTER, A. & VARGAFTIG, B.B. (1988). Eosinophil recruitment into guinea-pig lungs after PAF-acether and allergen administration. *Am. Rev. Respir. Dis.*, **137**, 948–954.
- MARSH, W.R., IRVIN, C.G., MURPHY, K.R., BEHRENS, L. & LARSEN, G.L. (1985). Increases in airway reactivity to histamine and inflammatory cells in bronchoalveolar lavage after the late asthmatic response in an animal model. *Am. Rev. Respir. Dis.*, **131**, 875–879.
- MAZZONI, L., MORLEY, J., PAGE, C.P. & SANJAR, S. (1985). Induction of airway hyperreactivity by platelet activating factor in the guinea-pig. *J. Physiol.*, **365**, P107.
- METZGER, W.J., SJOERDSMA, K., RICHERSON, H.B., MOSELEY, P., ZAVALA, K., MONICK, M. & HUNNINGHAKE, G.W. (1986). Platelets in bronchoalveolar lavage from asthmatic patients and allergic rabbits with allergen-induced late phase responses. *Agents Actions*, (Suppl.), **21**, 151–159.
- MURLAS, C.G. & ROUM, J.H. (1985). Bronchial hyperreactivity occurs in steroid-treated guinea-pigs depleted of leukocytes by cyclophosphamide. *J. Appl. Physiol.*, **58**, 1630–1637.
- MURPHY, K.R., WILSON, M.C., IRVIN, C.G., GLEZEN, L.S., MARSH, W.R., HASLETT, C., HENSON, P.M. & LARSEN, G.L. (1986). The

- requirement for polymorphonuclear leukocytes in the late asthmatic response and heightened airways reactivity in an animal model. *Am. Rev. Respir. Dis.*, **134**, 62–68.
- NIEMINEN, M.M., HENSON, P.M. & IRVIN, C.S. (1988). Bimodal effects of platelet activating factor (PAF) on airways responses in the rabbit. *Am. Rev. Respir. Dis.*, **137**, A308.
- NIEMINEN, M.M., IRVIN, C.G. & HENSON, P.M. (1989). Body temperature modulates the effect of platelet activating factor (PAF) on airway responsiveness in the rabbit. *Eur. J. Resp. Dis.*, **2**, S289.
- O'BYRNE, P.M., WALTERS, E.H., GOLD, B.D., AIZAWA, H.A., FABBRI, S.E., ALPERT, S.E., NADEL, J.A. & HOLTZMAN, M.J. (1984). Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. *Am. Rev. Respir. Dis.*, **130**, 214–219.
- PAGE, C.P. (1988). The role of platelet activating factor in asthma. *J. Allergy Clin. Immunol.*, **81**, 144–150.
- PAGE, C.P. (1989). Platelets as inflammatory cells. *Immunopharmacol.*, **17**, 51–59.
- PERSSON, C.G.A. (1986). Role of plasma exudation in asthmatic airways. *Lancet*, **ii**, 1126–1128.
- POPOVICH, K.J., SHELDON, G., MACK, M., MUNOZ, N.M., DENBERG, P., BLAKE, J., WHITE, S.R. & LEFF, A.R. (1988). Role of platelets in contraction of canine tracheal muscle elicited by PAF in vitro. *J. Appl. Physiol.*, **65**, 914–920.
- ROBERTS, N.M., McCUSKER, M., CHUNG, K.F. & BARNES, P.J. (1988). Effect of a PAF antagonist, BN 52063, on PAF-induced bronchoconstriction in normal subjects. *Br. J. Clin. Pharmacol.*, **26**, 65–72.
- ROBERTS, N.M. & BARNES, P.J. (1989). Relationship between bronchoconstriction, bronchial hyperresponsiveness and microvascular leakage induced by inhaled PAF and antigen. *Am. Rev. Respir. Dis.*, **139**, A463.
- ROBERTSON, D.N. & PAGE, C.P. (1987). Effects of platelet agonists on airway reactivity and intrathoracic platelet accumulation. *Br. J. Pharmacol.*, **92**, 105–111.
- ROBERTSON, D.N., COYLE, A.J., RHODEN, K.J., GRANDORDY, B., PAGE, C.P. & BARNES, P.J. (1988). The effect of platelet-activating factor on histamine and muscarinic receptor function in guinea-pig airways. *Am. Rev. Respir. Dis.*, **137**, 1317–1322.
- SANJAR, S., AOKI, S., BOUBEKEUR, K., CHAPMAN, I.D., SMITH, D., KINGS, M.A. & MORLEY, J. (1990). Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet activating factor: effect of anti-asthma drugs. *Br. J. Pharmacol.*, **99**, 267–272.
- SCHELLENBERG, R.R., WALKER, B. & SNYDER, F. (1983). Platelet dependent contraction of human bronchi by platelet activating factor. *J. Allergy Clin. Immunol.*, **71**, 145 (Abstract).
- SCHELLENBERG, R.R. & FOSTER, A. (1984). In vitro responses of human asthmatic airway and pulmonary vascular smooth muscle. *Int. Arch. Allergy Appl. Immunol.*, **75**, 237–241.
- SCHELLENBERG, R.R. (1987). Airways responses to platelet activating factor. *Am. Rev. Respir. Dis.*, **136**, S28–S32.
- SVEDMYR, N.L., LARSSON, S.A. & THIRINGER, G.K. (1977). Development of "resistance" in beta-adrenergic receptors of asthmatic patients. *Chest*, **69**, 479–483.
- SZCZEKLIK, A., MILNER, P.C., BIRCH, J., WATKINS, J. & MARTIN, J.F. (1986). Prolonged bleeding time, reduced platelet aggregation, altered Paf-acether sensitivity and increased platelet mass are a trait of asthma and hay fever. *Thromb. Haemostasis*, **56**, 283–287.
- TAMURA, N., AGRAWAL, D.K., SULIAMAN, F.A. & TOWNLEY, R.G. (1987). Effects of platelet activating factor on the chemotaxis of normodense eosinophils from normal subjects. *Biochem. Biophys. Res. Commun.*, **142**, 638–644.
- TAYTARD, A., GUENARD, H., VUILLEMIN, L., BOUROT, J.L., VERGERET, J., DUCASSOU, D., PIQUET, Y. & FREOUR, P. (1986). Platelet kinetics in stable asthmatic subjects. *Am. Rev. Respir. Dis.*, **134**, 983–985.
- THOMPSON, J.E., SCYPINSKI, L.A., GORDON, T. & SHEPPARD, D. (1986). Hydroxyurea inhibits airway hyperresponsiveness in guinea pigs by a granulocyte-independent mechanism. *Am. Rev. Respir. Dis.*, **134**, 1213–1218.
- TOUVAY, C., PFISTER, A., VILLAIN, B., COYLE, A.J., PAGE, C.P., LELLOUCH-TUBIANA, A., PIGNOL, B., MENCIA-HEURTA, J.M. & BRAQUET, P. (1990). Effect of long term infusion of platelet activating factor on pulmonary responsiveness and morphology in the guinea-pig. *Pulmon. Pharmacol.* (in press).
- VARGAFTIG, B.B., LEFORT, J., CHIGNARD, M. & BENVENISTE, J. (1980). Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. *Eur. J. Pharmacol.*, **65**, 185–192.
- WARDLAW, A.J., MOQBEL, R., CROMWELL, O. & KAY, A.B. (1986). Platelet activating factor: a potent chemotactic and chemokinetic factor for human eosinophils. *J. Clin. Invest.*, **78**, 1701–1706.
- WILKENS, J.H., WILKENS, H., UFFMAN, J., BOVERS, J., FABEL, H. & FROLICH, J.C. (1990). Effects of a PAF antagonist (BN 52063) on bronchoconstriction and platelet activation during exercise induced asthma. *Br. J. Clin. Pharmacol.*, **29**, 85–92.
- YASAKA, T., BOXER, L.A. & BAEHNER, R.C. (1982). Monocyte aggregation and superoxide anion response to formyl-methionyl-phenylalanine (FMLP) and platelet activating factor (PAF). *J. Immunol.*, **128**, 1939–1944.

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