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

A.J. Coyle, D. Spina & 1C.P. Page
Department of Pharmacology, King's College, University of London, Manresa Road, London SW3 6LX

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; Barondes 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 (Murias & 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; Lelouch-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 (Boros 00) and the flow determined by a Validyne pressure transducer. Pleural pressure was estimated by placing an osophageal 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 (RL) and dynamic compliance (Cdyn) 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 μm in diameter.

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

1 Author for correspondence.
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, or a 30% fall in C dyn. The provocation dose (PD) of histamine which produced a 100% increase in R, (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-anasthetized 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 μg 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 ± 0.13 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 citrate. 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 μg ml -1) for 1 h, were 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 saturated with 95% O 2, 5% CO 2 and maintained at 37°C. Changes in isometric tension were measured with a Grass force-displacement transducer (FT03C) and recorded on a 6 channel chart recorder. 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 μM). 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 naive 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 vitro studies are expressed as mean ± s.e.mean. In vivo histamine potency derived from measurements of resistance (PD 100 ) and dynamic compliance (PD 30 ) are expressed as geometric means ± s.e.mean. 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 100 = log 10 EC 10 ) 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 ± 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 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, 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, (+)-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, NaHCO3 25, d-glucose 11.1, KCl 5.4, MgSO4 0.57, KH2PO4 1.03 and CaCl2 2.5. Isoprenaline was dissolved in 0.9% NaCl solution containing 20 μg ml⁻¹ ascorbic acid.

Results

In vivo studies

PAF (80 μg ml⁻¹) induced acute bronchoconstriction in control animals, R₄ increased by 146.2 ± 40.7% (n = 6) and Cdyn decreased by 30.6 ± 4.7% (n = 6) (Figure 1). PAF-induced changes in pulmonary function diminished over the next hour, although the values for R₄ and Cdyn did not return completely to baseline (Figure 1). Exposure to 0.25% BSA induced a small but significant bronchoconstriction (R₄ + 10.7 ± 2.3%, Cdyn - 7.2 ± 3.2%, P < 0.05). Platelet depletion reduced the magnitude of PAF-induced bronchoconstriction compared with rabbits treated with NGPS (R₄ + 29.3 ± 5.5%, Cdyn - 11.8 ± 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 R₄ by 100% (PD₁₀₀) 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 (43.5–82.2), 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 Cdyn by 30% (PD₃₀) 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 (R₄) and dynamic compliance (Cdyn) recorded over a 1 h period following a 1 h aerosol exposure to histamine in rabbits. Each point is the mean from 6 animals and vertical lines show s.e.mean.

Figure 2 Changes in total lung resistance (R₄) and dynamic compliance (Cdyn) 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 μg 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; n = 5) following exposure to 0.25% PAF compared with untreated controls (Figure 2).

Figure 3 Airway responsiveness to histamine (a) log PD₁₀₀ resistance and (b) log PD₃₀ compliance, before and 6 h, 24 h and 72 h after aerosol exposure to 0.25% bovine serum albumin (BSA) or 80 μg ml⁻¹ PAF (shaded columns). Results 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 Rₐ and Cₕ₀ values were 15.8 ± 1.5 cmH₂O l⁻¹ s⁻¹ (n = 10) and 3.2 ± 0.4 ml cmH₂O l⁻¹ (n = 10), respectively. Furthermore, there was no significant difference in baseline Rₐ and Cₕ₀ 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 ± 0.35 × 10⁹ cells μl⁻¹; post-treatment: 3.50 ± 0.53 × 10⁹ cells μl⁻¹; n = 3) or leucocytes (pretreatment: 1.28 ± 0.25 × 10⁴ cells ml⁻¹; post-treatment: 1.08 ± 0.50 × 10⁴ 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 Pₐ PD₁₀₀ following histamine administration was 50.1 mg ml⁻¹ (42.7–58.9; n = 5). Administration of APS failed to modify histamine-induced bronchoconstriction (PD₁₀₀ 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 naive (pretreatment PD₁₀₀ 51.3 mg ml⁻¹ (43.2–60.8); 24 h post-treatment: PD₁₀₀ 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 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 is 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 (ANOVA 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, ANOVA). 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

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment</th>
<th>Post NGPS</th>
<th>Post APS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cells μl⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>381545 ± 56056</td>
<td>436800 ± 79867</td>
<td>29286 ± 4082*</td>
</tr>
<tr>
<td>White blood cells</td>
<td>13.2 ± 1.00</td>
<td>11.4 ± 1.30</td>
<td>12.2 ± 1.70</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>4.80 ± 0.46</td>
<td>6.43 ± 1.23*</td>
<td>5.89 ± 0.65†</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.25 ± 0.03</td>
<td>0.30 ± 0.05</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>6.60 ± 1.00</td>
<td>3.64 ± 0.37*</td>
<td>4.91 ± 1.00†</td>
</tr>
<tr>
<td>Basophils</td>
<td>1.00 ± 1.10</td>
<td>1.03 ± 0.22</td>
<td>1.07 ± 0.16</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± s.e.mean. n = 5–6 rabbits.

* Significant difference compared with pretreatment value (P < 0.05, paired t test).
† No significant difference compared with post NGPS value (P > 0.05, non-paired t test).
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 preparations from rabbits exposed to BSA or PAF. However, there was no significant difference in either the contractile potency (pD2) 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 (0.001–1 μM) itself failed to contract isolated bronchi from naive 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 ± 11% (n = 5) and 78 ± 22% (n = 4) in rabbits exposed to BSA and PAF, respectively. Similarly, for theophylline the maximal relaxant response was 154 ± 19% (n = 5) and 147 ± 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 spasmodgens. 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 occur 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 (Robert 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 6-24h 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 (Niiminen 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 (Jenkins et al., 1989).

The underlying mechanisms whereby PAF can induce an increase in airway responsiveness is unclear. However, 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 24h after exposure to PAF, which was small 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 (Nasadulla et al., 1982) and eosinophils (Wardlaw et al., 1986; Tamura et al., 1987). Furthermore, specific binding sites for PAF have recently been indentified 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 intratracheal administration in healthy individuals (Alving et al., 1985), whereas local administration of PAF to the skin of allergic subjects induces predominantly an eosinophil-rich infiltrate (Henocq & Vargafitig, 1986). Aerodol 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 24h 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 it is clear that 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 (Sanjari 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 lipoxygenase metabolites of arachidonic acid), eosinophils (PF4) and mononuclear cells (e.g. PF4, transforming growth factor-beta (TGF-beta)) (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 (Bohmendinger & Neumann, 1989). Nevertheless, platelet aggregates have recently been found in the lung vasculature (Coyle 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 beta-thromboglobulin were attenuated in asthmatics following treatment with the PAF-antagonist BN 52036 (Wilkens 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 spasmonens 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 beta-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 beta-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 beta-adrenoceptor function has been observed (Svedmyr et al., 1977). However, in severe asthma, beta-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 beta-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 (Touvay 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.

We would like to acknowledge the financial support of Institute Henri Bergonier. D.S. is a C.J. Martin fellow of the National Health and Medical Research Council of Australia. We would also like to thank Dr Anne Wozencraft, Dr Marie McKenniff and Claire Lloyd for helpful discussions.

References


(Received August 22, 1989 Revised December 10, 1989 Accepted May 3, 1990)