Mechanism of action of platelet activating factor in the pulmonary circulation: an investigation using a novel isotopic system in rabbit isolated lung

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1 Rabbit isolated lungs were perfused via the pulmonary artery with Tyrode solution containing 4.5% Ficoll and 0.1% bovine serum albumin at a constant rate of 20 ml min⁻¹. Lung perfusate was drawn for alternating 5 min periods from two reservoirs, one containing ¹²⁵I-albumin and the other unlabelled albumin to wash out the intravascular label. Microvascular ¹²⁵I-albumin leakage was determined from the count remaining at the end of the washout phase with an external gamma scintillation probe. In addition, perfusion pressure was monitored continuously. Each experiment comprised 6 cycles over a total period of 60 min.

2 Infusion of platelet activating factor (PAF, 3 nmol min⁻¹ for 10 min) resulted in microvascular ¹²⁵I-albumin leakage, whereas lyso-PAF was without effect. During PAF infusions there was also an increase in perfusion pressure. Both the permeability and pressor effects of PAF were inhibited by the PAF antagonist L-652731.

3 Infusion of the thromboxane analogue U-46619 (0.6 nmol min⁻¹ for 10 min) caused an increase in perfusion pressure but protein accumulation was not significantly different from that observed with control infusions.

4 Bolus injections of PAF (1 nmol) caused increases in perfusion pressure which were reduced by indomethacin, dazmegrel and BW 755C. Bolus injections of PAF, repeated at 30 min intervals caused reproducible pressor responses; however, repeated injections at 60 min intervals resulted in augmented responses. This augmentation did not occur in the presence of indomethacin.

5 Retrograde perfusion of PAF via the pulmonary vein induced increased perfusion pressure and microvascular ¹²⁵I-albumin leakage. The observed increase in leakage when compared with forward perfusion suggests that PAF produces predominantly arteriolar constriction i.e. proximal to the site of leakage during forward perfusion.

6 These results indicate that PAF is a vasoconstrictor in the rabbit pulmonary circulation and augmented responses occur with repeated injections at 60 min intervals. Cyclo-oxygenase inhibition abolished this vascular hyperresponsiveness induced by PAF. PAF also caused protein accumulation in the lungs. Both these actions of PAF appear to be receptor-mediated because they were inhibited by PAF antagonists. Another pulmonary vasoconstrictor, U-46619 did not cause protein accumulation suggesting that the extravasation of protein with PAF is not merely secondary to changes in vascular tone.

Keywords: PAF; PAF antagonists; thromboxane; oedema; perfused lung; inflammation

Introduction

Platelet activating factor (PAF) was originally described as a putative mediator of anaphylaxis released by rabbit basophils on exposure to antigen (Benveniste *et al.*, 1972). Subsequent investigations have revealed that PAF can be released from other cell types including platelets (Chignard *et al.*, 1980), neutrophils (Lynch *et al.*, 1979; Betz & Henson, 1980), monocytes and macrophages (Arnoux *et al.*, 1980) and endothelial cells (Camussi *et al.*, 1983b). In addition to its effects on platelets, PAF has been shown to be a potent stimulator of other target cells including neutrophils (Goetzl *et al.*, 1980; O'Flaherty *et al.*, 1981), vascular endothelial cells (Handley *et al.*, 1984; Bussolino *et al.*, 1987; Grigorian & Ryan, 1987), vascular smooth muscle cells and bronchial smooth muscle cells (Findlay *et al.*, 1981; Camussi *et al.*, 1983a; Stimler & O'Flaherty, 1983; Baranes *et al.*, 1986).

In vivo, PAF increases microvascular permeability resulting in oedema formation (Vargaftig & Ferreira, 1981; Wedmore & Williams, 1981; Humphrey *et al.*, 1982). In skin, PAF acts synergistically with vasodilator prostaglandins to induce local

oedema formation (Wedmore & Williams, 1981; Morley *et al.*, 1983; Archer *et al.*, 1984; Hellewell & Williams, 1986). This PAF-induced increase in vascular permeability appears to result from a direct effect of PAF on vascular endothelial cells since it is unaffected by depletion of circulating neutrophils or platelets (Wedmore & Williams, 1981; Pirotzky *et al.*, 1984). In addition to the effects of exogenously administered PAF, there is evidence that endogenous production of this phospholipid contributes to oedema formation. Thus, PAF antagonists partially suppress oedema in Arthus reactions initiated by immune complex deposition around vessel walls in rabbits (Hellewell & Williams, 1986; Hellewell, 1990) and rats (Camussi *et al.*, 1987; Warren *et al.*, 1989).

The effects of PAF in the lung mimic some of the major pathophysiological characteristics of lung injury. PAF causes pulmonary endothelial damage (Lewis *et al.*, 1983; McManus & Pinckard, 1985), pulmonary oedema and vasoconstriction (Heffner *et al.*, 1983; Hamasaki *et al.*, 1984; Burhop *et al.*, 1986; Christman *et al.*, 1988), increased airway resistance and decreased lung compliance (Halonen *et al.*, 1980, 1981; Christman *et al.*, 1988). Furthermore, PAF has been demonstrated to cause epithelial cell damage and an influx of inflammatory cells into airways and alveoli (Camussi *et al.*, 1983a; Arnoux *et al.*, 1988; Coyle *et al.*, 1988; Lellouch-Tubiana *et al.*, 1988; Sanjar *et al.*, 1990). In animal models of pulmonary dysfunction (associated with endotoxaemia), PAF antagonists are effective inhibitors of haemodynamic changes and lung injury

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(Chang *et al.*, 1986). Based on these observations, PAF has been proposed to be an important mediator of acute lung injury.

Although the phenomenon of PAF-induced pulmonary oedema is well documented, the precise mechanism by which this effect is mediated is contentious. For example, it is unclear whether oedema formation is due to a change in endothelial cell permeability or whether it is merely a consequence of increased hydrostatic pressure secondary to vasoconstriction (Heffner *et al.*, 1983; Hamasaki *et al.*, 1984; Burhop *et al.*, 1986; Christman *et al.*, 1988; Imai *et al.*, 1988). To investigate the effects of PAF and other inflammatory mediators in the lung under constant flow conditions, we have developed a novel model using *in vitro* lung perfusion. With this system, we have measured simultaneously changes in pulmonary vascular resistance and accurately measured pulmonary microvascular protein leakage. Our results demonstrate that PAF causes potent pulmonary vasoconstriction and increases the permeability of the pulmonary microvasculature and that these processes are dissociable. Parts of this work have been published in abstract form (Seale *et al.*, 1987).

Methods

Animals

Male New Zealand White specific pathogen-free rabbits (2.5–3.5 kg) were purchased from Froxfield Farm, Hampshire and Hacking and Churchill, Huntingdon, Cambridge.

Preparation of isolated perfused lungs

Rabbits were anaesthetized with i.v. sodium pentobarbitone (30 mg kg^{-1}). A tracheostomy was performed and the animals allowed to breathe room air. Heparin (100 iu kg^{-1}) was administered i.v. and the animal killed with an overdose of sodium pentobarbitone. A thoracotomy was immediately performed and a silicone cannula was inserted into the pulmonary artery. The lungs were fully inflated with room air and 100 ml of perfusate at 37°C was flushed through the pulmonary circulation. Effluent perfusate escaped freely through an incision in the left atrium. Perfusion fluid comprised Tyrode solution (pH 7.4) containing 4.5% (w/v) Ficoll 70 and 0.1% (w/v) BSA gassed with 100% oxygen.

Lungs and heart were removed en bloc and suspended by the tracheal cannula. Lungs were inflated with 50–70 ml air and perfusate pumped through the pulmonary circulation at a constant flow of 20 ml min^{-1} (model 5025, Watson-Marlow, Falmouth, Cornwall). Evans blue dye (10 mg ml^{-1} , final concentration) was added to the perfusate for short periods of time so that the distribution of lung perfusate could be visualized. Perfusion pressure (arterial) was recorded continuously with a pressure transducer (Bell and Howell, model 4/327-1, Glasgow, Scotland) connected to a Lectromed MX2P pre-amplifier and MX212 pen recorder. The lung and apparatus were maintained at 37°C in a thermostatically-controlled perspex box.

In some experiments the lungs were perfused in a retrograde direction. The rabbits were prepared as described but instead of incising the left atrium, a cannula (of the same dimension as the pulmonary artery cannula) was inserted into it through the mitral valve. The lungs were perfused initially in a forward direction (i.e. via the pulmonary artery) at 20 ml min^{-1} for 5 min. The direction of flow was then reversed such that the perfusate entered the pulmonary circulation via the left atrial cannula.

Measurement of extravascular ^{125}I -albumin accumulation

Lung perfusate was drawn for alternating periods of 5 min from two reservoirs, one containing ^{125}I -human serum albumin ($20 \mu\text{g ml}^{-1}$, $0.05 \mu\text{Ci ml}^{-1}$) and one containing normal buffer (see below). The change from one reservoir to another was achieved by a solenoid-controlled valve. A gamma detector probe (Nuclear Enterprises Ltd., model DM1-1, Edinburgh, Scotland) fitted with a 25 mm diameter $\times$ 3 mm thick NaI crystal was mounted in a lead collimator with a single cylindrical hole (diameter 20 mm $\times$ length 15 mm) and positioned adjacent to a segment of well perfused lung, as determined by the infusion of Evans blue dye. ^{125}I -counts were displayed on a scalar ratemeter (Nuclear Enterprises, model SR7) and printed out every 30 s. Before each experiment a volume of the radioactive perfusate containing 100 μg total albumin was placed in the position of the lung in front of the probe to determine the counts per second corresponding to 1 μg of albumin so that radioactivity within the lung could be expressed in terms of μg protein. As shown in Figure 1, the whole system was linked to an Apple IIe microcomputer (Apple Computer, Cupertino, California,

Figure 1 Schematic diagram of experimental apparatus. Lung perfusate was drawn for alternating periods of 5 min from two reservoirs, one of which contained ^{125}I -labelled human serum albumin (labelled perfusate). A gamma-detector probe was positioned adjacent to the pleural surface of the lung. An Apple IIe computer regulated the system and collected data.

Figure 2 Protein accumulation during six 10 min cycles. Each cycle comprised 5 min 'wash in' of labelled perfusate followed by 5 min 'wash out' with unlabelled buffer. Infusion of PAF during the 4th cycle (b) caused an increase in protein accumulation compared with saline infusion (a).

U.S.A.) which regulated the solenoid and the scalar ratemeter as well as collecting and tabulating the protein-leakage data.

In preliminary experiments with continuous perfusion of labelled albumin it was found that protein leakage could not be accurately measured when superimposed upon a large background signal due to intravascular label. Therefore, a cycling perfusion procedure was devised to wash out the intravascular component periodically as shown in Figure 2. During perfusion with radioactive solution ('wash-in' phase) counts in the lung steadily increased and reached a plateau by 5 min. After starting perfusion with non-radioactive buffer ('washout' phase), there was a rapid fall in counts until a plateau was reached by 5 min (Figure 2). The residual activity in the collimated region of the lung at the end of each 'wash-out' cycle was used as a measure of extravasated albumin. Three such 10 min cycles (comprising 5 min 'wash-in' and 5 min 'wash-out') were completed. At the start of the 4th cycle, PAF or lyso-PAF (3 nmol min⁻¹ in saline containing 0.25% BSA), or saline (containing 0.25% BSA) was infused for 10 minutes (i.e. during the whole of the 4th cycle). In some experiments the stable thromboxane mimetic U-46619 was infused at 0.6 nmol min⁻¹.

Drugs and materials

Bovine serum albumin (BSA, Cohn Fraction V, fatty acid-free), arachidonic acid and indomethacin were purchased from Sigma Chemical Co., Poole, Dorset; Evans blue dye was from BDH Chemicals, Poole, Dorset; platelet activating factor (PAF) was from Bachem, Saffron Walden, Essex; sodium pentobarbitone and mepyramine maleate were from May and Baker, Dagenham, Essex; Ficoll 70 was from Pharmacia Fine Chemicals, Uppsala, Sweden; sterile 0.9% saline (Steriflex) was from the Boots Company, Nottingham, Nottinghamshire; ¹²⁵I-human serum albumin (50 µCi ml⁻¹, 20 mg albumin ml⁻¹) was from Amersham International plc, Amersham, Buckinghamshire. Sterile Tyrode solution (composition mM: NaCl 148, KCl 2.7, CaCl₂ 1.16, MgCl₂ 0.5, NaH₂PO₄ 0.36, glucose 5.6, NaHCO₃ 1.2) was from the Clinical Research Centre media department.

The following were obtained as gifts: 48740 RP (3-(3-pyridyl)-1H,3H-pyrrolo [1,2-e]thiazole-7-carboxamide) from Dr P. Sedivy, Rhone-Poulenc Sante, Vitry sur Seine, France; L-652,731 (*trans*-2,5-bis (3,4,5-trimethoxy phenyl) tetrahydrofuran) from Dr J.C. Chabala, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey, U.S.A.;

WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno(3,2-f)(1,2,4-triazolo-(4,3-a)(1-4)-diazepine-2-yl]-1-(4-morpholinyl)-1-propanone) from Dr H. Heuer, Boehringer Ingelheim KG, Ingelheim am Rhein, Germany, BW 755C (3-amino-1-[M-(trifluoromethyl)-phenyl]-2-pyrasoline) was from Wellcome Research Laboratories, Beckenham, Kent; dazmegrel (3-(1H-imidazol-1-yl methyl)-2-methyl-1H-indole-1-propanoic acid) was from Pfizer Central Research, Sandwich, Kent; AH 23848 ([1α(z), 2β,5α]-(±)-7-[5-[[1,1'-biphenyl]-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4 heptenoic acid) was from Dr P.P.A. Humphrey, Glaxo, Ware, Herts; FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid) was from Fisons Pharmaceuticals, Loughborough, Leicestershire; U-46619 (11α,9α-epoxymethano-prostaglandin H₂) was from Dr J. Pike, The Upjohn Company, Kalamazoo, Michigan, U.S.A.

Statistical analysis

Data are presented as the mean ± s.e.mean for *n* lungs and have been analysed with Student's unpaired *t* test. A *P* value of <0.05 was considered statistically significant.

Results

PAF-induced pressor response and protein accumulation in the rabbit lung

As shown in Figure 2, there was little increase in extravascular ¹²⁵I-albumin over the first three cycles as indicated by the residual activity remaining at the end of the intravascular washout cycles (dashed line). A saline infusion had no detectable effect on label retention (Figure 2a). An infusion of PAF (3 nmol ml⁻¹ for 10 min) caused a marked increase in the label remaining at the end of the washout cycles indicating microvascular albumin leakage. Extravascular label continued to rise after stopping the PAF infusion, indicating that leakage persisted. At 60 min, protein accumulation in the collimated area was equivalent to 850 ± 170 µg (*n* = 10). Infusion of lyso-PAF had no effect on albumin accumulation (Figure 6b), (279 ± 27 µg at 60 min, *n* = 4 compared with saline controls 250 ± 33 µg, *n* = 6).

In addition to the enhanced protein leakage, infusion of PAF caused an increase in perfusion pressure. Figure 3 shows the pressor responses plotted against the superimposed ¹²⁵I wash-out curves from the same experiment. The mechanism of this pressor response was investigated further by use of bolus injections of PAF (1 nmol) in the pulmonary circulation (this resulted in pressor responses of shorter duration than those obtained during PAF infusion and therefore allowed several injections to be given in one lung preparation). In 11 experiments the mean increase in perfusion pressure with PAF injection was 10.5 ± 1.3 mmHg. The effects of a number of inhibitors were tested on the pressor response induced by a bolus (1 nmol) injection of PAF. PAF responses were measured before and after drug administration in the same lung with an interval of 30 min between PAF doses. The PAF-induced vasoconstriction was markedly reduced by indomethacin (at 10 µM, 74% inhibition in 2 experiments), the dual 5-lipoxygenase/cyclo-oxygenase, inhibitor BW 755C (at 50 µM, 57% inhibition in 2 experiments) and by the thromboxane synthesis inhibitor, dazmegrel (at 100 µM, 67% inhibition in 4 experiments). Responses were also inhibited by the thromboxane receptor antagonist, AH23848 (at 1 µM, 50% inhibition in 2 experiments). Injections of arachidonic acid (300 nmol) induced matching pressor responses to PAF at 1 nmol and these arachidonic acid-induced responses were totally inhibited by indomethacin, BW755C and dazmegrel (data not shown). However, the pressor responses to PAF and arachidonic acid were not affected by the sulphidopeptide leukotriene antagonist, FPL 55712 or by the H₁ receptor antagonist, mepyramine. The most effective inhibitors of the constrictor action of PAF were the PAF receptor antagonists 48740 RP,

Figure 3 Effects of PAF on perfusion pressure (upper panels) and protein accumulation (lower panels) in sequential 'wash out' cycles (numbered 1–6). During saline infusion there was no increase in pressure and small increments in protein accumulation. Infusion of PAF during 4th cycle caused an increase in perfusion pressure and large increments in protein accumulation (middle panel). These effects of PAF were inhibited by the PAF receptor antagonist, L-652731 (right panel). The traces are representative of four separate experiments.

L-652,731 and WEB 2086, the order of potency being WEB 2086 > L-652,731 > 48740 RP. Complete inhibition of the pressor response was obtained with $1\text{ }\mu\text{M}$ WEB 2086 ($n = 3$ lungs), while greater than 90% inhibition was obtained with $10\text{ }\mu\text{M}$ L-652,731 ($n = 4$ lungs) and $100\text{ }\mu\text{M}$ 48740 RP ($n = 2$ lungs). These results suggest that the constrictor effect of PAF in the pulmonary circulation is mediated partly by a cyclo-oxygenase independent mechanism.

In a series of experiments where protein accumulation was measured, PAF-induced vasoconstriction and protein leakage were almost abolished in the presence of $10\text{ }\mu\text{M}$ L652,731 (Figures 3 and 6).

PAF-induced vascular hyperreactivity

Repeated bolus injections of PAF (1 nmol) at 30 min intervals were found to cause reproducible increases in perfusion pressure in the isolated lung (Figure 4a). However, when injections were made at 1 h intervals then a progressively larger pressor response was observed on each injection (Figure 4b). In three experiments, the mean pressor response on the first injection was $6.0 \pm 1.2\text{ mmHg}$ which increased to $14.3 \pm 1.9\text{ mmHg}$ on the second and to $19.0 \pm 4.0\text{ mmHg}$ on the third, representing an overall increase of 220%. Such increased responsiveness was not observed with injection of histamine ($1\text{ }\mu\text{mol}$) which also induced vasoconstriction in this model (results not shown). To determine whether the PAF-induced vascular hyperreactivity was mediated by cyclo-oxygenase products, the experiment was repeated in the presence of $10\text{ }\mu\text{M}$ indomethacin. As shown in Figure 4c indomethacin blocked the hyperresponsiveness to repeated PAF injections. Similar findings were obtained in two further experiments.

A similar pattern of increased responsiveness as seen with PAF was also evident following injections of the stable thromboxane mimetic drug U-46619 (1 nmol) (Figure 4d); suggesting that the vascular hyperreactivity to PAF is mediated by cyclo-oxygenase products such as endoperoxides and thromboxane A_2 .

Figure 4 Responses to repeated bolus injections of PAF. Injections at 30 min intervals caused reproducible increases in perfusion pressure (a). Progressively greater pressure responses occurred with each injection of PAF at 60 min intervals (b). This augmentation did not occur in the presence of indomethacin (c). A similar pattern of augmented pressure responses was evident with injections of the thromboxane analogue, U-46619 (d). The results are representative of three separate experiments.

Comparison of responses to PAF and U-46619 in the rabbit lung

Figure 5 compares the pressor responses and protein leakage induced by PAF and U-46619. The pressor responses are plotted against the superimposed individual ^{125}I washout curves from the same experiments. In a series of experiments infusion of U-46619 ($0.6 \text{ nmol min}^{-1}$) induced comparable increases in perfusion pressure ($31.6 \pm 6.0 \text{ mmHg}$, $n = 6$) to those observed with 3 nmol min^{-1} PAF ($25 \pm 4.0 \text{ mmHg}$, $n = 10$). However only PAF infusions increased protein accumulation (Figure 6a) indicating that in this model protein leakage is not merely a consequence of increased hydrostatic pressure. Furthermore, these results suggest that endogenous thromboxane A_2 is unlikely to be mediating the PAF-induced protein leakage.

PAF-induced protein leakage with retrograde perfusion of lungs

During perfusion of the lungs via a left atrial cannula the PAF-induced increase in perfusion pressure ($12.9 \pm 2.0 \text{ mmHg}$, $n = 6$) was lower than that observed during forward perfusion (see above). Although background albumin accumulation during retrograde perfusion was marginally higher than during forward perfusion, infusion of PAF resulted in a considerably greater ($P < 0.05$) albumin accumulation than during forward perfusion (Figure 7).

Discussion

PAF is a potent inflammatory mediator with diverse biological actions, including the ability to enhance permeability in microvessels. However, the response of the pulmonary circulation to PAF is poorly understood. It has been suggested that, in rabbit isolated lungs, PAF-induced pulmonary oedema is mediated by thromboxane released from PAF-stimulated platelets (Heffner *et al.*, 1983). In a different species, the guinea-pig, Hamasaki and colleagues (1984) have reported that PAF-induced pulmonary oedema is independent of plate-

Figure 5 Effects of PAF (a) and U-46619 infusion (b) on perfusion pressure (upper panels) and protein accumulation (lower panels). U-46619 caused an increase in perfusion pressure of similar magnitude to that with PAF, without causing protein accumulation. The results are representative of six separate experiments.

Figure 6 Effects of PAF (a) and U-46619 (b) on protein accumulation. (a) Infusion of PAF ($\circ$, 3 nmol min^{-1} , $n = 10$) during the 4th cycle resulted in protein accumulation at 60 min which was significantly greater than control ($\blacktriangle$, $n = 6$) ($P < 0.05$). In the presence of the PAF antagonist, L-652731 ($\bullet$, $10 \mu\text{M}$, $n = 4$), the protein accumulation at 60 min was not significantly different from control values. (b) After infusion of U-46619 ($\circ$, $0.6 \text{ nmol min}^{-1}$, $n = 6$) during the 4th cycle, protein accumulation at 60 min was not significantly different from protein accumulation after infusion of lyso-PAF ($\blacksquare$, 3 nmol min^{-1} , $n = 4$). Each point represents the mean and vertical bars show s.e.mean.

lets. More recently, studies in sheep and rabbit lungs, have suggested that the PAF-mediated oedema is a result of vasoconstriction and is not due to any effect on microvascular permeability (Bolin *et al.*, 1986; Burhop *et al.*, 1986).

In order to investigate the effects of PAF on the pulmonary microvasculature we developed a novel technique for measuring changes in pulmonary vascular resistance and directly quantifying protein leakage under constant flow conditions in rabbit isolated perfused lungs. We have demonstrated that PAF is a potent vasoconstrictor in rabbit lungs perfused with cell-free Tyrode solution. The PAF-induced pressor response was substantially suppressed by cyclo-oxygenase and thromboxane synthetase inhibitors suggesting that thromboxane and/or endoperoxides contribute to the vasoconstriction. This effect of PAF appears to be receptor-mediated, as three selective antagonists, i.e. 48740 RP (Sedivy *et al.*, 1985), L-652,731 (Hwang *et al.*, 1985) and WEB 2086 (Casals-Stenzel *et al.*, 1987), effectively inhibited the vasoconstriction.

When injections of PAF were repeated at 60 min intervals and pressor responses were recorded, vascular hyperreactivity was detected. With intervals of 30 min between injections no changes in the magnitude of responses were seen, possibly because of a compensating tachyphylactic effect. Indeed, repeated injections of PAF at 15 min intervals resulted in progressively smaller pressor responses (results not shown). The increased vascular responsiveness to PAF seen with 60 min

Figure 7 Protein accumulation during retrograde and forward perfusion of PAF 3 nmol min^{-1} and saline/BSA. The closed symbols joined by solid lines represent responses to retrograde perfusion of PAF ($\blacksquare$, $n = 6$) and saline ($\blacktriangledown$, $n = 4$) whilst the open symbols joined by dashed lines represent responses to forward perfusion of PAF ($\square$, $n = 4$) and saline ($\triangledown$, $n = 3$). The protein accumulation at 60 min during retrograde perfusion of PAF was statistically significantly greater than during forward perfusion ($*P < 0.05$). Each point represents the mean and vertical bars show s.e.mean.

intervals was abolished by indomethacin, suggesting that it is mediated by cyclo-oxygenase products, probably thromboxane since U-46619 also induced the phenomenon. Although PAF-induced airway smooth muscle hyperresponsiveness may be a pathogenic mechanism in clinical asthma, the significance of PAF-induced vascular hyperreactivity is less clear.

As well as being a potent vasoconstrictor, PAF caused increased albumin accumulation in the lungs. To determine whether this effect was secondary to changes in hydrostatic pressure or due to a direct effect on vascular permeability, the stable thromboxane-mimetic, U-46619, was infused into lungs. U-46619 caused comparable increases in perfusion pressure to

those occurring with PAF but U-46619 failed to induce protein leakage. These results suggest that the PAF-induced protein leakage was due to an increase in microvascular permeability and not a direct consequence of increased hydrostatic pressure. In addition, these results suggest that although thromboxane endoperoxides contribute to the PAF-induced pulmonary vasoconstriction, they are not involved in the PAF-induced increase in pulmonary microvascular permeability.

During retrograde perfusion, PAF caused both vasoconstriction and protein leakage. However, the extent of the protein leakage was greater than during forward perfusion (despite a smaller increase in perfusion pressure). These findings could be explained if the PAF-induced vasoconstriction occurred predominantly in the pulmonary arterioles, i.e. distal to the site of leakage in the case of retrograde perfusion. Thus, the hydrostatic pressure within the microvessel lumen would be expected to be higher at the site of leakage during retrograde perfusion.

In the present experiments the lungs were perfused with Tyrode solution to eliminate blood elements from the pulmonary circulation. Thus, the vasoconstrictor and permeability effects of PAF probably result from a direct action on vascular smooth muscle and endothelial cells respectively.

In summary, PAF is a potent vasoconstrictor and mediator of increased microvascular permeability in rabbit isolated perfused lungs. The pulmonary vasoconstrictor response is partly mediated by cyclo-oxygenase products probably endoperoxides and/or thromboxane A_2 . Our results suggest that thromboxane A_2 or endoperoxides are unlikely to be mediating the protein leakage due to PAF and that protein leakage is due to an effect of PAF on the endothelium i.e. leakage is not secondary to vasoconstriction.

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