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EFFECT OF PLATELET ACTIVATING FACTOR ON FORMATION AND COMPOSITION OF AIRWAY FLUID IN THE GUINEA-PIG TRACHEA

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SUMMARY

1. We studied the effect of platelet activating factor (PAF) on leakage of albumin, and secretion of fucose (a marker for mucus glycoprotein) and protein into the tracheal lumen of the guinea-pig isolated *in situ*, and on bioelectric properties and fluxes of mannitol *in vitro*. We also studied the effect of PAF on mucus secretion in human bronchi *in vitro*.

2. In guinea-pig, intravenous PAF markedly increased the luminal concentration of protein but did not significantly increase fucose concentrations. Increased albumin leakage (274% above controls at a dose of 50 ng/kg PAF) was associated with the increased luminal content of protein (248% above controls at the same dose of PAF).

3. Leakage of albumin was maximal 10 min after PAF, was significantly reduced by 20 min and had returned to baseline by 30 min. This pattern of leakage could be repeated with successive administrations of PAF.

4. PAF induced small but significant biphasic changes in bioelectric properties *in vitro*. The initial response was rapid in onset and characterized by maximal increases in short-circuit current (I_{sc}) of 6·5% above controls at 7·5 min and in conductance (G) of 7% at 20 min. Both responses were blocked by the PAF receptor antagonist WEB 2086. Amiloride blocked the increase in I_{sc}. Permeability of the tissue to mannitol (P_{mann}) was unaltered. The delayed response was characterized by maximal increases in I_{sc} and G of 10% above controls at 60–90 min which were not significantly affected by WEB 2086 or amiloride. P_{mann} was increased by 38% at 90 min.

5. PAF increased fucose secretion in human bronchi *in vitro*.

6. Lyso-PAF *in vitro* caused changes similar to those induced by PAF on bioelectric properties and mucus secretion, but had no significant effects *in vivo*.

7. Light microscopy showed no evidence of epithelial disruption in animals given intravenous PAF at a dose causing significant albumin transudation.

8. We conclude that PAF increases the protein content of guinea-pig tracheal fluid principally by inducing plasma leakage rather than mucus secretion and that the small changes in ion transport and epithelial conductance may reduce the tendency to epithelial disruption during plasma leakage.

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INTRODUCTION

The shallow layer of fluid which overlies the epithelium of the airways is normally protective; it provides a physical barrier to inhaled irritants and particles, and facilitates the ciliary transport and removal of entrapped foreign materials. Airway fluid comprises a number of components including water, salts, enzymes, mucus glycoproteins and other proteins which in the correct proportions confer its protective characteristics (Widdicombe, 1989; Rogers, Alton & Barnes, 1990). For example mucus comprises only 1% of the fluid but is essential to the viscoelastic properties necessary for mucociliary clearance. Epithelial transport of ions is thought to contribute to the hydration of the airway by affecting water movement. The contribution of serum, extravasated from bronchial microvessels, to the fluid in healthy individuals is relatively unknown but may provide enzymes and mediators necessary for airway homeostasis. However, changes in amount and composition may cause airway fluid to cease to be protective and, instead, contribute to disease. For example, in asthma, mucus hypersecretion (Richardson & Somerville, 1988), plasma exudation (Chung, Rogers, Barnes & Evans, 1990) and defective epithelial control of the osmolality and ion concentration of airway fluid (Hogg & Eggleston, 1984) have all been suggested as contributing to pathogenesis. In the present study our aim was to integrate changes in mucus secretion, plasma exudation and ion transport in the guinea-pig trachea in response to the inflammatory mediator platelet activating factor (PAF). We chose PAF because it has been implicated in the pathogenesis of asthma (Barnes, Chung & Page, 1988) and may have effects on formation and composition of airway fluid. We used an *in situ* preparation of the trachea in the anaesthetized guinea-pig to study mucus secretion and plasma transudation using fucose as a specific marker for mucus glycoprotein, radioiodinated albumin as a specific marker for plasma leakage, and protein as a non-specific marker for secretion (Rogers, Turner, Marriott & Jeffery, 1987; Rogers, Boschetto & Barnes, 1989). Changes in ion transport were studied indirectly using bioelectric measurements of the trachea *in vitro*. We also determined the effect of PAF on mucus secretion in human bronchi *in vitro* which has a greater proportion of submucosal gland compared with the guinea-pig.

METHODS

Male, outbred Dunkin–Hartley guinea-pigs were premedicated with diazepam (6·5 mg/kg I.P.), and anaesthetized with fentanyl citrate and fluanisone (Hypnorm, 2·4 ml/kg I.M.).

In situ tracheal preparation

The *in situ* tracheal preparation for collection of airway secretions in small mammals has been described in detail (Rogers *et al.* 1987, 1989). Anaesthetized guinea-pigs (300–450 g body weight) were laid supine on a homeothermic blanket which maintained body temperature at 37 °C and the jugular veins were exposed for injection of drugs. The extrathoracic trachea was cannulated at either end for superfusion with 4 ml saline (0·9% (w/v) NaCl in distilled water), initially at 37 °C and pregassed with 5% CO_2 in O_2. The fluid was circulated through the trachea and the perfusion tubing at a rate of 1 ml/min via peristaltic pumps. The animal breathed spontaneously through a third cannula inserted into the lower trachea, pointing caudally. At unit time intervals (see below) the perfusion circuit was emptied, the saline (containing secretions and plasma) collected, the circuit refilled and further collections made.

Protocol and drug administration

To remove any blood remaining after surgery the perfusion circuit was washed with saline for 15 min after which six collections were taken. Preliminary studies indicated that equilibration and collection periods were optimal using an initial three collections taken after 15 min perfusion periods followed by a further three (collections 4–6) after 30 min periods. A 1 min interval during which the trachea remained unfilled with saline preceded each perfusion period.

In baseline studies, no drugs were administered. To determine the effect of PAF (1–200 ng/kg) or lyso-PAF (100 ng/kg) on protein and fucose secretion, each was injected I.V. at the start of collection 5 which allowed comparison with the basal values at collection 4 and an assessment of duration of effect into the following collection, number 6. For comparison, the effect of methacholine (100 mg/kg I.V.) on secretion was also determined. Collected samples were rapidly frozen by immersion in liquid nitrogen and were stored at −70 °C prior to analysis.

The effect of PAF or lyso-PAF (50 ng/kg I.V. each) on albumin transudation was investigated in different animals to those used for secretion. ^{125}I-labelled human serum albumin (HSA; 3 μCi/kg) was injected I.V. after the initial wash-out period. To study the duration and tachyphylaxis of the leakage response, 18 × 10 min collection periods were used. PAF (50 ng/kg) was injected at the start of collections 5, 10 and 15 (i.e. each separated by 50 min). Radioactivity in the collected fluid samples was determined directly (Wallace 80000 Gamma Sample Counter, LKB, Milton Keynes) and background (15–18 c.p.m.) subtracted from each sample.

In vitro measurement of bioelectric properties

Tracheae were removed from anaesthetized guinea-pigs (approximately 1 kg body weight and drained of blood by incising the left ventricle) and transferred to a dish containing KH buffer (see below). The tracheae were opened along the posterior membranous surface with scissors, and each one cut transversely into four equal portions which were mounted as sheets between the two halves of Ussing chambers of aperture 0·28 cm^2. All four pieces of tissue were mounted within 20 min of excision of the trachea from the animal. The tissue was bathed with 9 ml Krebs–Henseleit (KH) saline buffer solution, pH 7·4 after oxygenation, warmed to 37 °C and circulated by gas lift pumps of 5% CO_2 in O_2. The composition of the KH solution was (mM): NaCl, 120·8; KCl, 4·7; KH_2PO_4, 1·2; $MgSO_4.7H_2O$, 1·2; $NaHCO_3$, 24·9; $CaCl_2$, 2·4; glucose, 5·6. We studied bioelectric properties in a manner similar to that described previously (Welsh & Widdicombe, 1980; Knowles, Murray, Shallal, Askin, Ranga, Gatzy & Boucher, 1984). Potential difference (PD) and short-circuit current (I_{sc}) were measured using 1 M-KCl and NaCl–agar bridges respectively. Tissues were studied at open circuit except for 300 ms recordings of I_{sc} using an automatic voltage clamp (World Precision Instruments, New Haven, USA), and conductance (G) calculated from Ohm's law. Bronchi with a baseline $G > 14$ mS/cm^2 were not included in the analysis.

Tissue was equilibrated for approximately 45 min before drugs were added to the mucosal chamber. PAF and lyso-PAF were freshly prepared on each day of experimentation from the lyophilized powder in 0·35% bovine serum albumin (BSA) (w/v) in KH solution at a concentration of 10^{-3} M. Ninety microlitres was added to the fluid in the chambers to give a final concentration of 10^{-5} M. An equivalent volume of BSA solution was also tested and compared with no treatment. Drug addition to the four chambers derived from each animal was rotated to avoid pre-incubation bias. The effect of the drugs on bioelectric properties was followed for 2 h after addition. In certain experiments the PAF receptor antagonist WEB 2086 (10^{-4} M) or the sodium channel blocker amiloride (10^{-5} M) were added to the mucosal surface 10 min prior to PAF or lyso-PAF.

The contribution of paracellular permeability to changes in conductance was determined in separate animals using mannitol fluxes (Stutts, Schwab, Chen, Knowles & Boucher, 1986). After equilibration, 7·5 μCi [^{14}C]mannitol (Amersham International plc, Little Chalfont) was added to the fluid on the serosal side of the tissue (source). At this concentration of mannitol 95% of the values for duplicate samples were within 1–2% of the mean count. Four 1 ml samples of the KH solution were removed from the mucosal side of the tissue (sink) at 15 min intervals before no treatment or addition of BSA solution, PAF or lyso-PAF. A further 8 × 1 ml sink samples were taken over the following 2 h. One millilitre cold Krebs solution was returned to sink to maintain constant volume. In addition, a 50 μl sample was taken from source once during the baseline period and once during the period after drug administration. This volume was not replaced. Source samples were diluted to 1 ml in KH solution to allow for quench. Samples were mixed with 10 ml

scintillant (Ultima Gold, Canberra Packward Ltd, Pangbourne) and counted in a liquid scintillation analyser (2200CA Tri-Carb, Canberra Packard Ltd, Pangbourne). The uniformity of sample quench was monitored by the external standard channels ratios method (see Stutts *et al.* 1986).

Mucus secretion in human bronchi in vitro

Main bronchi macroscopically uninvolved in the tumour were obtained at resection for lung carcinoma and transported to the laboratory in ice-cold KH solution pregassed with 5% CO_2 in O_2 where they were cleared of adventitious tissue and kept overnight in fresh ice-cold oxygenated KH solution. The bronchi were opened longitudinally and cut into sheets which were mounted flat between the two halves of Ussing chambers (see above) so that the tissue separated the chambers into mucosal (i.e. mucus-producing) and serosal sides (Pack, Williams, Phipps, Richardson & Rich, 1984; Rogers & Barnes, 1989). Use of chambers of different cross-section, either round with an internal area of 1·44 cm^2 or rectangular with internal dimensions of 7 × 16 mm, enabled experiments in two to four chambers per patient. Forty-eight pieces of bronchi from seventeen patients were used. The fluid from both sides of the chamber was drained at the end of each 30 min incubation period and replaced by fresh Krebs solution. Fluid from the mucosal side (containing secretions) was plunged into liquid nitrogen and stored at −70 °C before analysis for mucus markers (see below). After equilibration for 1·5 h, KH solution alone was replaced by KH containing PAF, lyso-PAF (or vehicle at a dilution equivalent to 10^{-5} M-PAF), or methacholine (all at 10^{-5} M) on both sides of the chamber for incubation periods 2, 6 and 10 after baseline. Addition of the same drug was not repeated in any tissue and the order of addition was randomized.

Chemical analysis

The sample preparation and chemical analysis for mucus have been described in detail previously (Rogers *et al.* 1987). Frozen perfusion fluid from guinea-pig trachea or human bronchi was freeze dried and the proteins, including glycoproteins in the lysophilized residues precipitated in 85·5 (v/v) ethanol in distilled water at −20 °C for 16 h. Centrifuged pellets were reconstituted in sodium hydroxide, dispersed by ultra-sonication and aliquoted for analysis of fucose (Gibbons, 1955) and protein (Lowry, Rosebrough, Farr & Randall, 1951). The standard curves for each assay were linear over the range of concentrations determined in the present study with coefficients of variation (Armitage & Berry, 1987) for duplicate samples less than 7·5%. The minimum detectable concentrations for fucose and protein were of the order of 0·1 and 0·6 μg/collection respectively.

Histology

A 3 mm section of the lower trachea was excised from three guinea-pigs 10 min after administration of PAF (50 ng/kg I.V.) and immersed in fixative (2·5% glutaraldehyde in 0·1 M-sodium cacodylate buffer solution) for a minimum of 4 h and stored in cacodylate buffer at 4 °C. The specimens were post-fixed in 1% osmium tetroxide, dehydrated in ascending concentrations of methanol and embedded in epoxy resin (Araldite). Transverse sections 1 μm thick were stained with 1% Toluidine Blue in 1% borax and observed by light microscopy using a ×40 objective. Five different levels within each tissue block, separated by 30 μm, were studied for each animal with each level consisting of four to six consecutive sections per slide.

Drugs, chemicals and solutions

The drugs and chemicals used were diazepam (Phoenix Pharmaceuticals Ltd, Gloucester), Hypnorm (Janssen Pharmaceuticals, Oxford), C_{16}-PAF (MW 523·7) and C_{16}-lyso-PAF (Novabiochem, Nottingham); bovine serum albumin (BSA) and acetyl-β-methylcholine chloride (methacholine) (Sigma Chemical Co Ltd, Poole), 0·9% saline (Travenol Laboratories, Thetford), ^{125}I-labelled HSA (specific activity 2·5 μCi/mg) and D-[^{14}C]mannitol, 50 μCi/ml in absolute ethanol (Amersham International plc, Little Chalfont). All other chemicals were purchased at 'Analar' grade from BDH Chemicals, Poole. PAF and lyso-PAF (stock solutions of 1 mg/ml in absolute ethanol at −70 °C) were diluted in either KH or saline containing 0·35% BSA (w/v). Methacholine was dissolved directly in saline. Fresh solutions of drugs were prepared on each day of experimentation to give injection volumes of 1 ml/kg. Amiloride and WEB 2086 were generous gifts of Merck, Sharpe & Dohme Research Laboratories (Pennsylvania, USA) and Boehringer Ingelheim (FRG) respectively.

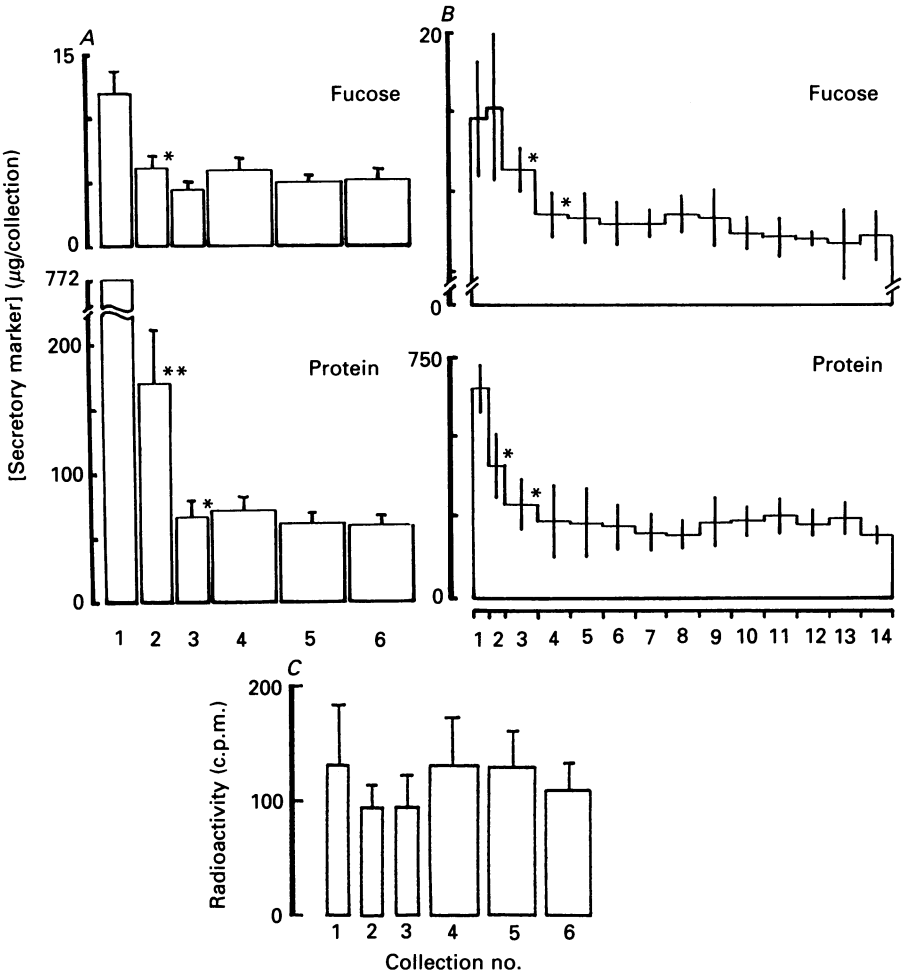

Fig. 1. Baseline secretion. *A*, superfusates collected from the tracheal lumen isolated *in situ* in anaesthetized guinea-pigs were analysed for the secretory markers fucose and protein. Collections 1–3 were taken after 15 min perfusion periods and 4–6 after 30 min periods. *B*, Krebs–Henseleit solution bathing the mucosal surface of human main bronchi mounted in Ussing chambers was collected and analysed for secretory markers. Collections 1 and 2 were taken after 15 min incubation periods and 3–14 after 30 min periods. *C*, superfusates collected as described in *A* were analysed for radioactivity in animals injected intravenously before collection 1 with ^{125}I-labelled human serum albumin. The height of each histogram represents the mean of five animals (*A* and *C*; vertical bars = S.E.M.) or pieces of bronchus (*B*, vertical bars = range). In *A* and *B*, * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ compared with previous collection. In *C* there were no significant differences between consecutive collections.

Data analysis

Data did not approximate a Gaussian distribution but showed positive skew and non-parametric analyses were applied (Armitage & Berry, 1987). Kruskal–Wallis analysis was used initially to determine the probability of differences between groups. The effects of drugs on secretion of mucus markers or of albumin leakage were assessed by comparing the concentrations of mucus or plasma

marker in the collections before and after administration, with significance tested using the Wilcoxon sign-ranked sum test (two-tailed). Concentrations of secretory markers *in vitro* were calculated as micrograms per square centimetre of tissue (to standardize for chambers of different cross-section) per collection. Because of variability in basal mucus secretion (presumably related

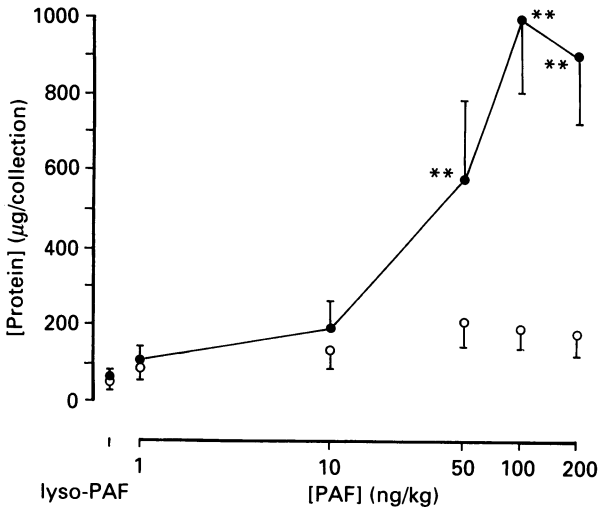

Fig. 2. Effect of intravenous platelet activating factor (PAF) or lyso-PAF (100 ng/kg) on the concentration of protein in tracheal perfusates collected *in situ* in anaesthetized guinea-pigs. PAF increased protein concentrations in the 30 min collection period immediately after injection (●) but not in the subsequent 30 min period (○); ** represents a significant change ($P < 0{\cdot}01$) compared with basal secretion. Each point is the mean of six animals (vertical bars = S.E.M.).

to the amount of submucosal gland present), the response by human bronchi was expressed as percentage change compared to the previous collection. For the bioelectric measurements, there was no significant difference between responses during the period of no treatment ($n = 5$) and responses to BSA solution ($n = 6$) and the data were combined. To correct for variability in baseline (for example the lowering in I_{sc} after amiloride), values for each animal were converted to a change (percentage or absolute) compared with their basal value and the mean for the group calculated. Permeability coefficients for mannitol fluxes (P_{mann}, cm/s) at each sampling period were calculated from conventional formulae (Stutts *et al.* 1986). For all analyses, the null hypothesis was rejected at $P < 0{\cdot}05$. The dose of PAF causing a half-maximal increase in the luminal concentration of protein *in situ* (ED_{50}) was calculated using a curve-fitting program (Graph Pad, H. J. Motulsky, ISI Software, Philadelphia 19104, USA) with a goodness of fit of the sigmoid curve of 0·97. Data in Results are means and S.E.M.

RESULTS

The profiles of basal secretion of fucose and protein and of leakage of albumin in either guinea-pig trachea or human bronchi are shown in Fig. 1.

Guinea-pig tracheal secretion in situ

Mean basal values of fucose and protein in tracheal perfusion fluid were 6 ± 1 and 72 ± 11 μg/30 min collection respectively. PAF had no significant effect on fucose concentrations (mean maximal increase of 20 ± 28% at a dose of 50 ng/kg) but increased the concentration of protein in the fluid in a dose-related manner (Fig. 2)

with significant mean increases of 274±75% above basal at the ED_{50} of 50 ng/kg and a mean maximal increase of 1076±75% at 100 ng/kg. The increase lasted only for the duration of one 30 min collection period with concentrations in the following collection not different to baseline. Lyso-PAF (50 ng/kg) had no significant effect on

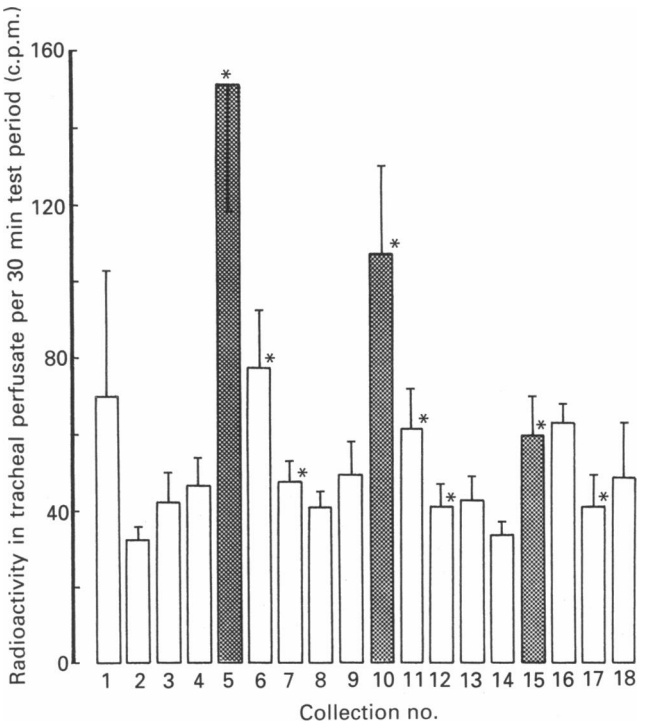

Fig. 3. Effect of repeated intravenous injection of platelet activating factor (PAF) on plasma transudation into the tracheal lumen isolated *in situ* in anaesthetized guinea-pigs. ^{125}I-labelled human serum albumin was injected 1 min before collection 1 and perfusion fluid collected every 10 min. PAF (50 ng/kg) was injected at the beginning of collections 5, 10 and 15 (indicated by stippled columns). The height of each histogram represents the mean of six animals (vertical bars = S.E.M.). * $P < 0.05$ compared with previous collection.

secretion of either marker. Methacholine (100 ng/kg) significantly ($P < 0.05$) increased secretion of fucose by 63% (±15%, $n = 6$) but not of protein (40±18%, $n = 6$).

Transudation of ^{125}I-labelled albumin

PAF (50 ng/kg I.V.) increased the mean radioactive count in the 30 min samples from 271±59 c.p.m. ($n = 6$) to 959±281 c.p.m. ($n = 6$); a mean increase of 248±75% ($n = 6$) above baseline. Lyso-PAF had no significant effect. Reducing the duration of the collection periods demonstrated maximal leakage of radioactivity during the 10 min period after administration (Fig. 3). Leakage, although reduced,

was still apparent during the following 10 min but had returned to baseline by the third 10 min period following administration. The response to PAF also demonstrated tachyphylaxis (Fig. 3). However, although reduced, there was still significant leakage of radioactivity in response to a second and third administration of PAF at intervals of 50 min.

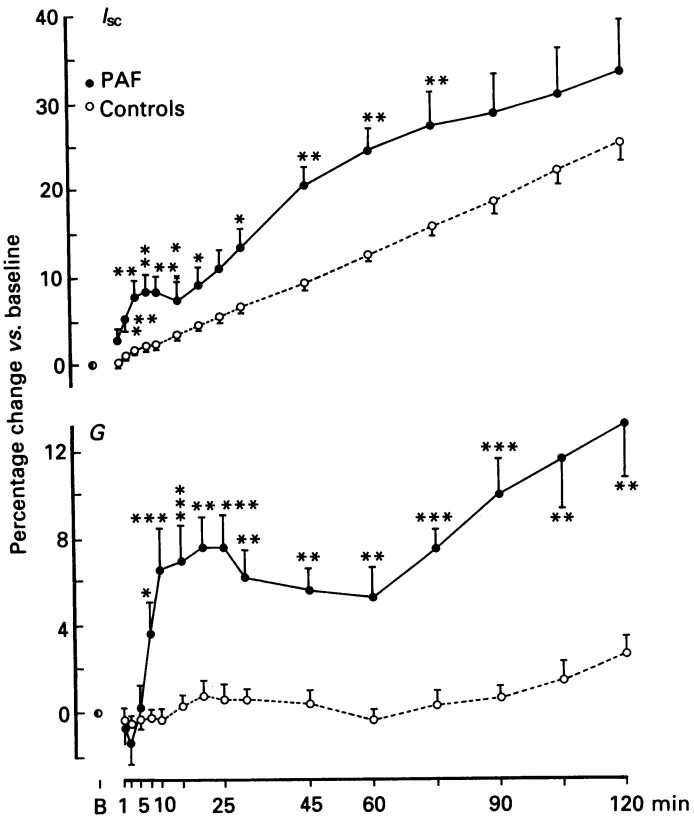

Fig. 4. Time course of platelet activating factor (PAF, 10^{-5} M) on short-circuit current (I_{sc}) and conductance (G) in guinea-pig trachea *in vitro*. Each point represents the mean percentage change (vertical bars = S.E.M.) for each group of animals after PAF (●, n = 10) or control intervention (○, n = 11) compared with their own baseline values (B; i.e. 0% change): mean basal I_{sc} and G = 55 μA/cm^2 and 8 mS/cm^2 respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with corresponding control value.

Bioelectric properties and mannitol fluxes

Mean basal PD, I_{sc} and G in fifty-one pieces of trachea from fourteen guinea-pigs were 7.0 ± 0.3 mV, 54.6 ± 2.3 μA/cm^2 and 8.1 ± 0.2 mS/cm^2 respectively. Mean basal P_{mann} was $3.6 \times 10^{-7} \pm 0.2$ cm/s (n = 23 pieces of tissue from six animals). Baseline values of I_{sc} increased steadily with time whereas values for G did not (Fig. 4). PAF (10^{-5} M mucosally over 120 min) induced small but significant biphasic increases in I_{sc} and G above baseline (Fig. 4). The initial response was characterized by maximal increases in I_{sc} of 6.5% at 7.5 min and in G of 6.9% at 20 min. P_{mann} was not

significantly altered by no treatment or BSA solution, nor by PAF during this time. The changes in I_{sc} and G were inhibited by the PAF receptor antagonist WEB 2086 by approximately 64% each (Fig. 5). WEB 2086 had no significant effect on baseline bioelectric parameters. Amiloride reduced baseline PD and I_{sc} by approximately

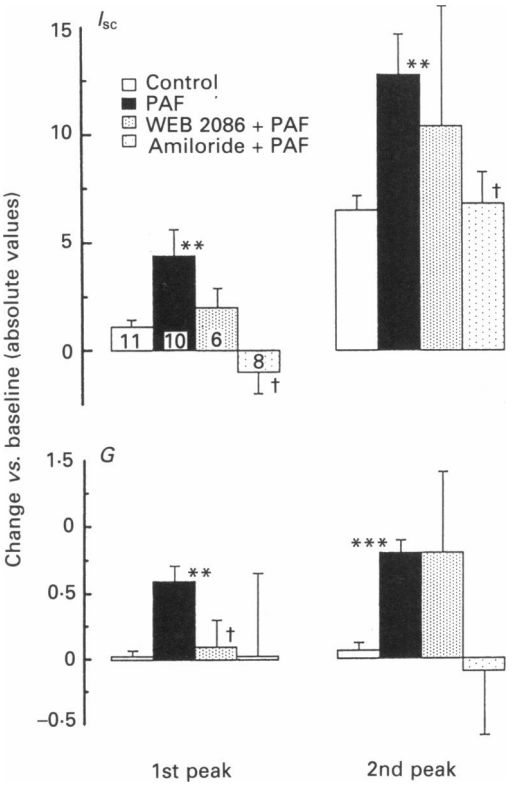

Fig. 5. Effect of WEB 2086 (10^{-4} M) or amiloride (10^{-5} M) on increases in short-circuit current (I_{sc}, μA/cm^2) and conductance (G, mS/cm^2) induced by PAF (10^{-5} M). Each histogram represents mean peak response (vertical bars = S.E.M., number of tissues indicated) for I_{sc} at 7·5 and 60 min and for G at 20 and 90 min after PAF. ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ compared with control, † $P < 0{\cdot}05$ compared with PAF.

37% ($\pm$7%, $n = 8$) with a small decrease in G (7$\pm$2%, $n = 8$). Following pre-treatment with amiloride PAF induced an initial transitory rise in I_{sc} of 6·5% above baseline at 2·5 min which was followed by complete loss of the maximal response at 7·5 min. Amiloride had no significant inhibitory effect on PAF-induced changes in G although, unlike the I_{sc} response, the response was variable in that five tissues exhibited inhibition and three did not. The variability did not correlate with the initial PD, I_{sc} or G nor with the percentage change in these parameters with amiloride ($r < 0{\cdot}62$, n.s., $n = 8$).

The delayed response was characterized by maximal increases in I_{sc} and G of 10% above controls at 60–90 min (Fig. 4). P_{mann} was significantly ($P < 0{\cdot}001$) increased above baseline by 27% at 60 min and by 38% at 90 min (Fig. 6). The increases in I_{sc} and G were not inhibited by WEB 2086 or by amiloride (Fig. 5). Lyso-PAF caused

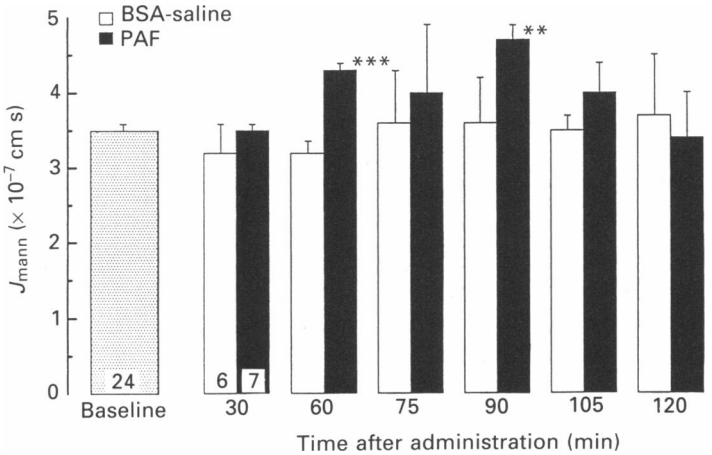

Fig. 6. Effect of platelet activating factor (PAF) or BSA-saline on mannitol fluxes (J_{mann}) across guinea-pig trachea *in vitro*. Baseline rates of flux were taken before addition of drugs. The height of each histogram represents the mean flux (vertical bars = S.E.M.) for the number of animals indicated in the columns. ** $P < 0.01$, *** $P < 0.001$ compared with value for BSA-saline at equivalent time point.

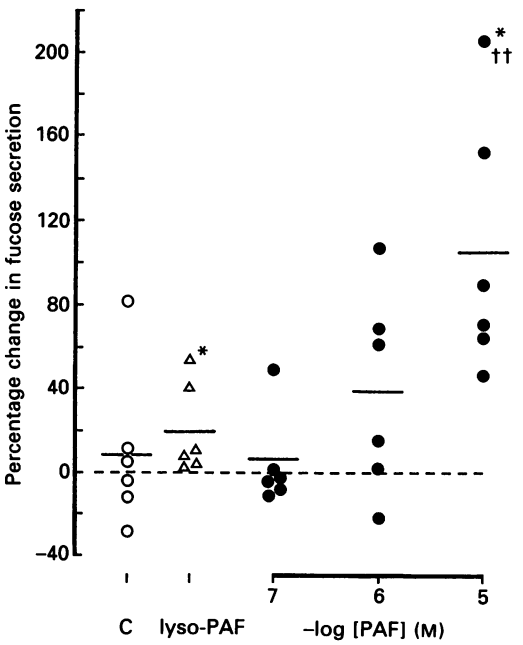

Fig. 7. Effect of platelet activating factor (PAF) on fucose secretion in human bronchi *in vitro*. Each point is the percentage change in fucose concentration in a 30 min incubation sample in the presence of control vehicle (C), lyso-PAF or PAF compared with the preceding sample without drug (dashed line). Horizontal lines, mean percentage change. Significant change: * $P < 0.05$; †† $P < 0.01$ compared with control.

initial increases in I_{sc} and G which were similar to those induced by PAF and which were blocked by WEB 2086. There was no secondary change in I_{sc} whereas both G and P_{mann} were increased.

Mucus secretion in human bronchi

Basal concentrations of fucose and protein in the KH samples were $9{\cdot}4 \pm 1{\cdot}4$ ($n = 46$) and 238 ± 11 ($n = 44$) μg/30 min collection. The highest concentration of PAF tested (i.e. 10^{-5} M) increased secretion of fucose (by $105 \pm 12\%$) (Fig. 7) but had no significant effect on protein (increase of $23 \pm 12\%$). An equivalent concentration of lyso-PAF also caused a small but significant increase in fucose secretion ($20 \pm 10\%$). Methacholine (10^{-5} M) significantly ($P < 0{\cdot}05$) increased secretion of fucose by 103% ($\pm 30\%$, $n = 13$) and protein by 75% ($\pm 42\%$, $n = 11$).

Histology

No pathological changes were observed in the tracheae of three guinea-pigs injected with PAF. The epithelium was intact and there was no evidence of oedema, either intercellularly or associated with the underlying connective tissue.

DISCUSSION

Effect of PAF on albumin leakage into the guinea-pig trachea

PAF increases plasma exudation into guinea-pig airway tissue (Evans, Chung, Rogers & Barnes, 1987; O'Donnell & Barnett, 1987) and, at high doses applied topically to the mucosa, induces plasma leakage into the tracheal lumen (Erjefält & Persson, 1989). In the present study we gave PAF I.V. to reproduce the finding of PAF-like activity in blood of asthmatics (Nakamura, Morita, Kuriyama, Ishihara, Ito & Miyamoto, 1987). High doses of PAF I.V. (i.e. 236 ng/kg) compromise breathing and induce pulmonary oedema (Erjefält & Persson, 1989) whereas the ED_{50} for protein leakage of 50 ng/kg did not induce adverse systemic effects. This dose is minimally effective for plasma leakage into tissues (Evans *et al.* 1987) and converts to a blood concentration of 1·3 nM-PAF based on a blood volume for guinea-pigs of 75 ml/kg (Archer, 1965).

Continuous sampling of tracheal superfusates showed that luminal leakage of albumin in response to PAF was quick in onset, of short duration and, although reduced with subsequent administrations, was repeatable. O'Donnell & Barnett (1987) also found no apparent tachyphylaxis of tissue leakage in response to PAF.

In contrast to knowledge of mechanisms underlying leakage of plasma out of bronchial microvessels (Chung *et al.* 1990), the mechanisms involved in the appearance of plasma in the airway lumen are less well understood. Albumin is actively transported across the whole trachea *in vitro* of ferret (Webber & Widdicombe, 1989) and rabbit (Price, Webber & Widdicombe, 1990). In the air-filled ferret trachea, basal transport rate of fluorescent BSA of 0·2 μg/min was increased to 6·8 μg/min by 200 μM-salbutamol. Basal transport in the equivalent fluid-filled trachea was 4·7 μg/min. In the fluid-filled rabbit trachea, basal rate of 2·0 μg/min was increased to 17·8 μg/min by 100 μM-salbutamol. Assuming I.V. radiolabelled HSA is handled similarly to the animal's, albumin transport rates in the present

experimental system may be calculated from the following: specific activity of HSA and amount injected (see Methods), the blood volume of guinea-pigs (see above) and its albumin concentration (26 g/l; Miturka & Rawnsley, 1979), the d.p.m. (for ^{125}I, c.p.m. = d.p.m.) in the collections under basal and PAF-stimulated conditions (see Results); and the equation 1 Ci = $2{\cdot}22 \times 10^{12}$ d.p.m. (Segel, 1976). Solving for a 350 g guinea-pig, basal transport was 8·0 μg/min and increased to 28·3 μg/min in response to PAF (50 ng/kg). The mean internal surface area of the tracheal segment used herein was 0·85 cm^2 ($\pm$0·05 cm^2, $n = 5$) compared with that of 7·5 cm^2 for a whole rabbit trachea of similar body weight to those used by Price *et al.* (1990). Accounting for airway size, our values in guinea-pig approximate a basal albumin transport in rabbit trachea of 71 μg/min rising to 250 μg/min after PAF. The higher transport rates compared to those in rabbit or ferret (Webber & Widdicombe, 1989; Price *et al.* 1990) may be due to differences between the *in vitro* and *in vivo* preparations, the initial concentration of albumin external to the tracheal lumen, or to differences in transport of radiolabelled HSA *versus* fluorescent BSA. In the absence of the use of diffusion markers, we do not know whether the appearance of albumin in the tracheal perfusates is active or not. The data of Webber & Widdicombe (1989) indicate that basal transport may be active. In contrast, the recent observation by Webber (1990) that PAF did not increase albumin transport across the ferret whole trachea *in vitro* suggests that PAF-induced transport in the present study was due primarily to passive diffusion.

Effect of PAF on bioelectric properties of guinea-pig trachea

PAF has been detected in bronchoalveolar lavage (BAL) of asthmatics at a concentration of 0·9 nM (Court, Goadby, Hendrick, Kelly, Kingston, Stenton & Walters, 1987). This value may be an underestimate because of loss of activity in the acetylation procedure generating PAF *ex vivo* from plasma lyso-PAF, and the lack of allowance for dilution factors in the BAL. The presence of PAF at the mucosal surface might affect ion transport across the epithelium and we studied the effect of PAF on bioelectric properties and permeability to mannitol. We used open-circuit conditions to simulate conditions *in vivo* where a transepithelial PD will affect movement of charged molecules. PAF (10^{-5} M, for comparison with effects on mucus secretion *in vitro*) induced initial small but significant increases in I_{sc} and G which were of short duration (10–15 min) and were blocked by the specific PAF antagonist WEB 2086 (Casals-Stenzel, Maucevic & Weber, 1987) indicating that the responses were mediated via specific PAF receptors. Mannitol permeability did not change during the initial response. Whether this represents a change in paracellular conductance not large enough to be detected by changes in P_{mann} or whether passage of mannitol through the relatively thick tracheal tissue did not allow short-lived changes to be seen cannot be determined from the present study.

Amiloride markedly decreased baseline I_{sc} which is in accordance with previous data which indicates that the guinea-pig tracheal epithelium is principally Na^+-absorbing (Boucher, Narvarte, Cotton, Stutts, Knowles, Finn & Gatzy, 1982). Following amiloride pre-treatment there was a rapid very short-lived increase in I_{sc} in response to PAF followed by complete inhibition suggesting that the early response to PAF comprised a brief secretion of Cl^- followed by Na^+ absorption. The

lack of inhibition of G indicates that changes in transcellular conductance are poorly reflected in overall measurement of tissue conductance.

A delayed and prolonged response to PAF was also seen, with neither increases in I_{sc} nor G inhibited by WEB 2086 or amiloride indicating that the effect was neither PAF-receptor specific nor related to transcellular Na^+ absorption. Mannitol permeability was increased which suggests that increased paracellular permeability contributed to the increased G.

We do not know whether similar changes in epithelial bioelectric properties in response to PAF occur *in vivo* nor what the significance of small changes might be. However, Welsh, Widdicombe & Nadel (1980) have shown in canine trachea *in vitro* that, under open-circuit conditions, a 30% increase in PD in response to aminophylline is associated with a greater than 200% increase in fluid movement across the epithelium.

The steady upward shift in the control I_{sc} over the period of the experiment was not associated with an increased G, at least not over the first 90 min after addition of drugs to other tissues (at least 2·25 h after mounting). The increasing I_{sc} is probably due to increasing activity of the basolateral Na^+–K^+-ATPase which presumably contributes predominantly to the magnitude of the I_{sc} in this Na^+-absorbing epithelium. The baseline G of the guinea-pig trachea *in vitro* approximates that of the airways of a number of animal species (for review, see Welsh, 1987) whilst the congruence of PD *in vitro* (-7 mV) with that of -8 mV *in vivo* (Boucher, Bromberg & Gatzy, 1980) argues against a substantial increase in tissue conductance due to incubation in suboptimal conditions.

The timing of the increase in G (7·5 min after PAF) and its initial peak effect (25 min after PAF) approximate the time course of plasma leakage induced by PAF *in vivo* (Evans *et al.* 1987). One route for clearance of tissue exudate is into the airway lumen where, in the present study, we found a similar time course. Increased G with increased paracellular permeability in response to an inflammatory mediator which also causes increased microvascular permeability may be a clearance mechanism for the exudate. This suggestion is consistent with our present finding of no gross epithelial disruption in animals in which plasma leakage had been induced by intravenous PAF. It is possible that leaked plasma proteins and other blood products, induced by PAF, would in turn have effects on ion transport (Welsh, 1987).

Effect of PAF on mucus secretion in guinea-pig trachea

Fucose is an endogenous marker for mucus but not serum glycoproteins which we have used to measure secretion in rat trachea *in situ* (Rogers *et al.* 1987) and human bronchi *in vitro* (Rogers & Barnes, 1989). In the guinea-pig trachea *in situ*, PAF increased protein but not fucose content of the luminal fluid whilst methacholine had the reverse effect (increased fucose but not protein content). Guinea-pig airways have little submucosal gland but abundant goblet cells (Jeffery, 1983) and methacholine induces goblet cell discharge in guinea-pig trachea (Barnes, Kuo, Rogers, Rohde & Tokuyama, 1990) with little effect on plasma leakage (Chung *et al.* 1990) suggesting that increased fucose secretion in response to methacholine is due mainly to goblet cell secretion. In contrast, PAF appears to have little effect on goblet cells which is consistent with the small increase in secretion of radiolabelled mucin in response to

high doses (10^{-4} M) of PAF in tracheal explants from rat, rabbit and guinea-pig (Adler, Schwarz, Anderson & Welton, 1987), species with little gland. The increased protein content in the tracheal fluid in the present study appears to be due primarily to PAF-induced leakage of plasma into the lumen. The albumin transport rates calculated above are consistent with this suggestion. However, topical PAF decreases the number of guinea-pig conjuctival goblet cells which may indicate mucus discharge (Woodward, Spada, Nieves, Hawley & Williams, 1989).

Effect of PAF on mucus secretion in human bronchi

In human bronchi *in vitro*, both PAF and methacholine at the same concentration (10^{-5} M) doubled fucose secretion. This same dose of methacholine induces secretion of radiolabelled mucin in human bronchi *in vitro* (Pack *et al.* 1984) whilst relatively high doses of PAF induce secretion from ferret trachea *in vivo* (Lang, Hansen & Hahn, 1987). Human and ferret airways have abundant submucosal glands which indicates that both drugs induce predominantly gland secretion. Methacholine may have additional effects on goblet cell discharge (Barnes *et al.* 1990). The low protein content of the PAF-induced secretion compared with that produced in response to methacholine may indicate an effect primarily on fluid rather than mucus secretion (Steiger, Bray & Subramanian, 1987).

Effect of lyso-PAF on airway secretion

A noteworthy finding in our present study is the activity of lyso-PAF *in vitro* which, as the precursor and metabolite of PAF (Barnes *et al.* 1988), is currently believed to represent an appropriate control for PAF as it is considered biologically inert. Our data indicate that this is not the case and are supported by reports of biological activity in other systems (Woodward *et al.* 1989; Yukawa, Read, Kroegel, Rutman, Chung, Wilson, Cole & Barnes, 1990). We found herein that effects of lyso-PAF on bioelectric properties were blocked by WEB 2086. Another PAF antagonist, CV6209, blocked the effect of lyso-PAF on microvascular permeability (Woodward *et al.* 1989). Lyso-PAF does not bind to PAF receptors (Dent, Ukena, Sybrecht & Barnes, 1989) indicating that enzymatic conversion to biologically active levels of PAF accounts for its effects.

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