Mechanisms of platelet activating factor-induced changes in sheep tracheal blood flow

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- 1 The effects of platelet activating factor (PAF) have been studied on the cervical tracheal vasculature and smooth muscle of anaesthetized sheep.
- 2 The predominant action of PAF (2 pmol–2 nmol) was a dose-dependent fall in tracheal vascular resistance. The maximum fall in resistance was -41.6% (-38.5 to -44.7% , 95% confidence interval) and the ED_{50} was 17 pmol (12–28 pmol). Lyso-PAF (200 pmol) did not change vascular resistance.
- 3 PAF had no effect on tracheal smooth muscle tone assessed by measuring changes in the external diameter of the trachea.
- 4 The fall in vascular resistance produced with PAF was unaffected by the anti-asthma drug nedocromil sodium (1 mg, i.a.), the cyclo-oxygenase inhibitor indomethacin (5 mg kg^{-1} , i.v.), the leukotriene receptor antagonist FPL55712 (2 mg kg^{-1} , i.v.), or a combination of the histamine H_1 - and H_2 -antagonists mepyramine (2 mg kg^{-1} , i.v.) with cimetidine (5 mg kg^{-1} , i.v.). The PAF antagonist WEB 2086 ($300 \mu\text{g kg}^{-1}$, i.v.) significantly reduced the vasodilatation produced by PAF (before, $-40.8 \pm 4.2\%$; after $-16.5 \pm 5.9\%$, $P < 0.05$).
- 5 Thus in this model, PAF is a potent vasodilator of the tracheal circulation but has no action on tracheal smooth muscle. The vasodilatation is mediated by specific PAF receptors and is not due to the release of prostanoids, leukotrienes or histamine.

Keywords: Platelet activating factor (PAF); airways blood flow; trachea

Introduction

Platelet activating factor (PAF) is released from a number of inflammatory cells and is likely to play an important role in the pathology of asthma (Barnes *et al.*, 1988; Spina *et al.*, 1989). *In vivo* it produces bronchoconstriction both in animals and man (Morley *et al.*, 1984; Cuss *et al.*, 1986) and it weakly stimulates tracheal submucosal gland secretions (Lang *et al.*, 1987). The most important action of PAF is to produce a non-specific hyperresponsiveness in the airways (Spina *et al.*, 1989). Many of the actions of PAF, including the development of hyperresponsiveness, may be indirect through its chemotactic action on inflammatory cells and its ability to produce secondary release of other inflammatory mediators such as 5-hydroxytryptamine (5-HT), prostaglandins, leukotrienes, bradykinin and oxygen radicals from these cells (Barnes *et al.*, 1988). More recently PAF has been shown to release weakly histamine from mast cells (Misawa & Huang, 1990). In man PAF-induced skin weal and erythema can be reduced by pretreatment with an H_1 -histamine antagonist (Fadel *et al.*, 1990).

Airway mucosal wall thickness produced by inflammation and oedema can have substantial effects on airway function (Moreno *et al.*, 1986; Hogg *et al.*, 1987). The tracheobronchial circulation will have an important role in modulating these actions. However, the actions of PAF on airways blood flow has been little studied (Laitinen *et al.*, 1987) and its mode of action is unknown. PAF induces microvascular leakage in the airways which may be due to a direct rather than indirect action on the vasculature since in guinea-pigs it is independent of cyclo-oxygenase or lipoxygenase products (Evans *et al.*, 1987).

In sheep the blood supply to the cervical trachea can be isolated and perfused *in vivo* (Webber *et al.*, 1988; 1989; 1990). Here this preparation has been used to study the actions of PAF on the circulation and on tracheal smooth muscle. The role of the secondary release of other mediators by PAF was tested with the cyclo-oxygenase inhibitor, indomethacin, the leukotriene receptor antagonist, FPL55712 and the H_1 - and

H_2 -antihistamines, mepyramine and cimetidine. The effects of the anti-asthma drug, nedocromil sodium, were also tested. The role of specific PAF receptors was examined by use of the PAF antagonist WEB 2086.

Methods

Experiments were performed on 12 female sheep (all approximately 1 year old and weighing 27–32 kg). All sheep were anaesthetized with sodium pentobarbitone (initially 20 mg kg^{-1} , i.v.). Additional anaesthetic was given as required to maintain surgical anaesthesia. One femoral artery was catheterized and connected to a pressure transducer (P23, Gould) for the measurement of systemic arterial pressure. An external jugular vein was also catheterized for administration of heparin, gallamine and supplemental doses of anaesthetic.

A low cervical tracheostomy was performed and a tracheal cannula inserted. All animals were paralysed with gallamine triethiodide (1 mg kg^{-1}) and ventilated with a tidal volume of $12\text{--}16 \text{ ml kg}^{-1}$ at a constant rate of 28 breaths per min. Inspired air was supplemented with 100% oxygen ($2\text{--}5 \text{ l min}^{-1}$). Anaesthetic was maintained during paralysis as previously described (Webber *et al.*, 1988).

Measurements of tracheal perfusion flow

The preparation of the tracheal arteries in sheep has been described previously (Webber *et al.*, 1988). The common carotid arteries were exposed on both sides of the trachea. In all the sheep, vessels supplying the trachea were predominantly from the left side. One or two arteries were isolated coming from the left common carotid artery in the middle of the neck. These generally divided into several branches going to trachea, skeletal muscle, oesophagus and glandular tissue. As far as possible, all branches which did not supply the trachea were tied off. The left carotid artery cranial to the isolated tracheal arteries was ligated so that blood perfused only the trachea. A square-wave electromagnetic flowmeter (1.6 mm internal diameter; Carolina Medical Electronics) was placed between two catheters inserted into the carotid artery

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below the tracheal arteries which allowed perfusion to this segment to be measured. The occluded left carotid artery was by-passed from the right carotid artery with a plastic Y-tube connection to ensure an intact blood supply to the brain and a normal pressure in the carotid sinuses. Thus, perfusion of the cranial trachea was via an isolated segment of the left common carotid artery. Heparin (50,000 units i.v.) was administered before the perfusion was connected. Distribution of the perfusion was assessed by injecting Evans blue dye in 0.15 M NaCl (saline) into the catheter inserted below the tracheal arteries. The arteries perfused the trachea on both sides over a length of between eight and twelve cartilagenous rings; a small amount of dye was occasionally seen in adjacent tissues such as the oesophagus.

Measurement of tracheal smooth muscle tone

In six of the sheep, changes in tracheal smooth muscle tone were measured by recording changes in the external diameter of the cranial trachea (Webber *et al.*, 1988). A fixed bar was placed on one side of the trachea and a thin lever connected to a force-displacement transducer (FT03C, Grass) was gently placed on the other side with a micromanipulator. Changes in force were calibrated to represent displacement of the lever tip (range ± 1.6 mm) indicating a change in external tracheal diameter.

Administration of agonists

Injections of PAF, lyso-PAF and methacholine were given directly into the arterial input of the perfusion circuit supplying the vascular bed, in 0.1 or 0.2 ml of saline. Drugs were injected at intervals of at least 10 min, allowing for complete recovery from the previous injection. Doses of PAF (2–2000 pmol) were given to 7 sheep to establish a dose-response relationship; each sheep received between 3–7 different doses of agonist. PAF and lyso-PAF, the inactive precursor, were given separately to a further 5 sheep to establish if there were any non-specific effects of the drugs on the circulation.

Control injections of 0.1 and 0.2 ml saline were given into the perfusion circuit. These produced brief falls in perfusion flow followed by small increases; these effects have previously been shown to be artefactual due to injection pressure and transient reduction in the viscosity of the blood respectively (Salonen *et al.*, 1988). All these effects were transient and were taken into account in assessing the responses to the drugs, where peak changes usually occurred and were measured after the artefactual effects of the injection had passed.

Administration of inhibitors

The concentrations of inhibitors used were: indomethacin (5 mg kg^{-1} , i.v., $n = 5$), FPL55712 (2 mg kg^{-1} , i.v., $n = 5$), mepyramine maleate (2 mg kg^{-1} , i.v., $n = 5$) combined with cimetidine (5 mg kg^{-1} , i.v., $n = 5$), nedocromil sodium (1 mg , i.a., $n = 5$) and WEB 2086 ($200 \mu\text{g kg}^{-1}$, i.v., $n = 4$).

In seven animals a single dose of PAF (between 100 and 1000 pmol) was chosen which produced approximately 70% of the maximum PAF-induced vasodilatation in each particular sheep. This dose was administered before and after each inhibitor. All the inhibitors except nedocromil sodium were administered intravenously 10–15 min before the test dose of PAF. Nedocromil sodium was administered intra-arterially in a 0.1 ml bolus 1 min before PAF. An average of 3–4 different inhibitors was tested in each sheep. The order of administration was random except for WEB 2086 which was always given as the final inhibitor.

Evaluation of results

The maximum change in each variable was measured for each intervention. In most sheep, PAF produced a simple increase in blood flow which returned to baseline within a few minutes.

In three animals the response was more complex. Blood flow rose to a peak, fell towards baseline and then peaked a second time before finally returning to baseline. The magnitude of the fall was not consistent and in some interventions the fall was transiently below the initial baseline. For these results the larger of the two peaks was measured; this was generally the earlier of the two.

All results are given as the mean $\pm$ standard error of the mean (mean $\pm$ s.e.mean). For the vascular responses, results were expressed as changes in vascular resistance where vascular resistance was calculated by dividing mean systemic arterial blood pressure (MAP) by tracheal blood flow, measured at the same instant. The log dose-response curve was obtained by pooling the results from all experiments. The ED_{50} value (the estimated dose producing 50% of the maximum response of the agonist), the maximum responses and the associated 95% confidence interval were estimated by a computerized non-linear least squares estimate.

Other statistical comparisons were made by the Wilcoxon signed rank non-parametric test for paired data.

Drugs

PAF, methacholine chloride, indomethacin, mepyramine maleate and cimetidine were obtained from Sigma Chemicals; lyso-PAF from Bachem; sodium pentobarbitone (Sagatal) and gallamine triethiodide from May and Baker; heparin sodium from CP Pharmaceuticals; and Evans blue dye from Aldrich Chemical Corporation. FPL55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid) and nedocromil sodium were kindly donated by Fisons and 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) by Pfizer.

Results

Vascular responses to PAF and lyso-PAF

The baseline values in the 12 sheep prior to any interventions were $5.3 \pm 0.6 \text{ ml min}^{-1}$ for tracheal blood flow, $27.0 \pm 2.3 \text{ mmHg ml}^{-1} \text{ min}^{-1}$ for tracheal vascular resistance and $130 \pm 5 \text{ mmHg}$ for mean arterial blood pressure (MAP).

In 3 sheep, following injection of PAF, tracheal blood flow rose initially indicating a fall in vascular resistance. Blood flow then fell towards baseline, rose again to a second peak and returned to baseline; on occasion the intermediate fall was to below baseline indicating a vasoconstriction. In the remaining sheep, PAF produced a simple increase in blood flow that reached a maximum and fell towards baseline; an example of one such response is shown in Figure 1. The falls in vascular resistance produced with PAF (2–2000 pmol) were dose-dependent (Figure 2). The calculated ED_{50} value was 17 pmol (12–28 pmol, 95% confidence limits) and the maximum fall in vascular resistance was -41.6% (-38.5 to -44.7%). The maximum changes in vascular resistance occurred between 20

Figure 1 The effects of platelet activating factor (200 pmol at arrow) on tracheal blood flow and systemic arterial blood pressure (BP) in one sheep. Following a brief injection artefact the drug produced a marked increase in blood flow with no effect on BP.

Figure 2 Dose-response curves showing the effects of platelet activating factor (PAF) on tracheal vascular resistance. Points are the means of 3–8 determinations in 7 sheep; the vertical lines represent s.e.mean.

and 84 s following injection. At the doses studied PAF had no systemic actions except at higher doses when on three occasions it produced a small (5–8%) and transient fall in MAP. The duration of each response was assessed by the time taken for the blood flow to return from the maximum to a value half way to the initial baseline value ($t_{0.5}$). The $t_{0.5}$ was weakly dose-related and ranged from 15 to 90 s (Figure 3).

In five separate sheep, PAF (200 pmol) produced a fall in vascular resistance of $-27.1 \pm 16.7\%$ while lyso-PAF (200 pmol) produced no significant change in vascular resistance ($-0.6 \pm 0.8\%$).

Actions on tracheal smooth muscle

PAF had no measurable effects on tracheal diameter at any dose in the six sheep where this was measured. In contrast methacholine (5 nmol) decreased tracheal diameter on 4 out of 5 occasions by -0.29 ± 0.19 mm ($n = 5$) indicating contraction of tracheal smooth muscle.

Effects of the inhibitors

Indomethacin was the only inhibitor to alter the baseline conditions; it produced a sustained increase in MAP ($36.8 \pm 5.2\%$) and in tracheal vascular resistance ($47.9 \pm 3.5\%$, Figure 4).

The fall in vascular resistance produced with a single dose of PAF was unchanged by nedocromil sodium, mepyramine and cimetidine, indomethacin and FPL55712 (Figure 5). The response was however substantially attenuated by WEB 2086 (before, $-40.8 \pm 4.2\%$; after, $-16.5 \pm 5.9\%$; Figure 5). The $t_{0.5}$ was not significantly altered by any of the inhibitors

Figure 3 Curve showing the duration of the response ($t_{0.5}$) in tracheal vasculature produced by platelet activating factor (PAF); s.e.mean shown by vertical lines.

Figure 4 The effects of the inhibitors on baseline vascular resistance. Indomethacin was the only inhibitor that significantly changed the baseline vascular resistance (* $P < 0.05$).

(Figure 6). Analysis of the 'area under the curve' of the change in blood flow produced by PAF was also performed. WEB 2086 was the only inhibitor that significantly reduced the response assessed in this way ($P < 0.05$).

Discussion

The effects of a number of vasoactive agents have now been studied on the tracheal circulation of dogs and sheep. These include histamine, methacholine, 5-HT, peptides and prostaglandins (Laitinen *et al.*, 1987; Salonen *et al.*, 1988; Webber *et al.*, 1988; 1989; 1990; Corfield *et al.*, 1991). Here PAF has been shown to be one of the most potent vasodilators yet

Figure 5 The change in tracheal vascular resistance produced by a dose of PAF before (solid columns) and after (open columns) five inhibitors. WEB 2086 was the only inhibitor that significantly reduced the response (* $P < 0.05$).

Figure 6 The duration of the PAF-induced vasodilatation ($t_{0.5}$) before (solid column) and after (open column) five inhibitors; vertical lines show s.e.mean.

studied in this system, second only to the other inflammatory mediator bradykinin which is also notable for its extremely long duration of action (Corfield *et al.*, 1991). Both drugs produce similar maximum falls in vascular resistance.

The other target tissue studied here was the tracheal smooth muscle; PAF had no action on this. However, in other preparations PAF contracts airways smooth muscle (Barnes *et al.*, 1988). In sheep, PAF increases airways resistance when instilled directly into the airway lumen, this effect being blocked both by a specific PAF antagonist and by nedocromil sodium (Soler *et al.*, 1990). PAF may have different effects on tracheal and bronchial smooth muscle or the intraluminal action may be outside the dose-range studied here. Further, the specific effects may depend on the route of administration.

The actions of PAF on the tracheal circulation may be produced by direct effects on the vascular endothelium and smooth muscle or indirectly through the release of further mediators from other target cells. PAF releases a variety of mediators including prostaglandins, leukotrienes (Barnes *et al.*, 1988; Spina *et al.*, 1989) and possibly histamine (Fadel *et al.*, 1990; Misawa & Huang, 1990). If PAF were to release these mediators in the airways they could produce a subsequent vasodilatation at the tracheal circulation. In the present study, the mechanism of the PAF-induced vasodilatation was tested by the administration of a number of potential inhibitors. The change in vascular resistance produced with PAF was not affected by a combination of H₁- and H₂-antihistamines, cyclo-oxygenase inhibition, lipoxygenase receptor blockade or the anti-asthma agent, nedocromil sodium. Of these, indomethacin, the cyclo-oxygenase inhibitor, was the only agent to alter baseline conditions and this action is therefore, perhaps, harder to interpret. However, the percentage fall in vascular resistance produced with methacholine is the same before and after indomethacin when there are similar changes in baseline conditions (Corfield *et al.*, 1991). Therefore it appears that indomethacin does not affect the PAF-induced vasodilatation. The doses of inhibitors administered here have been used successfully in the same preparation; mepyramine alone will inhibit the responses to exogenous histamine (Webber *et al.*, 1988); cimetidine is without effect indicating this action to be exclusively through H₁-histamine receptors. The vasodilatation produced with *Ascaris suum* antigen is largely attenuated by a combination of indomethacin and FPL55712 (Webber *et al.*, 1989) and is reduced slightly by intra-arterial nedocromil sodium (Corfield *et al.*, 1990b). Therefore it is unlikely that the PAF-induced vasodilatation here is mediated by these other mediators or, by implication, that the vasodilatation produced by *Ascaris suum* (Webber *et al.*, 1989) is mediated by PAF. Although nedocromil sodium was without effect, it reduces PAF-induced airway hyperresponsiveness (Soler *et al.*, 1990).

The vasodilatation was substantially attenuated by WEB 2086, a specific PAF antagonist. Lyso-PAF, the inactive pre-

cursor of PAF with the same detergent properties, was inactive. It is clear therefore that the vasodilatation is receptor mediated and not due to a nonspecific detergent action. PAF can dilate other vascular beds without the release of further mediators such as prostaglandins (Braquet *et al.*, 1987) and may act directly through the release of endothelium-derived relaxing factor (Kamata *et al.*, 1989).

In a few of the sheep, PAF both constricted and dilated the vascular bed. Unfortunately the vasoconstrictor action was inconsistent and possibly masked by the vasodilatation and could not be studied further. This dual action of a single mediator on the sheep tracheal circulation has also been seen for histamine (Webber *et al.*, 1988) and 5-HT (Webber *et al.*, 1990) although the mechanisms are not always clear. The vasoconstrictions seen here could be produced by the release of 5-HT from platelets by PAF.

In airways inflammation, hyperaemia will result from the actions of a number of mediators. This will affect airways function by increasing mucosal thickness, directly increasing the size of the blood volume compartment (Laitinen *et al.*, 1986; Corfield *et al.*, 1990a) and indirectly influencing the magnitude of oedema formation in the tissue. Tissue oedema and plasma exudation into the airway lumen will depend on the degree of vascular leakage and on blood flow to the tissue. A mediator that both increases blood flow and enhances leakage will have greater effects than an agent producing only one; PAF as well as being a potent vasodilator, produces vascular leakage in the airways (Evans *et al.*, 1987). In guinea-pigs, PAF-induced leakage, like the vasodilatation seen here, is not mediated by prostaglandins (Evans *et al.*, 1987). Interestingly, plasma leakage produced by bradykinin is, at least in part, mediated by PAF (Rogers *et al.*, 1990) while the plasma leakage produced with ovalbumin in sensitized guinea-pigs is not mediated by PAF (Evans *et al.*, 1988). The vascular actions of PAF may contribute to the nonspecific hyperresponsiveness produced by the mediator. Increasing blood flow to target tissues may enhance inflammatory cell infiltration; increasing blood volume and tissue oedema in the airway mucosa will enlarge the changes in airways resistance produced with smooth muscle contraction (Moreno *et al.*, 1986; Hogg *et al.*, 1987).

In conclusion, PAF in this model has potent actions on the tracheal circulation but no action on tracheal smooth muscle. The vascular action is predominantly a vasodilatation; this is mediated via specific PAF receptors and does not involve the secondary release of histamine, prostaglandins or leukotrienes. The response is not attenuated by nedocromil sodium. The actions of PAF on the vasculature are of particular interest because of the interaction between blood flow and plasma leakage which influences oedema formation in the airways.

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