Abstracts

The role of platelet-activating factor in airway microvascular permeability

Timothy W. Evans

Experimental and clinical evidence suggests that inflammation plays an important role in the airway hyperresponsiveness that characterizes asthma. Increased microvascular permeability with leakage of plasma, neutrophils, eosinophils and monocytes is the hallmark of the acute inflammatory reaction. Because of the interest in platelet-activating factor (PAF) as a putative mediator of asthma, we have examined its role in generating increased microvascular permeability in the guinea-pig airway. PAF causes a dose-dependent rise in vascular leak at all airway levels in doses as low as 1 ng/kg, an effect not mediated via the products of arachidonic acid metabolism or platelets. The effects of PAF are greatest in the trachea and main bronchi and are comparable in distribution to those of histamine, but with a potency over 10,000 times greater. In sensitized guinea-pigs challenged with antigen, increased microvascular permeability occurs throughout the airways, although this is not inhibited by the PAF-receptor antagonist BN 52063, suggesting that PAF is not responsible for the airway oedema seen in anaphylaxis. However, the increase in microvascular leakage induced in guinea-pig airways by Salmonella enteritidis endotoxin is abolished by BN 52063, indicating that the airway inflammation and bronchial hyperresponsiveness that can be induced by endotoxin may be mediated via PAF.

References


Effects of platelet activating factor on airways in animals

C.P. Page

Platelet-activating factor (PAF) has been described as having a number of effects on the airways of animals of relevance to the aetiology of respiratory diseases such as bronchial asthma. PAF is one of the most potent spasmogens yet described although it has little direct effect on airway smooth muscle. PAF-induced bronchoconstriction in the guinea-pig is known to be secondary to platelet activation following administration, although it is platelet independent following aerosolized PAF. PAF will also induce increased vascular permeability in both the pulmonary and bronchial circulation associated with leakage of plasma proteins into extravascular tissues such as the airways. Of potential interest to our understanding of the pathogenesis of asthma are the recent observations that PAF will induce a long-lasting non-selective increase in bronchial responsiveness following both systemic and aerosol administration in a number of animal species. This bronchial hyperresponsiveness is not secondary to changes in identity affinity or post-receptor transduction of muscarinic, histamine (H₁) or beta-adrenoceptors in airway tissue. However, bronchial hyperresponsiveness following aerosolized PAF in the guinea-pig is associated with a recruitment of eosinophils into the airways as assessed by bronchoalveolar lavage. It is not yet known whether PAF-induced airway eosinophilia contributes to the observed bronchial hyperresponsiveness but it is of interest that recent studies indicate that selective platelet deletion will reduce PAF-induced airway eosinophilia as well as abrogating the bronchial hyperresponsiveness. These results suggest that a complex interaction involving platelets as well as eosinophils may be involved in PAF-induced bronchial hyperresponsiveness. Recent investigations with selective PAF antagonists suggest that PAF may be a central determinant of allergen-induced airway eosinophilia and bronchial hyperresponsiveness.

References


Platelet-activating factor and asthma

K.F. Chung

Platelet-activating factor (PAF) is attracting a lot of attention as a putative inflammatory mediator in asthma capable of mimicking some of the features of the asthmatic airway. PAF induces eosinophil chemotaxis, increases airway microvascular and epithelial permeability, slows mucociliary clearance and causes bronchoconstriction and enhanced airway responses to methacholine and histamine. PAF also stimulates oxidative metabolism and peroxidase release from...
Platelet activating factor and pulmonary vessels

D. McCormack

Platelet-activating factor (PAF), a product of cleavage of membrane phospholipids, has been shown to possess marked vasoactive properties. In 1986, McMurtry from Denver first reported that PAF at very low concentrations was an endothelial vasodilator in the pulmonary vasculature of the rat. Subsequently the suggestion has been put forth that PAF may function as an endogenous vasodilator in the pulmonary circulation, perhaps contributing to the maintenance of low pulmonary vascular resistance, a unique yet constant finding in normal man. PAF has also been shown to be a pulmonary vasoconstrictor in several species. Work in our laboratory has shown that when injected into the pulmonary circulation of the pig, doses from 0.05 to 1.0 μg PAF cause dose-dependent increases in pulmonary vascular resistance.

We have used an isolated perfused and ventilated in situ rat lung model to assess the role of PAF in hypoxic pulmonary vasoconstriction (HPV). We have found that the potent and specific PAF antagonist WEB 2086 is able to attenuate HPV in a dose-dependent manner. Further, when we evaluated the effects of WEB 2086 on the pressor response to angiotensin II we found that doses of WEB 2086 which significantly (>50%) attenuated HPV did not have any effect on the response to angiotensin II suggesting that WEB 2086 is able to selectively inhibit the pressor response to hypoxia. Additionally, when evaluated using isolated rat pulmonary artery rings in an organ bath, we have found that WEB 2086 does not significantly relax vessels precontracted with phenylephrine, supporting the fact that WEB 2086 is not acting as a nonspecific vasodilator. These data suggest that PAF may be a mediator of HPV, at least in the rat and experiments are now underway to test this hypothesis in other species. If PAF is important in HPV then the source of PAF must be found. It has been shown that systemic endothelial cells are capable of producing PAF in response to a variety of stimuli and work is progressing in our laboratory to evaluate if PAF is produced by pulmonary endothelial cells when stimulated with hypoxia.

Therefore whether the physiological role of PAF is as an endogenous pulmonary vasodilator, a mediator of hypoxic pulmonary vasoconstriction, or has another role in the pulmonary circulation is not yet known but with the increasing availability of specific PAF antagonists, this question will likely be answered in the next few years.

References


Platelet activating factor
Cardiac anaphylaxis and the coronary circulation

Priscilla J. Piper, A.W.B. Stanton and H.B. Yaacob,

Platelet-activating factor (PAF) has potent actions in the cardiovascular system in a number of species. For example, in the open chested anaesthetized pig, intravenous bolus injections of PAF cause a fall in blood pressure, coronary blood flow, cardiac output and increase pulmonary arterial and intracardiac pressure. Responses to PAF given intravenously are repeatable and do not exhibit tachyphylaxis. These changes are similar to those produced by intravenous injection of rabbit anti-pig IgG (anti-IgG). Reproducible responses to anti-IgG can be obtained, and effects of PAF and anti-IgG are inhibited by intravenous administration of indomethacin or BN 52021, both at 5 mg/kg.

Severe cardiac dysfunction occurs in cardiac anaphylaxis in guinea-pig perfused hearts in vitro. This is characterized by sustained increase in coronary perfusion pressure (CPP) or reduction in coronary flow, arrhythmias, sinus tachycardia, reduction in cardiac developed tension (CDT), which reflects contractility, with eventual heart failure.

The possible involvement of PAF in cardiac anaphylaxis was investigated in isolated perfused hearts from actively sensitized guinea pigs. PAF (50 pmol) injected into coronary circulation of guinea-pig perfused hearts could mimic many of the features of cardiac anaphylaxis which include biphasic and prolonged increase in perfusion pressure, decreases in CDT and the concomitant release of leukotriene (LT)C4, LTB4 and thromboxane (TX)A2 (assayed as TXB2) into the
cardiac effluent. A 5-lipoxygenase inhibitor CGS 8515 (1 μM) inhibits the PAF-induced increase in CPP but does not significantly affect the reduction in CDT. In addition, CGS 8515 inhibits the PAF-induced release of LTC₄ showing that LTC₄ contributes to part of the cardiac dysfunction produced by PAF.

PAF antagonists L652,731, BN 52021 and WEB 2086 inhibit PAF-induced increase in CPP and CDT. WEB 2086 completely inhibits the PAF-induced increase in CPP while L652,731 and BN 52021 only block the late phase but not the early phase of PAF response.

The same antagonists partially inhibit cardiac anaphylaxis. They all inhibit the late phase of increase in CPP. Investigations with WEB 2086 showed that it also significantly reduced the reduction in CDT and the antigen-induced release of LTC₄ but not of TXB₂.

The findings described above show that PAF has a role in cardiovascular reactions in response to immunological challenge in the pig and in actively sensitized guinea-pig hearts, and suggest that PAF antagonists may have therapeutic value for the treatment of systemic anaphylaxis in man.

References


Biochemistry and analysis of platelet activating factor

A. Mallett

The use of physico-chemical methods, especially mass spectrometry, has been central to the elucidation of the structure and metabolism of PAF. This potent mediator of inflammation can occur in several molecular forms with variants including differing lengths and degrees of unsaturation in the alkyl chain on C-1 and in the substitution of a propionyl function for the more common acetyl group on C-2 of the glycerol backbone.

The immediate precursor of PAF, most commonly described, is lyso-PAF but it is possible that an alkyl-acetyl-glycerol molecule could also be converted to PAF in some systems. Lyso-PAF is also the major catabolite of PAF and the rapid hydrolysis of PAF to the product, especially in matrices such as blood, makes for considerable difficulty in the quantitation of PAF.

The two major approaches to this problem have involved (a) a bioassay using a marker of platelet aggregation, and (b) a combination of chromatography and mass spectrometry. The former method is sensitive and cheap but suffers from limited dynamic range and sample capacity and lack of internal standards. The mass spectrometric method is lengthy and expensive but can use internal standards, describe the molecular speciation and it can analyse lyso-PAF at the same time. Recent developments in the mass spectrometric methods can provide more than sufficient sensitivity.

The release and bioassay of platelet activating factor

M.F. Fitzgerald

Platelet-activating factor (PAF) is an ether-linked phospholipid with profound biological activities that are consistent with it being a mediator of inflammation and anaphylaxis.

We have previously shown that anaphylactic challenge of sensitized guinea-pig lungs perfused through the airways provokes the release of PAF. The release of PAF was not detected when perfusion and challenge was through the vasculature. This suggests that cells situated close to or on the alveolar surface of the lungs, probably alveolar macrophages, are involved in the synthesis and release of PAF.

In this study the effects of selective inhibition of either cyclooxygenase or lipoxygenase on the release of PAF and eicosanoids from two in vitro models have been investigated. In order to achieve selective inhibition of 5-lipoxygenase the novel inhibitor BW A137C was used.

Anaphylactic challenge of sensitized guinea-pig lungs perfused through the airways provoked the release of PAF, TXB₂ and LTB₄, all reaching a maximum within 6 minutes of starting the challenge. Indomethacin (5.6 μM) completely suppressed the release of TXB₂ (maximal release, 64 ± 12 ng/ ml, n = 7) but significantly increased the maximal release of LTB₄ from 0.6 ± 0.1 ng/ml (n = 7) to 1.4 ± 0.2 ng/ml (n = 5). BW A137C (1μM) inhibited the maximal release of LTB₄ from 1.6 ± 0.4 ng/ml (n = 4) in control lungs to 0.5 ± 0.1 ng/ml (n = 5) in treated lungs without affecting maximal release of TXB₂. Neither drug had a marked action on the release of PAF.

Guinea-pig alveolar macrophages incubated with FMLP
(1 μM) for 5 minutes released PAF, immunoreactive (i)-LTB₄ and TXB₂. Indomethacin (1 μM) completely suppressed the release of TXB₂ whilst enhancing the release of i-LTB₄ from 0.32 ± 0.09 ng/10⁶/cells (n = 3) to 1.6 ± 0.32 ng/10⁶/cells (n = 3). BW A1377C (1 μM) completely suppressed the release of i-LTB₄ without affecting the release of TXB₂. Neither drug affected the release of PAF.

These results suggest that the release of PAF from these two in vitro models is not secondary to the release of TXB₂ or LTB₄.

References


Cell priming and platelet-activating factor (PAF)/cytokine autogenated feedback networks: fundamental mechanisms in the induction and evolution of microvascular damage

Pierre Braquet, Monique Paubert-Braquet and David Hosford.

The amplification of neutrophil (PMN) responses to a stimulus by prior exposure of cells to an agonist is known as 'priming'. Recent studies have shown that both PAF and TNF can prime PMN responses. For example, PAF primes the release of superoxide anions from PMN induced by TNF or chemotactic peptides, similarly TNF primes PMN responses to various agonists. We have recently demonstrated that TNF induces a dose-dependent generation of superoxides from PMN. In this PMN system, while very low concentrations of PAF per se cannot increase superoxide release, PAF [1 pmol–0.1 nmol] added to cells previously incubated with TNF causes a significant dose-dependent amplification of superoxide production relative to that induced by TNF alone. This effect is blocked by the specific PAF antagonists BN 52021, kadsurenone, BN 52111 and WEB 2086. PAF also activates TNF production from peripheral blood derived monocytes and at very low doses [10 pmol–0.1 nmol] significantly amplifies lipopolysaccharide (LPS)-induced TNF production, effects inhibited by BN 52021. Cell priming may have important physiological consequences; we have recently proposed that PAF and TNF play pivotal roles in the generation of deleterious feedback cycles, which if unregulated may lead to the endothelial damage and microvascular leakage observed in various pathologies such as shock, sepsis, ischaemia and asthma. In addition to free radicals, PAF is also able to elicit the release of major basic protein (MBP) and leukotriene C₄ (LTC₄) which are damaging to microvascular integrity, while endothelial cells (EC) produce PAF under TNF stimulation. Other vectors in this 'cross talk' system between EC and blood cells may include interleukins (IL) 1, 2,5 and 6, granulocyte/macrophage-colony stimulating factor (GM-CSF), gamma-interferon, lymphotoxin, thrombin and ATP. PAF activates platelets to form these two latter agents which in turn, as IL 1 and TNF, act on EC to produce more PAF resulting in increased PMN chemotaxis, degranulation and EC injury. As PAF antagonists inhibit PAF-induced priming and the PAF-dependent components of the feedback cycles, they may be of therapeutic value in the above conditions due to their ability to decouple deleterious autogenated mediator networks which would otherwise lead to vascular leakage and circulatory collapse.

References


The effect of platelet activating factor on eosinophils

A.B. Kay

In a previous study we showed that platelet-activating factor (PAF) induced directional locomotion of eosinophils, in a time- and dose-dependent fashion, at concentrations from 10⁻³ to 10⁻⁷ M; lyso-PAF had minimal activity over the same dose range. When PAF was compared with several other documented chemoattractants for eosinophil chemotaxis the rank order of potency (using optimal eosinophilactotic concentrations) was PAF (10⁻⁶ M) > C5a (10⁻⁹ M) > C5a des arg (10⁻⁷ M) = LTB₄ (10⁻³ M) > fMLP (10⁻⁷ M). In fact, compared with PAF the eosinophil locomotory responses to LTB₄, histamine and the valyl and alanyl eosinophil tetrapeptides were negligible. We recently established that PAF-induced eosinophil locomotion can be inhibited by specific PAF antagonist derived form Gingko biloba (BN 52021). In response to an
optimal concentration of PAF (10^{-6} \text{ M}) the drug was significantly more potent \((P<0.001)\) in inhibiting eosinophil as compared with neutrophil locomotion. These inhibitory effects were observed in a dose-dependent manner with an IC_{50} of 7.0 (± 2.2) \times 10^{-6} \text{ M} and 2.3 (± 0.2) \times 10^{-5} \text{ M}, for eosinophils and neutrophils, respectively. Sodium cromoglycate, nedocromil sodium, salbutamol and dexamethasone (preincubated with cells for up to 6 hours) had no effect. Inhibition by BN 52021 was specific for PAF, in that it had no effect on chemotaxis induced by either LTB_{4}, N-formylmethionyl-leucyl-phenylalanine (fMLP) or a purified human mononuclear cell-derived neutrophil chemotactic factor. BN 52021 also inhibited the specific binding of ^3H-PAF (10^{-8} \text{ M}) to eosinophils and neutrophils, in a concentration-dependent fashion.

PAF (at an optimal concentration of 10^{-7} \text{ M}) gave dose- and time-dependent increases in the expression of membrane receptors as measured by IgE, IgG and complement rosettes on normal density eosinophils. Fc-epsilon RII expression was also studied by FACS using an excess of native human myeloma IgE and a fluorescein-conjugated polyclonal anti-IgE to detect bound IgE. Receptors could be upregulated since preincubation of eosinophils with PAF (10^{-7} \text{ M}) gave a time- and dose-dependent increase in IgE binding. A smaller, but significant difference was also observed with LTB_{4} (10^{-7} \text{ M}) and histamine (10^{-5} \text{ M}). Lyso-PAF or diluent alone had no effect at any of the concentrations used.

The effector function of eosinophils, as measured by their ability to adhere to and kill appropriately-opsonized helminthic targets, in vitro, was also enhanced by prior stimulation of cells with PAF. Using preparations of IgG- and IgE-rich fractions of immune serum PAF (at an optimal dose of 10^{-8} \text{ M}), but not lyso-PAF produced 185%, 106% and 100% enhancement of eosinophil cytotoxicity against schistosomula of Schistosoma mansoni coated with IgE, IgG and complement, respectively.6

References

5. Walsh, G.M., Nagakura, T., Moqbel, R. Flow cytometric analysis of eosinophil IgE receptor (Fc-epsilon RII); enhancement by platelet activating factor. Submitted.