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The anaphylactic release of platelet-activating factor from perfused guinea-pig lungs

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1 The release of platelet-activating factor (Paf-acether) and of its inactive precursor/metabolite lyso-platelet activating factor (lyso-Paf) from control and sensitized guinea-pig isolated lungs challenged with antigen was investigated.

2 Control guinea-pig lungs perfused either through the pulmonary circulation or through the airways and challenged with antigen did not release Paf-acether.

3 Sensitized guinea-pig isolated lungs perfused through the pulmonary circulation and challenged with antigen released lyso-Paf but not Paf-acether.

4 Sensitized guinea-pig isolated lungs perfused through the airways and challenged with antigen released three times more lyso-Paf and also Paf-acether.

5 These results support a possible role for Paf-acether in respiratory anaphylaxis in the guinea-pig.

Introduction

Platelet-activating factor (Paf-acether) is an ether phospholipid with profound biological activities which suggest a role as a putative mediator of anaphylaxis and inflammation (Benveniste & Arnoux, 1983; Page *et al.*, 1984).

Paf-acether has been detected when released from chopped lungs from sensitized rabbits (Kravis & Henson, 1975) and guinea-pigs (Andersson *et al.*, 1984) challenged with antigen *in vitro*. However, sensitized guinea-pig lungs perfused through the pulmonary circulation released only lyso-platelet-activating factor (lyso-Paf), which is the inactive precursor and metabolite of Paf-acether (Rotilio *et al.*, 1983).

We have recently shown that lyso-Paf is released by normal guinea-pig lungs challenged with calcium ionophore A23187 and perfused through the airways, but not through the pulmonary circulation, suggesting the involvement of cells close to the alveolar surface (Parente *et al.*, 1985).

We now report that sensitized guinea-pig lungs perfused through the pulmonary circulation and challenged with antigen release only lyso-Paf, whilst sensitized lungs perfused through the airways release both lyso-Paf and Paf-acether after antigen challenge. Control lungs do not release Paf-acether following antigen challenge.

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Methods

Male Dunkin-Hartley guinea-pigs were sensitized by injecting ovalbumin 50 mg subcutaneously and 50 mg intraperitoneally. Control animals received subcutaneous and intraperitoneal injections of saline (4 ml kg^{-1}). Three weeks later the animals were killed by intraperitoneal injections of pentobarbitone, the lungs were removed and placed in a heated chamber (37°C).

Guinea-pig lung perfusion

In the first series of experiments, the lungs from control and sensitized animals were perfused at a flow rate of 5 ml min^{-1} through the pulmonary artery with Krebs bicarbonate solution containing 0.25% bovine serum albumin (hereafter referred to as Krebs-BSA) warmed (37°C) and gassed (95% O_2 + 5% CO_2). The trachea was also cannulated and the cannula connected to a syringe which was used to inflate the lungs with

20 ml of air (Piper & Vane, 1969). After 30 min of perfusion, the antigen (100 ng ml^{-1}) was infused through the pulmonary circulation, for 5 min.

In the second series of experiments the lungs from control and sensitized animals were perfused through the pulmonary artery for the first 10 min, after which the perfusion was changed to the tracheal cannula in order to perfuse the airways as previously described (Parente *et al.*, 1985). After a further 20 min the antigen (100 ng ml^{-1}) was infused through the airways for 5 min.

In both series of experiments one sample was collected before antigen infusion and then 1 min (5 ml) samples of the lung effluent were collected from the beginning of the antigen infusion every other min for 20 min.

In the same way, samples of the effluent were also collected for 20 min from sensitized lungs perfused either through the vascular bed or through the airways without antigen challenge.

Paf-acether and lyso-Paf were extracted from the lung effluent and bioassayed as previously described in detail (Parente & Flower, 1985; Parente *et al.*, 1985). The extracted Paf-acether fulfilled the identification criteria established by Benveniste *et al.* (1977); (i) same R_F (0.46) as synthetic Paf-acether on thin layer chromatography (chloroform, methanol, water 65:35:6 v/v); (ii) inactivation by phospholipase A_2 (0.1 mg ml^{-1} for 60 min at 37°C); (iii) insensitivity to a lipase (0.1 mg ml^{-1} for 60 min at 37°C); (iv) aggregation of rabbit platelet rich plasma in presence of a cyclo-oxygenase inhibitor (indomethacin 1 μM) and ADP scavengers (creatine phosphate 31.25 μg ml^{-1}, creatine phosphokinase 15.25 μg ml^{-1}).

Recovery of [^{14}C]-Paf-acether

Control and sensitized guinea-pig lungs were perfused either through the pulmonary circulation or the airways with Krebs-BSA as described above. In a separate group of experiments control lungs were perfused either through the vasculature or through the airways with Krebs alone. A solution containing Paf-acether and [^{14}C]-Paf-acether (1 μg and 0.3 μCi ml^{-1} respectively) was prepared in Krebs-BSA; 1 ml was injected as a bolus either through the vasculature or the airways at the beginning of the antigen infusion in both control and sensitized lungs. The effluent was collected for 15 min and extracted as previously described (Parente & Flower, 1985; Parente *et al.*, 1985). The organic residue resuspended in chloroform/methanol (1:1) was applied to a thin layer chromatography plate together with [^{14}C]-Paf-acether and [^{14}C]-lyso-Paf and developed in chloroform:methanol:water (65:35:6 v/v). The label was then visualized by autoradiography, the radioactive segments on the plate were scraped off and counted by conventional liquid scintillation techniques. Initial experiments showed that after 15 min of perfusion there was no further detection of the label (less than1% of total radioactivity administered).

Statistical analysis

Results are expressed as the means ± s.e. mean. Unpaired Student's t test was used to assess the significance of the experimental results. Significance was accepted when $P < 0.05$.

Materials

The Krebs bicarbonate solution had the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4,7H_2O$ 1.17, $CaCl_2,6H_2O$ 0.25, $NaHCO_3$ 25 and glucose 8.4. Other compounds used were: chicken egg albumin, grade V; bovine serum albumin fraction V; Paf-acether (1-0-alkyl-2-acetyl-sn-glycero-3-phosphoryl-choline, mixture of C_{16}/C_{18}; phospholipase A_2 from porcine pancreas; lipase from *Rhizopus arrhizus*; indomethacin, creatine phosphate, creatine phosphokinase (Sigma). Pentobarbitone sodium (Sagatal, May & Baker). Platelet-activating factor (N-methyl-^{14}C) and lyso-platelet-activating factor (N-methyl-^{14}C) 59 mCi mmol^{-1} (Amersham International). All other reagents were of 'Analar' grade.

Results

Release of Paf-acether and lyso-Paf from control and sensitized lungs

Sensitized guinea-pig lungs perfused through the pulmonary circulation in absence of antigen challenge released a basal amount of lyso-Paf which did not change over the 20 min observation period (3.13 ± 0.33 ng min^{-1}, $n = 3$). This release was similar to the pre-challenge levels observed in the experiments where antigen was used. The pre-challenge levels of lyso-Paf were 2.77 ± 0.33 ng min^{-1} ($n = 6$) (Figure 1), following the challenge the lyso-Paf concentration in the lung effluent rapidly increased to levels which were significantly higher than the pre-challenge concentrations between the 2nd and the 16th min of the observation period. The mean stimulated release of lyso-Paf over 20 min from sensitized lungs perfused through the vascular bed was 4.86 ± 0.25 ng min^{-1} ($n = 6$) (Figure 1). In these experiments no Paf- acether was detected in the lung effluent.

A basal release of lyso-Paf was also observed in the effluent of sensitized lungs perfused through the airways. This release was three times higher than the one detected from the pulmonary circulation (10.6 ± 0.7 ng min^{-1}; $n = 3$, $P < 0.001$). The amounts

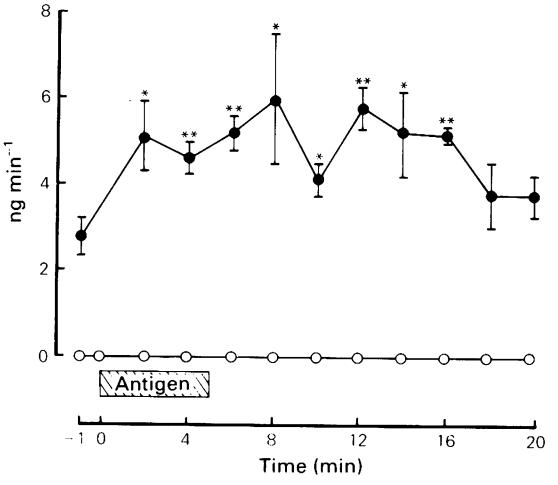

Figure 1 Release of Paf-acether (○) and lyso-Paf (●) from sensitized guinea-pig lungs perfused and challenged through the pulmonary circulation. Results represent the mean, with vertical lines showing s.e. mean, of 6 experiments. $*P<0.05$, $**P<0.01$.

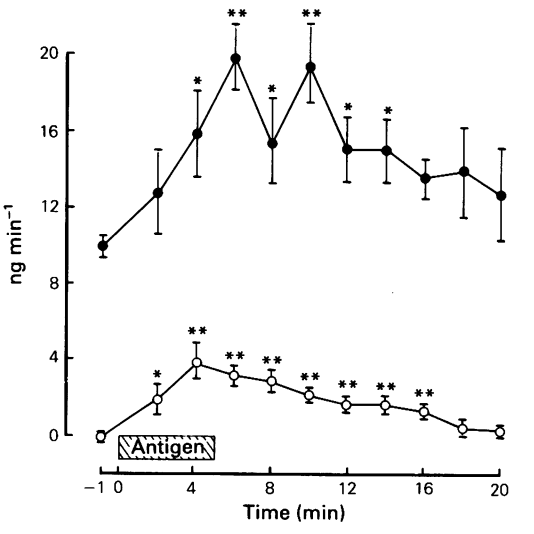

Figure 1 Release of Paf-acether (○) and lyso-Paf (●) from sensitized guinea-pig lungs perfused and challenged through the airways. Results represent the mean, with vertical lines showing s.e. mean, of 5 experiments. $*P<0.05$, $**P<0.01$.

of lyso-Paf released in absence of antigen challenge did not change over the 20 min observation period. No Paf-acether was detected under basal conditions. After antigen challenge the levels of lyso-Paf were statistically higher than pre-challenge levels between the 4th and the 14th min (Figure 2). Moreover, after antigen challenge Paf-acether was detected in the lung effluent. The levels of Paf-acether became significantly higher than controls between the 2nd and 16th min of the observation period. The mean release of lyso-Paf and Paf-acether over 20 min from sensitized lungs perfused through the airways was 15.5 ± 0.8 and 1.97 ± 0.37 ng min^{-1} respectively ($n=5$) (Figure 2).

Control lungs perfused either through the vasculature or the airways did not release Paf-acether after antigen challenge ($n=5$). In these experiments the levels of lyso-Paf were not measured.

Recovery of [^{14}C]-Paf-acether

Table 1 shows that the total recovery of the exogenously added label from control lungs perfused with Krebs alone was 29.0 ± 6.7% from the pulmonary circulation and 10.6 ± 2.4% from the airways. Paf-acether accounted for about 90% of the total label recovered from both vasculature and airways.

The total recovery of the label from control lungs perfused with Krebs-BSA was about 80% from either the vascular bed or the airways. More than 98% of the radioactivity co-chromatographed with synthetic Paf-acether. The extent of recovery and metabolism was not significantly changed in sensitized lungs perfused with Krebs-BSA.

Table 1 Percentage recovery of the [^{14}C]-Paf-acether (0.3 μCi, 59 mCi mmol^{-1}, see Methods) injected in control and sensitized guinea-pig lungs either through the pulmonary circulation (PC) or through the airways.

	Lungs perfused with Krebs	*Lungs perfused with Krebs-BSA*	
	Control ($n=3$)	*Control* ($n=3$)	*Sensitized* ($n=3$)
PC (total recovery)	29.0 ± 6.7†	80.5 ± 3.6*	87.0 ± 1.6
% Paf-acether	90.1 ± 3.2	98.7 ± 0.1	98.4 ± 0.3
% lyso-Paf	9.9 ± 3.2	1.3 ± 0.1	1.6 ± 0.3
Airways (total recovery)	10.6 ± 2.4	76.4 ± 7.7*	76.5 ± 3.5
% Paf-acether	87.3 ± 1.7	98.3 ± 0.2	98.1 ± 0.4
% lyso-Paf	12.7 ± 1.7	1.7 ± 0.2	1.9 ± 0.4

*$P<0.01$ vs control lungs perfused with Krebs.
†Not significant vs corresponding airways. Differences between control and sensitized lungs perfused with Krebs-BSA were not significant.

Discussion

Sensitized guinea-pig lungs perfused through the vascular bed and challenged with antigen release a variety of anaphylactic mediators including histamine, prostaglandins and leukotrienes which may contribute to the bronchospasm observed during anaphylactic shock (Piper & Vane, 1969). Paf-acether is a new putative mediator whose release has been demonstrated in rabbit serum during IgE-mediated anaphylaxis (Pinckard *et al.*, 1979; Camussi *et al.*, 1983) and in guinea-pig cardiac anaphylaxis (Levi *et al.*, 1984). However, Paf-acether was not detected from sensitized guinea-pig lungs perfused and challenged through the vascular bed (Rotilio *et al.*, 1983). These results are confirmed by the present experiments which show that lyso-Paf but not Paf-acether can be detected after challenge of sensitized lungs perfused through the pulmonary circulation. As lyso-Paf is the inactive metabolite of Paf-acether, its presence in the lung effluent might indicate that Paf-acether was generated in small amounts and rapidly de-acetylated by a specific acetylhydrolase present in high concentration in the lungs (Blank *et al.*, 1981).

In our experiments no significant change was observed in the recovery and metabolism of Paf-acether in control and sensitized lungs perfused with Krebs-BSA. It is then likely that when given as a bolus through the pulmonary artery or the trachea Paf-acether cannot reach the acetylhydrolase. This situation is similar to the metabolism in the lung of prostacyclin (PGI_2) and prostaglandin A_1 (PGA_1) which are substrates for the pulmonary 15-hydroxyprostaglandin dehydrogenase (PGDH) (McGuire & Sun, 1978), but are not inactivated in passage across the lungs (Dusting *et al.*, 1978; Horton & Jones, 1969). However, the observed lack of conversion of Paf-acether to lyso-Paf and the fact that the latter can also be the precursor indicates that the presence of lyso-Paf does not necessarily imply the release of Paf-acether itself.

The recovery of radioactive Paf-acether was significantly decreased from lungs perfused with Krebs alone. This confirms previous data obtained in rat isolated lungs (Lichey *et al.*, 1984) and stresses the importance of the presence in the perfusion fluid of the albumin which is able to sequestrate Paf-acether from cellular membranes.

Paf-acether was not detected in the effluent from control lungs challenged with antigen, but it was detected in significant amounts when sensitized lungs were perfused and challenged through the airways. Moreover, in these experiments the amounts of lyso-Paf released by the lungs were three times higher than the corresponding levels released by lungs perfused through the pulmonary circulation. The same pattern has been demonstrated in sensitized guinea-pig lungs perfused and challenged through the airways for the release of arachidonic acid metabolites. Under these conditions the lungs release three times more cyclooxygenase and ten times more lipoxygenase products than when they were perfused and challenged through the vascular bed (Bakhle *et al.*, 1985). The detection of Paf-acether through the airways is, therefore, likely to reflect an increased biosynthesis of the mediator. It is possible that the antigen given through the airways is able to stimulate cells close to the alveolar surface which can synthesize large amounts of Paf-acether.

Several cells could contribute to the release of Paf-acether from lungs including basophils, macrophages and mast cells. The available experimental evidence, however, suggests that alveolar macrophages are the main source of the mediator in our experiments. Indeed, the anaphylactic reaction we have used is an IgG-dependent process (Andersson, 1980) and basophils and mast cells seem to release Paf-acether only via IgE-dependent mechanisms (Betz *et al.*, 1980; Mencia-Huerta *et al.*, 1983). Moreover, the evidence for mast cells synthesizing the mediator is controversial (Mencia-Huerta & Benveniste, 1979). On the other hand alveolar macrophages can release Paf-acether either via direct antigen stimulation (Arnoux *et al.*, 1983) or by phagocytosing immune complexes (Mencia-Huerta & Benveniste, 1981). It has been shown that immune phagocytosis is greatly enhanced in lungs previously exposed to antigen (Harmsen *et al.*, 1985).

In conclusion, our results document for the first time the release of Paf-acether from sensitized and perfused guinea-pig lungs. Paf-acether is a potent bronchoconstrictor agent (Lefort *et al.*, 1984) and can stimulate the release of thromboxane A_2 (Lefort *et al.*, 1984) and leukotrienes C_4 and D_4 (Voelkel *et al.*, 1982) from lungs. However, further work is needed to clarify the role of Paf-acether in respiratory anaphylaxis.

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