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Inflammatory actions of platelet activating factor (Paf-acether) in guinea-pig skin

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1 Cutaneous responses to synthetic platelet activating factor (Paf-acether) have been studied in guinea-pigs by means of radioisotopic marker techniques.

2 Intradermal injection of Paf-acether elicited increased plasma protein extravasation (IPPE) (0.2–200 pmol/site), platelet accumulation (PA) (20–200 pmol/site) and red blood cell accumulation (RBCA) (200 pmol/site), whereas lyso-Paf (up to 2 nmol/site) was inactive in all these respects.

3 Following intradermal injection, the IPPE responses to Paf-acether (2 and 20 pmol/site) were complete within 15 and 30 min respectively, although in response to 200 pmol/site, IPPE was detectable up to 1.5 h. The PA and RBCA responses to Paf-acether (200 pmol/site) were complete within 1 h.

4 IPPE induced by Paf-acether (3 pmol/site) was potentiated by concomitant intradermal injection of a cutaneous vasodilator prostaglandin E_2 (PGE_2, 1 nmol/site) and inhibited by the β-adrenoceptor agonist, isoprenaline (4.5 nmol/site) or the α-adrenoceptor agonist, phenylephrine (6 nmol/site). Such observations are consistent with Paf-acether effecting increased vessel wall permeability.

5 Intradermal injection of PGE_1 (3 nmol/site) significantly reduced PA in response to Paf-acether (200 pmol/site), whilst significantly enhancing IPPE. This dissociation of increased vascular permeability from PA is consistent with Paf-acether eliciting IPPE via a platelet-independent mechanism.

6 These results indicate that a direct effect on vessel wall permeability contributes to the inflammatory response to Paf-acether in guinea-pig skin. It is suggested that Paf-acether is a potential mediator of allergy and inflammation.

Introduction

Platelet activating factor (Paf-acether) is one of several biologically active materials generated in allergic responses. Originally described as platelet activating factor (PAF), a soluble intermediate released following exposure of sensitized rabbit basophils to specific antigens (Benveniste, Henson & Cochrane, 1972), this active material is now known to be a series of phospholipids, 1-0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine, and is referred to as AGEPC (Demopoulos, Pinckard & Hanahan, 1979,) or Paf-acether (Benveniste, Tence, Varrene, Bidault, Boullet & Polonsky, 1979). Like many other materials released in allergic reactions, Paf-acether elicits inflammatory responses, as has been demonstrated in the skin (Stimler, Bloor, Hugli, Wykle, McCall & O'Flaherty, 1981; Wedmore & Williams, 1981a; Humphrey, McManus, Satouchi, Hanahan & Pinckard, 1982; Paul & Page, 1982) and paw (Bonnet, Loisseau, Orvoen & Bessin, 1981; Vargaftig & Ferreira, 1981; Page, Paul & Morley, 1983) of experimental animals and in the skin of man (Pinckard, Kniker, Lee, Hanahan & McManus, 1981; Basran, Page, Paul & Morley, 1983). Acute inflammatory responses are enhanced by vasodilators such as prostaglandin E_1 (PGE_1) and PGE_2, inhibited by vasoconstrictors such as the α-adrenoceptor agonist, phenylephrine, and also by β-adrenoceptor agonists, such as isoprenaline; which observations support the hypothesis that inflammatory mediators produce increased plasma protein extravasation (IPPE) as a consequence of interaction between vasodilator and permeability effects (Williams & Peck, 1977). In the present study, the effects of vasodilator, vasoconstrictor and β-adrenoceptor drugs have been defined in order to ascertain whether Paf-acether behaves as a permeability factor and PGE_1 has been used to establish whether IPPE induced by Paf-acether is associated with platelet activation.

Animals

Male Dunkin-Hartley guinea-pigs (400–500 g body weight) were used throughout this study.

Intradermal injections

The flank skin of animals was shaved at least 2 h before experiments and intradermal injections (0.1 ml) were allocated to marked sites according to balanced Latin square designs. The diluent for all intradermal injections was Dulbecco's phosphate buffered saline (PBS; Gibco Europe) containing 0.25% bovine serum albumin (BSA; Sigma).

Increased plasma protein extravasation (IPPE)

IPPE was measured by means of isotopically labelled albumin (Beets & Paul, 1980). Each animal was injected intravenously with 0.5 ml Evans blue dye containing 1.5 μCi ^{125}I-human serum albumin ([^{125}*T*]-HSA; Amersham International); 40 min after the intradermal injections, animals were killed by a blow to the head, bled (1 ml blood was collected) and skinned. Discs of skin containing the whole of each lesion were removed with a metal wad punch and counted in an automatic gamma spectrometer (Nuclear Enterprises). Extravasation in each lesion was calculated by expressing responses as equivalent μl of whole blood (^{125}I counts in lesion/^{125}I counts in 1 μl whole blood) and subtracting the values obtained at sites injected with diluent alone.

The time course of IPPE was determined by making a series of intradermal injections of Paf-acether at regular intervals before, and immediately after, intravenous injection of ^{125}I-HSA. Animals were killed 15 or 30 min after the intravenous injection.

Accumulation of platelets and red blood cells

Guinea-pig platelets or red blood cells were labelled using ^{111}Indium oxine (^{111}In; Amersham International) (Page, Paul & Morley, 1982). In brief, platelets, or red blood cells, were obtained from citrated arterial blood, washed, incubated with ^{111}In (25–50 μCi), re-washed to remove unbound label and injected intravenously into recipient animals. Animals were killed 40 min after skin testing and 5 ml of blood was collected and heparinised (10 iu ml^{-1}) for determination of the proportion of ^{111}In free in plasma. Animals were skinned and discs of skin containing the entire lesion were removed with a metal wad punch. Skin discs, whole blood (1 ml) and plasma samples (0.5 ml) were counted in an automa-

Table 1 Effects of platelet activating factor (Paf-acether) and lyso-Paf in guinea-pig skin

a

Treatment (dose/site)	*Plasma protein extravasation* (μl)
Paf-acether (0.2 pmol)	63 ± 9
Paf-acether (2 pmol)	126 ± 9
Paf-acether (20 pmol)	176 ± 15

b

Treatment (dose/site)	*Plasma protein extravasation* (μl)	*Platelet accumulation* (μl)
Paf-acether (20 pmol)	126 ± 5	13 ± 1
Paf-acether (200 pmol)	275 ± 33	57 ± 11
Lyso-Paf (2 nmol)	6 ± 3	0 ± 1

c

Treatment (dose/site)	*Plasma protein extravasation* (μl)	*Red blood cell accumulation* (μl)
Paf-acether (20 pmol)	177 ± 23	2 ± 0.1
Paf-acether (200 pmol)	273 ± 34	4.5 ± 0.6
Lyso-Paf (200 pmol)	−3 ± 3	0 ± 0.2

Increased plasma protein extravasation (IPPE) (a, b and c), platelet accumulation (PA) (b) and red blood cell accumulation (RBCA) (c) have been measured following intradermal injection of Paf-acether (0.2–200 pmol/site) or lyso-Paf (200 pmol or 2 nmol/site). IPPE, PA and RBCA have been expressed as equivalent μl of whole blood: IPPE (^{125}I counts in skin/^{125}I counts in 1 μl whole blood), RBCA and PA (^{111}In counts in skin/^{111}In counts in 1 μl whole blood). Values in the table represent the mean response ± 1 s.e.mean (n=4) and have been corrected by subtracting the response obtained following injection of diluent alone.

tic gamma spectrometer. Accumulation of platelets, or red blood cells, has been expressed as equivalent ul of whole blood, as described for IPPE. At the end of an experiment, free ^{111}In never exceeded 7.5% (platelets) or 2% (red blood cells). When both ^{125}I and ^{111}In were used in the same animals, correction was made for ^{111}In activity which registered in the ^{125}I channel (3.3%).

The time course of platelet, or red blood cell, accumulation was determined by making a series of intradermal injections of Paf-acether at regular intervals prior to, and immediately following, intravenous injection of ^{111}In-labelled platelets or red blood cells. Animals were killed 30 min (platelets) or 1 h (red blood cells) after the intravenous injection.

Drugs

Drugs used were: Batches of Paf-acether (gifts from Prof. J.J. Godfroid and Dr J. Benveniste, INSERM U200, Clamart, France); lyso-Paf (Bachem); prostaglandin E_1 (PGE_1); prostaglandin E_2 (PGE_2); (±)-isoprenaline sulphate and L-phenylephrine hydrochloride (Sigma). Stock solutions (1 mg ml^{-1}) of Paf-acether, lyso-Paf. PGE_1 and PGE_2 were prepared in absolute ethanol. All solutions were prepared in PBS containing 0.25% BSA (Sigma), immediately before use.

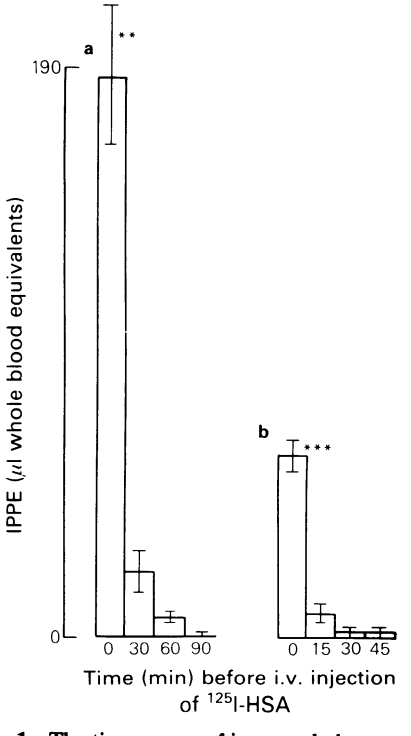

Figure 1 The time course of increased plasma protein extravasation (IPPE) in guinea-pig skin following intradermal injection of Paf-acether. In (a), intradermal injections of 20 pmol Paf-acether were made 90, 60, 30 and 0 min before intravenous injection of ^{125}I-HSA. Animals were killed 30 min later. In (b), intradermal injections of 2 pmol Paf-acether were made 45, 30, 15 and 0 min before intravenous injection of isotope and animals were killed 15 min later. IPPE responses have been expressed as equivalent μl of whole blood (^{125}I counts in skin sites/^{125}I counts in 1 μl whole blood). Each histogram column represents the mean IPPE response occurring in the specified time interval and vertical bars show ± 1 s.e.mean (n=4). The significance of the IPPE responses is indicated by ** ($P<0.025$; ***($P<0.005$).

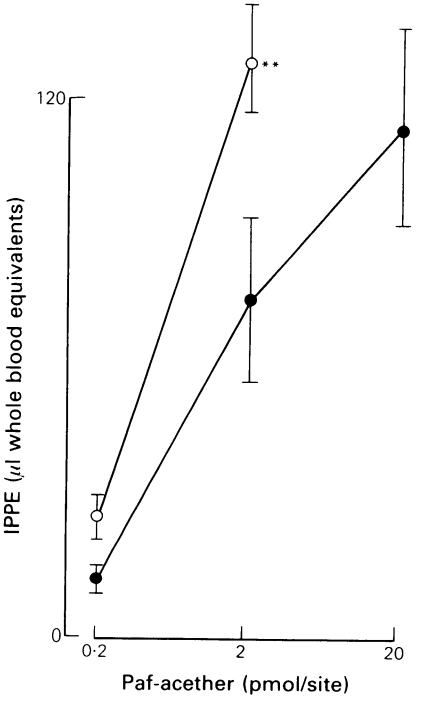

Figure 2 Increased plasma protein extravasation (IPPE) in guinea-pig skin following intradermal injection of Paf-acether alone (●) or admixed with prostaglandin E_2 (PGE_2, 1 nmol/site) (○). IPPE responses are expressed as equivalent μl of whole blood (^{125}I counts in skin sites/^{125}I counts in 1 μl whole blood). Each point represents the mean IPPE response and vertical bars show ± 1 s.e.mean (n=4). Significant potentiation of the IPPE response to Paf-acether is indicated by ** $P<0.025$. All values have been corrected by subtracting the response obtained following injection of diluent alone.

Results

Increased plasma protein extravasation

Over the dose range 0.2 to 200 pmol/site, Paf-acether elicited acute (0–40 min) IPPE in guinea-pig skin (Table 1a, b), whereas lyso-PAF (2 nmol/site) was inactive (Table 1b). With intermediate doses of Paf-acether (2 and 20 pmol/site), IPPE was complete within 15 and 30 min, respectively, after intradermal injection (Figure 1). However, in sites injected with 200 pmol Paf-acether, IPPE remained detectable until 90 min after injection (Figure 4). PGE_2 (1 nmol/site), admixed with Paf-acether potentiated the acute IPPE response (Figure 2), whilst local administration of isoprenaline (4.5 nmol/site) or phenylephrine (6 nmol/site) caused significant reduction of acute IPPE (Figure 3).

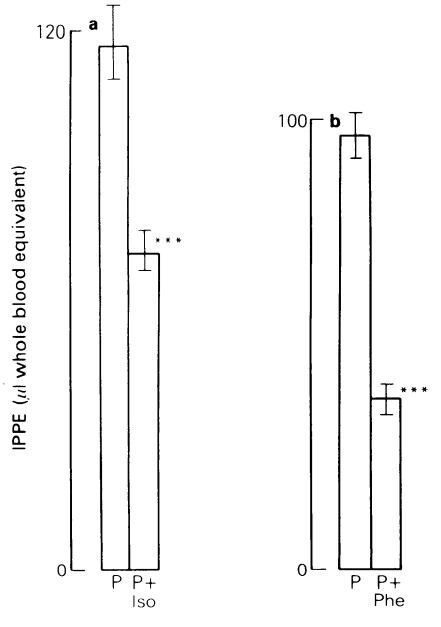

Figure 3 Increased plasma protein extravasation (IPPE) in guinea-pig skin in response to Paf-acether (P; 3 pmol/site) alone or admixed with (a) isoprenaline (Iso; 4.5 nmol/site) or (b) phenylephrine (Phe; 6 nmol/site). IPPE responses are expressed as equivalent μl of whole blood (^{125}I counts in skin/^{125}I counts in 1 μl whole blood). Each histogram column represents the mean IPPE response and the vertical bars indicate ±1 s.e.mean (n=4). Significant inhibition of IPPE induced by Paf-acether is indicated by *** $P<0.001$. All values have been corrected by subtracting the response obtained following injection of diluent alone.

Platelet accumulation

Paf-acether caused dose-related (20 to 200 pmol/site) accumulation of 111-In labelled platelets into guinea-pig skin, whereas lyso-Paf (2 nmol/site) was ineffective (Table 1b). The kinetics of PA, in response to 200 pmol Paf-acether, showed that this response was complete within 1 h of intradermal injection (Figure 4). Local administration of PGE_1 (3 nmol/site) produced only a slight IPPE response, but no significant PA (Figure 5). However, a mixture of Paf-acether (200 pmol/site) and PGE_1 (3 nmol/site) induced a significantly potentiated

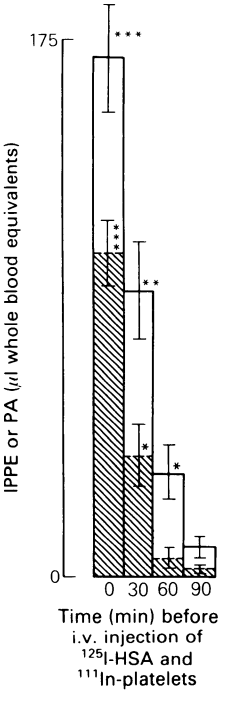

Figure 4 The time course of increased plasma protein extravasation (IPPE) and platelet accumulation (PA) in guinea-pig skin, following intradermal injection of Paf-acether (200 pmol/site). Intradermal injections of Paf-acether were made 90, 60, 30 and 0 min before intravenous injection of ^{125}I-HSA (IPPE) and ^{111}In platelets (PA). Animals were killed 30 min after the double isotope pulse. IPPE and PA responses have been expressed as equivalent μl of whole blood (^{125}I or ^{111}In counts in skin sites/^{125}I or ^{111}In counts in 1 μl whole blood). Each histogram column represents the mean IPPE (open columns) and PA (hatched columns) response occurring in the particular time interval and vertical bars show ±1 s.e.mean (n=4). The significance of IPPE and PA responses is indicated by * $P<0.05$; ** $P<0.025$; *** $P<0.005$. All values have been corrected by subtracting the response obtained following injection of diluent alone.

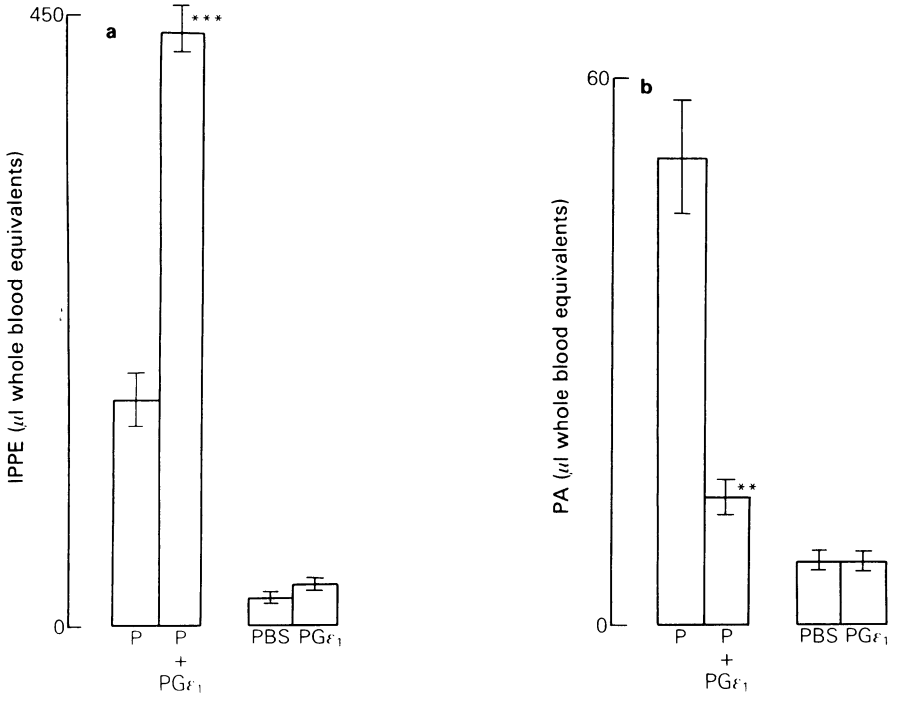

Figure 5 Increased plasma protein extravasation (IPPE) (a) and platelet accumulation (PA) (b) have been measured simultaneously in guinea-pig skin, in response to Paf-acether (P; 200 pmol/site) alone or admixed with prostaglandin E_1 PGE_1, 3 nmol/site). IPPE and PA responses have been expressed as equivalent μl of whole blood (^{125}I or ^{111}In counts in skin/^{125}I or ^{111}In counts in 1 μl whole blood). Each histogram column shows the mean IPPE response (a) or PA response (b) and the vertical bars represent ± 1 s.e.mean ($n=4$). Significant potentiation of IPPE is indicated by *** $P<0.001$ and significant inhibition of PA by ** $P<0.005$. Responses to injection of diluent (PBS) or PGE_1 alone are also shown.

IPPE response, accompanied by substantially reduced PA in the same sites (Figure 5).

Red blood cell accumulation (RBCA)

At sites injected with 200 pmol Paf-acether, modest haemorrhage was noted within 40 min of injection and remained apparent for over 24 h. Paf-acether produced significant RBCA, a response not observed following intradermal injection of lyso-Paf (200 pmol/site) (Table 1c). No significant RBCA occurred beyond the first hour (Table 2).

Table 2 Kinetics of platelet activating factor (Paf-acether) induced red blood cell accumulation (RBCA) in guinea-pig skin

Time interval (h)	*Red blood cell accumulation* (μl)
0–1	5.9 ± 0.5
1–2	0.7 ± 0.3
3–4	0 ± 0.3
7–8	0 ± 0.4
23–24	0 ± 0.4

The time course of RBCA in guinea-pig skin has been measured following intradermal injection of Paf-acether (200 pmol), 0–23 h before intravenous injection of ^{111}In RBC. Animals were killed 1 h after the intravenous injection. RBCA has been expressed as equivalent μl of whole blood (^{111}In counts in skin sites/^{111}In counts in 1 μl whole blood). Values given represent mean RBCA ± 1 s.e.mean ($n=4$) and have been corrected by subtracting the response obtained following injection of diluent alone (3.4 ± 0.2 μl).

Discussion

Paf-acether is one of the most potent platelet aggregating agents yet identified; it is produced by, and has specific effects on, various inflammatory cells, both *in vitro* and *in vivo* (Vargaftig, Chignard, Benveniste, Lefort & Wal, 1981). It induces IPPE after intradermal injection and, in the guinea-pig, is ex-

ceedingly potent, being approximately 1000 times more potent than histamine and more than 100 times more potent than bradykinin. We have observed variation between batches of Paf-acether in their capacity to induce IPPE. This may reflect differences in either batches of guinea-pigs or the chemical composition of Paf-acether preparations. Like histamine or bradykinin, Paf-acether produces a transient IPPE, except at higher doses, when modest extravasation is evident beyond the first 60 min. In addition to IPPE, intermediate doses of Paf-acether produce an accumulation of isotopically labelled platelets and erythrocytes. Histological examination of skin sites fixed 30 min after Paf-acether injection has confirmed the presence of extravasated RBCs as well as an abundance of platelet aggregates. Histological examination of sites fixed 24 h after Paf-acether injection has confirmed the presence of RBCs and revealed an infiltrate of inflammatory cells, including both mononuclear phagocytes and neutrophils (Basran, Morley, Page & Paul, 1982a).

IPPE in response to Paf-acether is significantly potentiated by concomitant administration of the cutaneous vasodilator prostaglandins, PGE_1 or PGE_2, is inhibited by concomitant administration of the β-adrenoceptor agonist, isoprenaline, or by concomitant administration of the α-adrenoceptor agonist, phenylephrine. These observations are consistent with an interpretation that Paf-acether acts at the level of the vascular endothelium to cause increased vessel wall permeability. The inference that Paf-acether produces disjunction of endothelial cells has been confirmed by transmission electron microscopy, which demonstrates such separation associated with passage of colloidal carbon particles across the endothelial lining of post capillary venules at sites of intradermal Paf-acether administration (Dewar, Morley, Page & Paul, 1983).

Since Paf-acether has considerable potency as a platelet aggregating agent, the possibility must be considered that effects of Paf-acether on the vascular endothelium are secondary to platelet activation and subsequent mediator release or formation. PGE_1 is both anti-aggregatory and vasodilator and thus provides an incisive test of the contribution of platelet accumulation to IPPE in response to Paf-acether. The ability of PGE_1 to potentiate significantly IPPE induced by Paf-acether, whilst causing a concomitant reduction of PA within the same site, implies a mechanism for IPPE that is not related to platelet activation. There are other indications that Paf-acether exerts a direct effect on vascular endothelium, since it induces IPPE at much lower concentrations than are necessary to elicit PA (Paul & Page, 1983) and elicits IPPE in rats, in which the platelet release reaction and the accumulation of platelets into Paf-acether-treated skin sites has been inhibited by pretreatment with a drug combination of indomethacin, methysergide and mepyramine (Pirotzky, Page, Roubin, Pfister, Paul, Bonnet, & Benveniste, 1983).

As Paf-acether also attracts and activates polymorphonuclear leucocytes *in vitro* (Vargaftig *et al.*, 1981), and as IPPE to C5a in rabbit skin has been shown to be dependent upon leucocytes (Wedmore & Williams, 1981b), it is possible that IPPE in response to Paf-acether is secondary to leucocyte accumulation. However, IPPE in response to Paf-acether in both rats and rabbits is not influenced by pretreatment with nitrogen mustard (Wedmore & Williams, 1981a; Pirotzky *et al.*, 1983). In addition, isolated perfused preparations of guinea-pig lung which can be assumed to be essentially free of inflammatory cells and platelets, show substantial IPPE following bolus injections of Paf-acether (Basran, Dewar, Morley, Page, Paul & Wood, 1982b). It may be concluded, therefore, that Paf-acether has a direct effect on vessel wall permeability.

The inflammatory properties of Paf-acether do not appear to depend upon its physicochemical properties, since lyso-Paf, whose physicochemical properties closely resemble Paf-acether, produces no IPPE, PA or RBCA following intradermal injection at relatively high concentrations. Because of its considerable potency in aggregating platelets and leucocytes, there has been a tendency to assume that the inflammatory effects induced by Paf-acether are indirect. The present study indicates, rather, that Paf-acether can elicit substantial inflammatory effects by a direct effect on vascular endothelium. Since Paf-acether is produced by a range of inflammatory cells, this material should be considered as a potential mediator of both allergic and non-allergic inflammation.

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