Effect of Platelet-Activating Factor on Microvascular Permselectivity: Dose-Response Relations and Pathways of Action in the Hamster Cheek Pouch Microcirculation

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Platelet-activating factor (PAF) has recently been described as a mediator of inflammatory processes. In this study, we quantitated the dose-response effects of topically applied PAF on microvascular permselectivity and investigated the biochemical pathways of this compound. Permselectivity alterations were assessed by measuring the clearance of macromolecules with fluorescein isothiocyanate dextran 150 (FITC-dx 150) as a tracer. The microvascular bed of the hamster cheek pouch served as a model. PAF was found to induce leakage of macromolecules from postcapillary venules. Control FITC-dx 150 plasma clearance (± SEM) was 72 ± 10 nl/90 min. Clearances of 61 ± 10, 47 ± 14, 62 ± 5, 30 ± 8, and 14 ± 3 nl/90 min were obtained at PAF concentrations of 10⁻², 10⁻⁴, 10⁻⁷, 10⁻⁵, and 10⁻⁷ M, respectively. A one-way analysis of variance showed that the population means were not equal. Multiple comparison by the Student-Newman-Keuls test demonstrated that the clearances obtained with 10⁻², 10⁻⁴, and 10⁻⁷ M were significantly greater than controls. Significant differences existed between 10⁻⁷ M PAF and 10⁻², 10⁻⁴, and 10⁻⁵ M PAF. In an effort to elucidate the biochemical pathways of PAF activity, several inhibitors of the arachidonic acid cascade and receptor blockers were used. Dexamethasone and kadsurenone attenuated the clearance response to PAF in a statistically significant manner, while indomethacin, OKY-046, and chlorpheniramine were without effect. These experiments demonstrate that 1) PAF produces a dose-related extravasation of macromolecules, 2) histamine does not contribute significantly to the leakage response, 3) leukotrienes may be responsible for the increased plasma clearance of macromolecules caused by PAF, and 4) clearance responses are mediated by PAF-receptor interactions. (Circulation Research 1988;62:732-740)

Platelet-activating factor (PAF, 1-O-alkyl-2-O-acetyl-sn-3-phosphorylcholine) has recently been implicated as an important mediator of inflammation. PAF is an endogenous polar lipid that is derived from the membrane phospholipids of various cell types, including many of the blood elements. After intravascular administration, PAF has been shown to induce detrimental pulmonary and cardiac alterations. Increased vascular permeability is another serious consequence of PAF administration. In baboons, hematocrit values increased, and total plasma protein decreased concomitantly after intravenous administration of PAF. Increased hematocrit values were similarly found after intravenous injection of PAF in guinea pigs and dogs. PAF has also been shown to be active when administered locally. After intradermal injection, PAF increased vascular permeability in humans, guinea pigs, and rats.

Since edema is a hallmark of inflammation, the relation between PAF and increased plasma protein extravasation is of significant interest. In this study, the direct effects of PAF on a microvascular bed were examined. Using the hamster cheek pouch preparation as our model, we characterized the dose-response relation between PAF and macromolecular extravasation. We also investigated the pathways through which PAF could be acting by using a series of enzyme inhibitors and receptor blockers.

Materials and Methods

Anesthesia and Surgery

Anesthesia and surgical procedures were similar for all experiments. Male golden Syrian hamsters, weighing 80–110 g, were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Tracheotomy was performed to ensure clear airway passages. The left jugular vein was cannulated for the administration of fluorochrome, supplemental doses of anesthetic, and other drugs when appropriate. The left carotid artery was cannulated for collection of blood samples and monitoring of blood pressure. Arterial pressure was measured and recorded with a Statham P23 series pressure transducer (Hato Rey, Puerto Rico) coupled to a Beckman R511A dynograph (Schiller Park, Illinois). Blood samples were collected for determination of hematocrit and
fluorometric analysis of the plasma. Animals were kept on a heating pad throughout the experiment to maintain body temperature at 37°C.

The right hamster cheek pouch was prepared for direct visual observation and intervention according to the methods of Mayhan and Joyner.13 Briefly, a two-piece Lucite chamber with a 1-ml reservoir capacity was attached to a single layer of the pouch, delineating a 2.3-cm² area for intravital observation. The chamber reservoir was filled with bicarbonate buffer and tested for leakage. The millimolar composition of the buffer was 131.9 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, and 18.0 NaHCO₃. The buffer was adjusted to pH 7.35 and equilibrated with 95% N₂-5% CO₂.

Characterization of the Dose-Response Effects of PAF on Macromolecular Clearance

The protocols followed in this investigation are illustrated in Figure 1. After surgical preparation, the hamster was positioned on a Lucite board and placed on the stage of a Nikon Optiphot microscope. A 1-hour stabilization period ensued, during which the pouch was continuously suffused with bicarbonate buffer solution at a rate of 1 ml/min. A borosilicate glass suffusion system equipped with heat and gas exchangers maintained suffusate temperature at 35°C with the aid of a constant temperature circulating bath (WVR Scientific, San Francisco, California).

Fluorescein isothiocyanate dextran 150 (FITC-dx 150) was used as the macromolecular tracer. The fluorochrome was prepared as a 5% solution in saline with a concentration of 0.005 mg/ml. A borosilicate glass suffusion system equipped with heat and gas exchangers maintained suffusate temperature at 35°C with the aid of a constant temperature circulating bath (WVR Scientific, San Francisco, California).

The calculated clearances represent the plasma clearances of FITC-dx 150 per 75 mg superfused pouch per collection period. Cumulative clearance values for the complete experimental periods were computed by simple addition of the individual clearances. Preparations showing poor blood flow and/or spontaneous macromolecular leakage were discarded at this point. The suffusate was then discontinued, and PAF (10⁻⁵-10⁻³ M) was topically applied to the pouch for 3 minutes. PAF (Sigma Chemical, St. Louis, Missouri) was dissolved in dimethyl sulfoxide (DMSO, Sigma) to a concentration of 10⁻² M and subsequently diluted to the appropriate concentration with a mixture of 1.5% bovine serum albumin (BSA, Sigma) and bicarbonate buffer solution. Each animal received only one dose of PAF. Suffusion was reestablished, and the effluent was collected for an additional 90 minutes. Blood samples, taken through the arterial cannula, were collected for plasma analysis of FITC-dx 150 concentration 5 minutes after FITC-dx 150 administration and then at 30-minute intervals. Both untreated pouches and pouches that received the DMSO/BSA/bicarbonate buffer vehicle served as controls.

For computation of FITC-dx 150 clearance values, both suffusate and plasma FITC-dx 150 concentrations were determined. The plasma fraction was diluted in 10 ml bicarbonate buffer, and the FITC-dx 150 concentration of the diluted sample was fluorometrically determined. The FITC-dx 150 concentration of the plasma was calculated from the plasma dilution factor and the concentration of the diluted sample as follows:

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\text{plasma dilution factor} = \frac{10,000 \, \mu l \text{buffer}}{\mu l \text{plasma}}
\] (1)

\[
\text{plasma concentration of FITC-dx 150} = \text{plasma dilution factor} \times \text{concentration diluted sample}
\] (2)

Clearance (Cl) values for FITC-dx 150 were determined from the ratio of suffusate (s, ng/ml) to plasma (p, mg/ml) concentration multiplied by suffusion flow rate: s/p x 1 ml/min = Cl (ml/min x 10⁻⁴) = Cl (nl/min).

The calculated clearances represent the plasma clearances of FITC-dx 150 per 75 mg superfused pouch per collection period. Cumulative clearance values for the complete experimental periods were computed by simple addition of the individual clearance values. Then, for statistical analysis, the mean clearance values for each experimental group and the controls were calculated. Mean clearance values were compared by a one-way analysis of variance followed by the Student-Newman-Keuls test.

A Perkin-Elmer LS-3 spectrofluorometer (South Plainfield, New Jersey) was used for the fluorometric analysis of suffusion and plasma samples. Fluorescence
was converted to tracer concentration by use of standard curves run in every experiment.

Investigation of the Pathways of PAF Action

The animal was surgically prepared and allowed to stabilize for 1 hour. PAF $10^{-7}$ M was topically applied. The selected inhibitor was administered in an appropriate manner, and FITC-dx 150 clearance was determined as described above. A brief summary for each inhibitor used follows.

Dexamethasone sodium phosphate (Decadron; Merck Sharp & Dohme, Rahway, New Jersey) was administered 45 minutes before application of PAF at a dose of 5 mg/kg i.p. Indomethacin was dissolved in 0.1 M Tris-buffer (pH 8.5) and given 2 hours before topical application of PAF at a dose of 1 or 10 mg/kg i.p. OKY-046 [sodium (E)-3-4-(1-imidazolylmethyl) phenyl-2-propanoate; Ono Pharmaceutical, Osaka, Japan] was dissolved in 0.9% NaCl saline solution and administered at a dose of 1.5 mg/kg i.p. 30 minutes before the PAF challenge. BW755C (3-amino-1-m-trifluoro methyl phenyl-2-pyrazoline hydrochloride; Burroughs Wellcome, Research Triangle Park, North Carolina) was tested at a dose of 1.5 mg/kg i.p. and dissolved in 0.9% NaCl saline solution. It was given 15 minutes before the topical application of PAF. Chlorpheniramine maleate was diluted in saline and given 15 minutes before PAF challenge at a concentration of 2 mg/kg i.v. Kadsurenone [2-[(3,4-dimethoxyphenyl)-2B, 3-dihydro-3a, methoxy-35-methyl-5-(allyl)-6-2H-oxobenzofuran; Merck Sharp & Dohme] was initially dissolved in DMSO to a 100-mM stock solution and subsequently diluted to a 0.5-MM solution in phosphate buffer. It was administered 30 minutes before challenge with PAF at a dose of 100 μg/kg i.v. None of the inhibitors per se, at the administered doses, produced any effect on systemic blood pressure.

Microscopy

Observations were made with a Nikon Optiphot microscope with 6.3×, 10×, 20×, and 32× long-working distance Leitz objectives with 10× Nikon oculars. The microscope is equipped for both transillumination and epi-illumination studies. Brightfield transillumination was provided by a 100 W halogen lamp. Light was delivered to the pouch through a fiber optic system placed in the animal's mouth. An episcopic-fluorescent Ploem attachment was used for fluorescent microscopy. Epi-illumination was provided by a 50 W mercury arc lamp in conjunction with the appropriate filters for fluorescein. An exciter filter for fluorescein (488 nm) was inserted between the mercury lamp and the dichroic mirror, while a barrier filter (515 nm) was positioned between the dichroic mirror and the oculars. The recording system was composed of a Cohu 4410 silicon intensified target (SIT) television camera (San Diego, California) coupled to an RCA time generator, a Sony VO 5858 videotape recorder, and an RCA monochrome video monitor. Still photographs were taken either from the monitor screen with a Nikon EL 2 camera or through the trinocular head of the Nikon microscope with a Nikon Photomicrographic Attachment Microflex PFX. Kodak Tri-X-Pan film was used.

Results

Characterization of the Dose-Response Effects of PAF

Thirty-three hamster cheek pouch preparations were used to fluorometrically quantitate the changes in FITC-dx 150 clearance resulting from topical application of $10^{-9}$ to $10^{-6}$ M PAF. Clearance of FITC-dx 150 was increased above control values with concentrations of PAF between $10^{-8}$ and $10^{-5}$ M. PAF at $10^{-6}$ M did not affect tracer clearance values.

Effect of PAF on FITC-dx 150. A representative time course of FITC-dx 150 clearance for a single experiment with $10^{-7}$ M PAF is presented in Figure 2. After a 20-minute control period wherein baseline fluorescence was determined, PAF was topically applied to the pouch for 3 minutes. Increased FITC-dx 150 was detected fluorometrically in the suffusate 5–10 minutes after initial application. Peak fluorescence occurred 15–20 minutes after application, and the duration of altered permeability was approximately 65 minutes. Visually, leakage began as a number of discrete sites emanating from postcapillary venules. These discrete sites eventually fused together, lending a greenish hue to the pouch. With time, extravasated FITC-dx 150 was washed out (Figure 3).

The visual and fluorometric patterns of FITC-dx 150 clearance for $10^{-8}$, $10^{-7}$, and $10^{-6}$ M PAF were qualitatively similar to that described for $10^{-5}$ M. There was a slight time shift in the mean responses that was not significant. For completeness of information, these data are presented in Table 1.

A dose-response histogram for FITC-dx 150 clearance versus PAF concentration is presented in Figure 4.
In control animals that received the PAF vehicle, DMSO/BSA/bicarbonate buffer, mean clearance was 72 ± 10 (SEM) nl/90 min. This value did not differ significantly from control animals that had no vehicle applied (data not shown). Application of 10⁻⁹ M PAF did not affect plasma clearance of the tracer molecule, resulting in a mean clearance value of 60 ± 10 nl/90 min. Four experiments with 10⁻¹⁰ M PAF yielded a mean clearance value of 78 ± 12 nl/90 min. In single experiments in which lower concentrations of PAF (10⁻¹¹, 10⁻¹², and 10⁻¹³ M) were used, clearance was similarly not increased above control. PAF 10⁻⁸ M was the lowest concentration at which plasma clearance of FITC-dx 150 increased above control values. Mean clearance for this concentration was 474 ± 161 nl/90 min. A maximal clearance value of 622 ± 62 nl/90 min was obtained with topical application of 10⁻⁷ M PAF. Plasma clearance decreased with application of concentrations of PAF greater than 10⁻⁷ M. Mean clearance obtained with 10⁻⁶ M was 301 ± 96 nl/90 min, while that resulting from 10⁻⁵ M was 142 ± 3 nl/90 min.

A one-way analysis of variance on this clearance data indicated that the population means were not equal. Multiple comparison by the Student-Newman-Keuls test demonstrated that the clearances obtained with application of 10⁻⁸, 10⁻⁹, and 10⁻¹⁰ M PAF were significantly greater than control, while the clearance obtained with 10⁻¹³ M was not. PAF 10⁻⁷ M yielded clearance values that were significantly greater than those obtained with 10⁻⁹, 10⁻⁸, and 10⁻⁷ M PAF.

**Effect of PAF on hematocrit and blood pressure.**

Hematocrits ranged between 47% and 59% in individual animals and did not change significantly during any one experiment as determined by the Student’s paired t test. Systemic blood pressures, ranging between 85 and 110 mm Hg, were also not influenced by topical application of PAF.

**Effect of Pharmacological Antagonists on PAF-Induced Clearance of Macromolecules**

Effect of pharmacological antagonists on plasma clearance of FITC-dx 150. The effects of the individual inhibitors and receptor blockers on microvascular clearance were first analyzed. In separate experiments, clearance of FITC-dx 150 was monitored for 1 hour after administration of the test substance. No substance that was tested increased clearance of macromolecules above control values. It was therefore concluded that the inhibitors and receptor blockers were not contributing to the leakages observed after application of PAF. BW755C had an adverse effect on flow in all preparations observed. No other test substance decreased flow. Dexamethasone, indomethacin, BW755C, and OKY-046 had a slight vasodilatory effect on arterioles. No inhibitor altered hematocrit values or affected systemic blood pressures.

**Attenuation of PAF-induced increased clearance of macromolecules by dexamethasone—failure of indomethacin and OKY-046 to alter the response.**

The permselectivity-altering effects of PAF 10⁻⁷ M were attenuated by dexamethasone, a phospholipase A₂ inhibitor. One-way analysis of variance followed by the Student-Newman-Keuls test demonstrated that the clearance obtained with dexamethasone in conjunction with PAF was significantly less than that resulting from

| Table 1. Time Course of Clearance of FITC-dx 150 Induced by PAF |
|------------------|------------------|------------------|
| Concentration (M) | Average onset time (min) | Average application to peak time (min) | Duration of increased clearance (min) |
| Control (7) | ... | ... | ... |
| 10⁻⁹ (6) | ... | ... | ... |
| 10⁻⁸ (5) | 8 ± 4 | 23 ± 8 | 57 ± 12 |
| 10⁻⁷ (6) | 9 ± 7 | 27 ± 11 | 64 ± 2 |
| 10⁻⁶ (5) | 11 ± 4 | 25 ± 11 | 56 ± 10 |
| 10⁻⁵ (4) | 13 ± 5 | 19 ± 11 | 54 ± 14 |

Clearance was considered elevated at values greater than twice baseline. Numbers in parentheses indicate number of animals. Values are given as mean ± SD.

FITC-dx 150, fluorescein isothiocyanate dextran 150; PAF, platelet-activating factor.
PAF 10^-7 M alone but significantly greater than the clearance values obtained in the control situation wherein no PAF was applied. This result suggested that arachidonate metabolites were involved in the clearance response. To investigate the involvement of cyclooxygenase products, animals were pretreated with indomethacin before PAF challenge. Indomethacin pretreatment did not alter the clearance response to topically applied PAF (Figure 5). OKY-046, the specific thromboxane synthetase inhibitor, similarly did not influence the permselectivity-altering effects of PAF (Figure 5). In one experiment with BW755C, which is an equipotent inhibitor of cyclooxygenase and lipoxygenase enzymes, the clearance response was attenuated to the same extent as with dexamethasone.

Ineffectiveness of chlorpheniramine maleate, the H1-receptor antagonist, to alter PAF-induced increased clearance. The histamine H1-receptor antagonist, chlorpheniramine maleate, did not alter the increased clearance response to PAF (Figure 5). The same concentration of chlorpheniramine completely blocked the vasodilation and increased permeability caused by topical application of 50 μg/ml histamine, demonstrating the efficacy of the administered dose.

Attenuation of PAF-induced increased clearance by the specific PAF-receptor antagonist kadsurenone. To test whether PAF-induced increased clearance was receptor mediated, kadsurenone, a newly synthesized receptor blocker of PAF, was administered before PAF application. Kadsurenone pretreatment attenuated the permeability-altering effects of PAF (Figure 5). One-way analysis of variance followed by the Student-Newman-Keuls test demonstrated that the clearances obtained with kadsurenone were significantly less than those resulting from PAF 10^-7 M alone and significantly greater than the clearance values obtained in the control wherein no PAF was applied.

Discussion

Our study focused on the direct effects of PAF on a microvascular bed. We characterized the dose-response relations between PAF and macromolecular clearance and investigated possible pathways of PAF activity. Our findings demonstrate that 1) PAF significantly increases the plasma clearance of molecules at concentrations of 10^-8-10^-6 M; 2) maximal clearance is obtained at 10^-7 M, after which there is a decline toward control; 3) leukotrienes may be responsible for the increased clearance of macromolecules caused by PAF; 4) histamine does not contribute significantly to the increased macromolecular clearance; and 5) clearance responses are mediated at least partially by PAF-receptor interactions. Our results demonstrate that the effects of PAF on the microvasculature bear a number of similarities to the microvascular phenomena observed in inflammation, and these lend further support to the contention that PAF is an important inflammatory mediator.

Characterization of the Dose-Response Microvascular Effects of PAF on Macromolecular Clearance

Clearance of macromolecules in our experimental conditions is dependent upon microvascular permeability, microvascular surface area available for exchange, transport driving forces across the microvascular wall, tissue diffusion characteristics, and concentration differences between tissue and suffusate. Normally, it is difficult to clearly determine the relative contributions of these factors to the measured overall blood-tissue transport. For several reasons, we believe that the changes in FITC-dx 150 clearances reported here largely reflect alterations in microvascular permselectivity. First, our experimental evidence shows that 10^-4 M PAF produces a comparable increase in FITC-dx 150 clearance regardless of whether that same concentr-
tion induces vasoconstriction or vasodilation. These opposite hemodynamic effects, which should lead to different changes in blood flow, microvascular pressure distributions, and possibly surface area, produce a similar clearance increment. Second, vasodilation per se is not sufficient to induce the observed magnitude of clearance changes in the hamster cheek pouch. Third, increased clearance is intimately associated with the visualization, under the fluorescence microscope, of leakage sites, which in this tissue have been related to increased separation of interendothelial gaps and, thus, to changes in permeability. Finally, the experimental conditions, except for pretreatment with inhibitors, were always the same. For these reasons, we interpret our measured increased clearances as an index of increased microvascular permeability.

Possible mechanisms of increased macromolecular permselectivity. Majno and Palade reported that the increased permeability of blood vessels after treatment with histamine and serotonin was due to partial dissociation of the endothelial sheet resulting from gaps between endothelial cells. Contractions of endothelial cells were also found after bradykinin administration, and contractile proteins have been demonstrated in endothelial cells. It is possible that PAF acts upon the vascular endothelium in a similar manner and increases macromolecular clearance by inducing endothelial cell contraction.

Microvascular permeability could also be increased by a polymorphonuclear-dependent mechanism. With this mechanism, neutrophils adhere to venular walls and in an unknown way induce vascular leakage. This mechanism has been postulated for several permeability-altering agents such as LTC₄, C₅a, and FMLP. Björk et al reported that pretreatment with neutrophil antiserum reduced the number of leakage sites resulting from topical application of PAF at 2×10⁻⁵ M but not at 5×10⁻⁶ M PAF. Humphrey et al described an early and late-phase permeability response to PAF in rabbits and attributed the late phase to neutrophil recruitment and activity. The two phases overlapped in skin-blueing models in guinea pigs. In contrast to studies supporting a role for PMNs in PAF-induced leakage, Pirotsky et al found no difference in permeability due to PAF after treatment with nitrogen mustard in rats.

While we found that PAF induced white cell adhesion, it is not possible to define the role, if any, that neutrophils played in increasing permeability in our experiments. Visually and fluorometrically, only one fluorescent peak was detected after application of PAF. Adhering leukocytes may contribute to a second permeability phase that is masked by our collection methods. In vitro, PAF was found to have profound effects on neutrophil activity in the dose range tested, 10⁻⁸ to 10⁻⁵ M. Enzyme secretion, superoxide production, migration, aggregation, and increased turnover of arachidonic acid by polymorphonuclear leukocytes (PMNs) are among the events that could be of importance in increasing vascular permeability.

Nature of the clearance response. Increased clearance of FITC-dx 150 induced by 3-minute topical applications of PAF were of a transient nature. Elevated levels of FITC-dx 150 were detected fluorometrically in the suffusate 5-10 minutes after initial application, peaked at 15-20 minutes after application, and declined toward control thereafter. Several factors could explain the transience of the response. First, the half-life of PAF in plasma is very short, ranging between 5 and 30 seconds. Thus, biologically active PAF disappears from the system shortly after the period of application terminates. Second, if PAF effects are receptor-mediated, the duration of the response could depend on the period of receptor interaction. Finally, if endothelial cell contraction is the mechanism underlying increased clearance of macromolecules, endothelial relaxation would reverse this process, restoring the natural sieving characteristics of the endothelial barrier. Even with concentrations of PAF as high as 10⁻³ M, the natural sieving characteristics of the endothelial membrane are eventually restored, indicating that the PAF-induced increases are reversible and not the result of irreversible damage.

Sites of PAF-induced leakage. PAF-induced leakage sites in the vascular bed originated mainly from post-capillary venules. This finding agrees with previous reports for PAF and is consistent with studies of other permeability-altering agents such as histamine, serotonin, LTC₄, and LTD₄. The preponderance of leakage from postcapillary venules could be due to higher receptor densities for permeability-altering agents or morphological predisposition.

Dose-response histogram: Decrease in tracer clearance with higher concentrations of PAF. Direct application of PAF to the hamster cheek pouch microcirculation increases macromolecular extravasation in a dose-dependent manner. An interesting aspect of the dose-response curve for PAF-induced clearance of macromolecules is the decrease in FITC-dx 150 extravasation that occurs with 10⁻⁶ and 10⁻⁵ M PAF. This decrease in clearance may reflect the vasoconstrictor properties of PAF as well as its impact on flow. If endothelial cell contraction and formation of interendothelial junctions is the mechanism by which PAF increases macromolecular clearance, concentrations that maximally open gaps for the longest period of time would have the greatest potential for increased clearance. Once gaps were opened, leakage would be dependent upon both the rate of blood flow past endothelial junctions and the relative capillary and interstitial hydrostatic pressures. With the lower doses of PAF tested, 10⁻⁸ and 10⁻⁷ M, blood flow did not appear to be greatly affected. However, with the higher doses, 10⁻⁶ and 10⁻⁵ M, flow stopped for 1-15 minutes. If PAF affects gap widening in a time-dependent manner, as bradykinin does, it is possible that there was no flow for much of the time that junctions were open. This would explain the decreased clearance in preparations where flow was stopped compared with those where flow was unaffected. Support for a flow-related explanation of the biphasic curve can be found with the indomethacin and OKY-046 clearance data. These inhibitors prevented PAF-
induced vasoconstriction and alterations in flow. When PAF $10^{-7}$ M was administered after indomethacin or OKY-046 pretreatment, flow was not compromised, and clearance values increased.

The reduction in flow caused by high concentrations of PAF could also protect against leakage if PMNs are involved in increasing FITC-dx 150 clearance. By lowering the number of neutrophils exposed to PAF, the total amount of permeability-altering agents released from these cells would be minimized. It is noteworthy that in vitro, PMNs' enzyme secretion induced by PAF was decreased when they were exposed to PAF concentrations between $10^{-6}$ and $10^{-5}$ M. These are the exact concentrations at which a reduction in permeability occurs. The investigators hypothesized that the decrease in secretion was due to simultaneous desensitization of the PMN to PAF.

Higher doses of PAF could also decrease plasma clearance of FITC-dx 150 by inducing production of materials that oppose endothelial cell contraction. Vasopressin, serotonin, and high doses of glucocorticoids antagonize the protein efflux induced by arteriolar infusion of histamine. The mechanism of action for these other agents, thought to be direct on the microvascular membrane, is not known. The decrease is independent of histamine receptor blockade, blood flow, microvascular pressure, and perfused surface area.

**Pathways of PAF Activity**

To investigate the pathways through which PAF increased clearance of macromolecules, four inhibitors of enzymes in the arachidonic acid cascade, a histamine receptor blocker, and kadsurenone, a receptor blocker for PAF, were tested. Dexamethasone, BW755C, and kadsurenone attenuated but did not prevent the increase in clearance of macromolecules resulting from topical application of PAF. Indomethacin, OKY-046, and chlorpheniramine were without effect on the clearance response.

**Role of enzymes in the arachidonic acid cascade.**

Metabolites of arachidonic acid are suspect in PAF activities for several reasons. First, in rats, PAF-stimulated platelets and neutrophils have increased phospholipase A$_2$ activity, producing a greater turnover of arachidonic acid and thus increasing the potential to release both cyclooxygenase and lipoxygenase products. Second, after intravascular administration of PAF, plasma levels of thromboxane B$_2$ are increased in dogs and baboons. Finally, in isolated lung preparations, PAF infusion results in increased thromboxane A$_2$ and leukotriene production.

To test whether the arachidonic pathway was in any way involved in PAF activity, the effects of dexamethasone were first assessed. Dexamethasone decreases the amount of arachidonic acid available for conversion to cyclooxygenase and lipoxygenase products. Dexamethasone pretreatment significantly reduced the amount of FITC-dx 150 cleared after topical application of PAF. These results implicated arachidonic metabolites as mediators of PAF-induced activities, but they indicated nothing of the relative contributions of the cyclooxygenase and lipoxygenase pathways.

Animals were next pretreated with indomethacin, a nonsteroidal anti-inflammatory drug that inhibits cyclooxygenase activity. Indomethacin did not reduce the amount of FITC-dx 150 cleared after topical application of PAF. This finding is supported by other investigators. Using a skin-bluing model in rats, Pirotzky et al reported similar increases in plasma protein extravasation after intradermal injection of PAF in both indomethacin pretreated and nontreated animals, while Bjork et al counted similar numbers of leakage sites from postcapillary venules in treated and untreated hamsters after topical application of PAF to the hamster cheek pouch.

The fact that dexamethasone decreases PAF-induced clearance of macromolecules, while indomethacin does not, implicates leukotrienes in the leakage process. Leukotrienes B$_4$, C$_4$, and D$_{4,23,24,30}$ have been shown to increase protein plasma extravasation in a variety of models. Other possible actions of dexamethasone should be considered. Dexamethasone inhibits vascular smooth muscle contraction, and by a similar mechanism, it may inhibit endothelial cell contraction. This could prevent gap formation and thereby reduce the amount of leakage incurred. Detsouli et al found that inhibitors of PLA$_2$, inhibit PAF-induced contraction of lung parenchymal strips. Dexamethasone is also a membrane-stabilizing drug. It inhibits platelet aggregation and release, as well as the release of lysosomal hydrolase, presumably through increasing levels of cyclic adenosine monophosphate (cAMP). When cAMP is elevated, calcium moves into storage areas. Bennett et al found that after subplantar injection in the rat paw, prednisolone, L-cysteine, anticalcic drugs, theophylline, and prostaglandin I$_1$, decrease PAF-induced edema. All of these drugs increase cAMP activity.

To determine whether the effects of dexamethasone were due to inhibition of phospholipase A$_2$ or some other influence, animals were pretreated with BW755C. Unfortunately, after administration of BW755C, flow in the pouch diminished and never recovered to preadministration rates. In one experiment in which clearance was determined, clearance was lower compared with PAF alone, which was comparable to the results obtained with dexamethasone. This finding supports the involvement of leukotrienes in increased clearance, but absolute clarification of this issue is dependent upon the development of a specific lipoxygenase inhibitor.

**Role of histamine.** Chlorpheniramine maleate, a competitive inhibitor of histamine at H$_1$-receptor sites, did not reduce the amount of FITC-dx 150 cleared after topical application of PAF to the hamster cheek pouch. The same concentration of chlorpheniramine completely blocked histamine-induced increases in clearance and vasodilation. This finding is consistent with other studies. Humphrey et al reported that histamine was not involved in PAF-induced increased clearance.
vasomotor tone and vascular permeability may be modulated according to the particular needs of the tissue.

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References
16. Gawlowski DM, Duran WN: Dose-related effects of adenosine and bradykinin on microvascular permeability to macro-

in rabbit and guinea pig skin, while Piotrzkzy et al11 reported similar findings in the rat. Also, histaminergic receptors reportedly are not involved in the hypotension produced in rats after intravenous administration of PAF.24 These initial findings adequately demonstrated that PAF effects were not mediated through histamine.

Role of kadsurenone. Kadsurenone attenuated the leakage response and inhibited the vasoconstrictor response to PAF. This finding, coupled with the fact that both neutrophils and platelets have been found to possess specific receptor sites for PAF, indicates that PAF actions are at least partially receptor mediated.

Sites of mediator production and release. While potential substances involved in mediating PAF activity have been identified in this study, the source of the mediators has not yet been established. Several studies have found that platelets12,25,26 and neutrophils13,12,27 are not involved in increasing plasma protein extravasation after PAF administration. Doebber et al14 found that the increase in plasma protein extravasation was concomitant with increased levels of lysosomal hydrolase (glucosaminidase) but independent of neutrophils. Platelets and neutrophils were similarly found not to be involved in PAF-induced edema and vasoconstriction in rat isolated lung preparations.25,26 Further testing is required to determine which cells are responsible for mediator production and release.

Overall Assessment of PAF Significance in the Microcirculation
A better understanding of inflammatory processes is of immediate significance to both basic and clinical sciences. The results of our work support the hypothesis that PAF is involved in inflammation. We contribute a quantitative characterization of the dose relations between PAF concentration and macromolecular leakage as well as vasomotor activity.15 The elucidation of the pathways of PAF action is also important for the development and evaluation of new therapeutical modalities in the treatment of inflammation.

The physiological significance of this work resides in the demonstration of the close interactions between PAF and the arachidonic acid cascade in the microcirculation, which strongly suggests that PAF may play an important role in microcirculatory control mechanisms. PAF may serve as either a mediator or a modulator of microcirculatory dynamics. In either case, its very short half-life makes it a potential candidate for the moment-to-moment control of the microvascular environment. Since microcirculatory dynamics appear to be controlled by a redundant system of regulatory substances, more work will be needed to elucidate the specific interactions of PAF with other microvascular control systems. Release of PAF triggers a series of reactions that elevate cellular and plasma concentrations of arachidonic acid derivatives. In turn, the levels of these compounds provide a feedback mechanism that reduces the synthesis or release of PAF. As the result of these interactions, vasomotor tone and vascular permeability may be


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