Vasoconstrictor Effects of Platelet-Activating Factor in the Hamster Cheek Pouch Microcirculation: Dose-Related Relations and Pathways of Action

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Platelet-activating factor (PAF) has been implicated as a potential mediator of inflammatory processes. In this study, we quantified the effects of PAF on vessel diameter in a microvascular bed and investigated the biochemical pathways of this compound. The hamster cheek pouch microcirculation was observed with intravital microscopy. Experiments were video-recorded and analyzed with an image shearing device. Vasoconstriction was the predominant vasomotor response to PAF. PAF (10^{-10} - 10^{-7} M) was applied topically to the pouch for 3 minutes. Arterioles ranging in size from 8 to 15 μm were the most sensitive, and they constricted completely in response to PAF 10^{-7} and 10^{-6} M. Arterioles 21–40 μm in diameter constricted to 12-17% of control after PAF at 10^{-7} and 10^{-6} M, respectively; they reopened to about 70% of their control value after a few minutes and remained near that size throughout the experiment. Arterioles 41–60 μm in diameter constricted to about 20% control size in response to 10^{-7} and 10^{-6} M PAF, and by the end of the experiment, these vessels had returned to about 90% control size. To determine the pathways of PAF actions, inhibitors of the arachidonic acid cascade and receptor blockers were used. Dexamethasone, indomethacin, OKY-046 (a thromboxane A2 synthetase inhibitor), and kadsurenone (a PAF-receptor blocker) blocked the vasoconstrictor response to PAF. Our experiments demonstrate that PAF-produced arteriolar constriction in a microvascular bed is 1) dose-related, 2) dependent upon vessel size, 3) largely due to thromboxane A2 activity, and 4) mediated by PAF-receptor interactions. (Circulation Research 1988;62:722–731)

Platelet-activating factor (PAF, 1-0-alkyl-2-0-acetyl-sn-3-phosphoryl-choline), a polar phospholipid derived from membrane phospholipids, is a putative mediator of inflammatory processes. It is produced by a variety of inflammatory cell types in both animals and humans, activates the cells involved in inflammation, and has been implicated in specific inflammatory processes such as immunoglobulin E-induced anaphylaxis and immune complex disease. PAF elicits a diversity of hemodynamic effects in vivo. When administered intravascularly, it induces pulmonary hypertension, systemic hypotension, detrimental cardiac alterations, transient neutropenia and thrombocytopenia, and increased vascular permeability. Plasma levels of thromboxane B2, platelet factor 4, and 6-keto PGF_2α are increased subsequent to intravascular administration of PAF, and the substance also causes platelets and polymorphonuclear leukocytes (PMNs) to sequester in the small blood vessels of various organs. In contrast with reports of hypotension after intravascular administration, intradermal injection of PAF induces localized vasoconstriction in human and guinea pig skin.

The mechanisms or pathways through which PAF alters vascular caliber are not known. Vasoactivity after intravascular administration could be mediated centrally, while the vasoconstriction induced by topical application or intradermal administration of PAF is most likely a local event. Since PAF is released by many blood-borne elements, an understanding of the local effects of PAF on microvascular variables is of importance.

In the present study, we characterized the effects of PAF on arteriolar diameter in the microvascular bed of the hamster cheek pouch. Possible pathways through which PAF could be exerting its effects were then examined using pharmacological inhibitors of enzymes in the arachidonic acid cascade as well as a receptor blocker for PAF.

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 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. 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 Greenblatt et al20 and Click et al.21 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 solution 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 with a Cole Palmer 5987 digiphase pH meter (Chicago, Illinois) and equilibrated with 95% N₂-5% CO₂.

Characterization of the Dose-Response Effects of PAF on Arteriolar Diameter

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 (VWR Scientific, San Francisco, California).

Fifteen minutes before and immediately preceding application of PAF, the transilluminated cheek pouch was scanned, and selected vessels were video-recorded for determination of control diameters. The suffusate was then discontinued, and PAF (10⁻⁴, 10⁻⁵, and 10⁻⁶ 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. After 3 minutes of PAF application, suffusion was reestablished. The video record was kept on continuously during PAF application and for 10 minutes after initial application. Vessels were recorded again at 20, 30, 40, 50, and 60 minutes after initial application of PAF. Both untreated pouches and pouches that received the DMSO/BSA/bicarbonate buffer solution served as controls. Vessel diameters were measured from a replay of the videotape with a video image shearing monitor model 907 (Instrument for Physiology and Medicine, San Diego, California). The instrument was calibrated with a slide micrometer.

Analysis

Vessels were categorized into one of three size groups: 8-15 μm, 21-40 μm, and 41-60 μm. Control diameters were normalized to a value of one, and experimental diameters were expressed as a ratio of control. The relative luminal diameters for each time point were averaged and plotted as relative luminal diameter versus time. Regression equations for the various time segments were determined for the curves within each size group according to dose, and the slopes of the curves were compared for statistical differences with the IBM ANOVA statistical analysis package.

Determination of Blood Pressure and Hematocrit

Blood samples were collected in duplicate from the arterial cannula 5 minutes before PAF application and then at 30-minute intervals. Hematocrits were determined after centrifugation of blood samples. Arterial pressures were continuously recorded on a Beckman dynograph (Schiller Park, Illinois) with a Statham pressure transducer.

Microscopy

Observations were made with a Nikon Optiphot microscope with 6.3 x, 10 x, 20 x, and 32 x long-working distance Leitz objectives with 10 x Nikon oculars. The microscope is equipped for both transillumination and epi-illumination. Bright-field 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. 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 video tape recorder, and an RCA monochrome video monitor TC1217. 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.

Investigation of the Pathways of PAF Action

The select inhibitor or receptor blocker was administered in an appropriate manner, and vessel diameter was determined as described above. PAF 10⁻⁷ M was topically applied, and vessels with control diameters ranging between 21 and 40 μm were observed. A brief summary for each inhibitor used follows.

Dexamethasone sodium phosphate (Decadron; Merck Sharp & Dohme, Ralway, New Jersey) was injected at 10 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 was 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 and dissolved in 0.9% NaCl saline solution. It was given intraperitoneally 15 minutes before the topical application of PAF. Kadsurenone [2-(3,4-dimethoxyphenyl)-2B, 3-dihydro-3a, methoxy-3B-methyl-5-(allyl)-6-2H-oxobenzofuran; Merck Sharp & Dohme] was used to test the hypothesis that PAF actions are receptor mediated. Kadsurenone was initially dissolved in DMSO to a 100 mM stock solution and subsequently diluted to a 0.5 mM solution in phosphate buffer. Kadsurenone was administered intravenously 30 minutes before challenge with PAF at a dose of 100 µg/kg i.v. At the administered doses, none of the inhibitors affected systemic blood pressure.

Results
Characterization of the Dose-Response Effects of PAF on Arteriolar Diameter

Twenty-six hamster cheek pouch preparations were used to assess the effects of PAF 10^{-5}, 10^{-7}, and 10^{-9} M on arteriolar diameter in a microvascular bed. The three doses tested induced vasoconstriction of the small arterioles observed (Figure 1). The time of onset, intensity, and duration of the vasoconstrictor response varied with both vessel size and the concentration of PAF applied. Arteriolar diameters were not significantly altered by topical application of the PAF vehicle, DMSO/BSA/bicarbonate buffer.

The time courses of the response of arterioles to PAF are presented in Figures 2–4. Figure 2 illustrates the effects of PAF on the smallest group of arterioles observed, those ranging in size from 8 to 15 µm. PAF 10^{-5} M had the most profound effect on these vessels, causing an immediate constriction that lasted for 9 minutes. The constriction was so severe in these vessels that the lumen was not detectable. Between 10 and 30 minutes after application, the vessels underwent a great deal of vasomotion, varying between 30% and 100% control size. At 40 minutes after PAF, arterioles assumed a diameter approximately 70% of control and remained that size through the end of the experiment. The effect of PAF 10^{-7} M on these vessels was less severe. The average initial response of arterioles was

![Figure 1](image1)

**FIGURE 1.** Arteriolar constriction induced by topical application of 10^{-7} M PAF. Top panels depict arteriole with epi-illumination: A, before PAF application; B, 2 minutes after application. Bottom panels depict an arteriole with transillumination: A, before PAF application, the arteriole is approximately 40 µm in diameter; B, after PAF application, the arteriole constricted to approximately 20 µm.

![Figure 2](image2)

**FIGURE 2.** Time course of PAF-induced constriction for arterioles between 8 and 15 µm in diameter. For PAF 10^{-5} M, 22 vessels were observed in 10 animals; average luminal diameter was 12.7 ± 2.4 µm. For PAF 10^{-7} M, 25 vessels were observed in 6 animals; average luminal diameter was 10.1 ± 2.3 µm. For PAF 10^{-9} M, 20 vessels were observed in 5 animals; average luminal diameter was 11.6 ± 2.7 µm. Data presented as mean ± SD.
a slight vasodilation that occurred 1 minute after application and was of very short duration. The vessels then constricted completely for 2 minutes. Five to 8 minutes after initial application, vessels opened to approximately 50% of their control diameters. At 10 minutes, vessels were at or slightly greater than control size. Twenty minutes after application, vessels decreased to approximately 80% of their control diameter and remained this size throughout the 60-minute experimental period. After application of 10^-5 M PAF, arterioles on the average retained their control sizes for 2 minutes. Constriction began 3 minutes after application. Maximal constriction occurred at 7 minutes, averaging 12% of control size. Arterioles opened to an average value of 70% control at 8 minutes. From this time to the end of the experimental period, arterioles underwent a great deal of vasomotion, ranging in size from 65% to 125% control diameter.

Arterioles 21-40 \( \mu m \) in diameter also constricted after exposure to PAF (Figure 3). In this size range, the responses to 10^-4 and 10^-3 M PAF were similar. Vessels began to constrict immediately upon application and reached maximal constriction at 3 minutes for 10^-7 M and at 4 minutes for 10^-5 M PAF. Maximal constriction values averaged 12% and 17% control size for PAF 10^-7 and 10^-5 M, respectively. The vessels then began to reopen. With 10^-3 M PAF, vessels opened to an average of 70% control value 7 minutes after initial application and remained near that size throughout the experiment. Arterioles also opened to approximately 70% of their control diameter after 7 minutes and 20-60 minutes after application of 10^-3 M PAF. However, between 8 and 20 minutes, vessels constricted to 20-40% control size. After application of 10^-4 M PAF, arterioles 21-40 \( \mu m \) initially underwent a wide variation in response. Between the time of application and 8 minutes afterward, some vessels constricted, others dilated, and still others appeared unaffected. The mean of these varied responses was an initial constriction to 70% control followed by a 2-minute dilation to 120% control. Between 3 and 7 minutes, there was a second vasoconstriction (80% control), which was followed at 8 minutes by another dilation (120% control). The vessels returned to approximate control sizes 10 minutes after the initial application and remained at these diameters throughout the experiment.

PAF 10^-7 and 10^-5 M also induced vasoconstriction in the largest group of arterioles tested, those ranging between 41 and 60 \( \mu m \) in diameter (Figure 4). As with the 21-40-\( \mu m \) group, the response profiles for these two doses were similar, with 10^-5 M slightly more effective. After topical application of 10^-5 M PAF, arterioles immediately began to constrict. Maximal constriction was obtained 2-4 minutes after initial application, decreasing to approximately 20% control diameter. The vessels began to open 5 minutes after application and hovered at control size between 7 and 20 minutes. The vessels closed again slightly to 75% control at 30-40 minutes after PAF, then opened to 90% control diameter by the end of the experiment. Vessels did not respond to 10^-7 M PAF the first minute after application. They began to constrict 2 minutes afterward and reached a maximal constriction value of 20% control 4 minutes after application. The vessels started to reopen 5 minutes after PAF and remained at approximately 50% control between 5 and 8 minutes. The vessels then opened farther to approximately 75% control size 10 minutes after application and remained near this size through the end of the experiment. After application of 10^-9 M PAF, vessels 41-60 \( \mu m \) in diameter underwent some initial vasomotion that subsided at 9 minutes. Throughout most of the experiment, the diameters for this size vessel ranged between 90% and 110% control.

For purposes of statistical analysis, every curve showing vasomotor response was divided into three time periods: 1 to 4 minutes, 4 to 10 minutes, and 10 to 60 minutes. These time periods were chosen because, in general, they reflected the three major
phases each vessel experienced when subjected to PAF. These phases consisted of 1) a time interval to maximal vasoconstriction; 2) a time interval for vessel reopening (rapid rate of recovery); and 3) a time interval of slow recovery and stabilization, normally below control level. Regression equations for each of the curves were determined. The slopes and 95% confidence intervals of the curves within each vessel-size group for each time interval were then compared for statistical differences. The slope of each curve is a measure of the rate change of vessel diameter over each time interval for each dose of PAF. The 95% confidence interval is a measure of the relative size of the vessel over each time interval. This is clearly shown in Figure 2.

The regression analyses, slope comparisons, and graphs can be used to identify trends in the responses of different sized vessels to increasing concentrations of PAF. For every vessel-size group and time interval, the curves obtained with PAF $10^{-3}$ M differed significantly from control with respect to either slope, 95% confidence interval, or both. With two exceptions, the same differences hold true for PAF $10^{-7}$ M, while PAF $10^{-9}$ M rarely induced curves that differed significantly from control.

Within each size group and time interval, the slopes of the curves for PAF $10^{-7}$ and $10^{-8}$ M differed significantly from each other only once, between 1 and 4 minutes for 8–15-μm vessels. The graphs show that, in fact, the response of vessels to these two concentrations of PAF are very similar. In most cases, smaller arterioles were more strongly affected by PAF than larger ones. In all cases, higher concentrations of PAF constricted the arterioles to an equal or greater extent than lower concentrations.

In separate experiments, $10^{-6}$ and $10^{-7}$ M PAF were applied to the pouch. Both concentrations produced arteriolar constrictions similar to $10^{-7}$ and $10^{-8}$ M PAF. Arteriolar responded to PAF $10^{-9}$–$10^{-10}$ M with slight vasodilation but no notable vasoconstriction. A limited study demonstrated that venular size was not significantly affected by topical application of PAF. Venular flow became sluggish after PAF challenge, but size remained fairly constant.

### Table 1. Comparison of Regression Lines for PAF-Induced Constriction

<table>
<thead>
<tr>
<th>PAF concentration</th>
<th>1–4 min</th>
<th>4–10 min</th>
<th>10–60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>8–15 μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.946 + 0.010 t†§</td>
<td>0.952 - 0.004 t*§</td>
<td>0.978 + 0.001 t§</td>
</tr>
<tr>
<td>$10^{-9}$ M</td>
<td>1.498 - 0.262 t*§</td>
<td>-0.225 + 0.114 t</td>
<td>0.812 + 0.001 t</td>
</tr>
<tr>
<td>$10^{-7}$ M</td>
<td>1.168 - 0.328 t*</td>
<td>-0.053 + 0.096 t</td>
<td>0.837 + 0.001 t</td>
</tr>
<tr>
<td>$10^{-5}$ M</td>
<td>0.0</td>
<td>-1.11 + 0.169 t</td>
<td>0.803 - 0.002 t</td>
</tr>
<tr>
<td>21–40 μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.046 - 0.001 t†§</td>
<td>0.917 + 0.013 t§</td>
<td>1.136 - 0.004 t†§</td>
</tr>
<tr>
<td>$10^{-9}$ M</td>
<td>1.078 - 0.066 t</td>
<td>0.623 + 0.046 t§</td>
<td>0.879 + 0.001 t*</td>
</tr>
<tr>
<td>$10^{-7}$ M</td>
<td>0.916 - 0.159 t</td>
<td>-0.168 + 0.096 t</td>
<td>0.919 - 0.006 t</td>
</tr>
<tr>
<td>$10^{-5}$ M</td>
<td>0.423 - 0.081 t</td>
<td>0.299 + 0.020 t</td>
<td>1.005 - 0.006 t</td>
</tr>
<tr>
<td>41–60 μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.006 - 0.004 t*†§</td>
<td>1.005 - 0.004 t§</td>
<td>0.671 - 0.066 t*†</td>
</tr>
<tr>
<td>$10^{-9}$ M</td>
<td>0.965 + 0.013 t*†§</td>
<td>1.104 - 0.016 t*§</td>
<td>0.735 + 0.007 t</td>
</tr>
<tr>
<td>$10^{-7}$ M</td>
<td>1.126 - 0.248 t</td>
<td>0.120 + 0.063 t</td>
<td>0.871 - 0.003 t</td>
</tr>
<tr>
<td>$10^{-5}$ M</td>
<td>0.648 - 0.129 t</td>
<td>0.121 + 0.100 t</td>
<td>0.826 + 0.001 t</td>
</tr>
</tbody>
</table>

All diameters normalized to control for each time interval. Vessels are grouped according to control diameter in micrometers. All table entries are the regression equation for the change in diameter with time during the time interval. *Slope is significantly different from $10^{-5}$ M PAF ($p<0.01$); †slope is significantly different from $10^{-9}$ M PAF ($p<0.01$); and ‡slope is significantly different from that obtained with $10^{-7}$ M PAF ($p<0.01$). §Diameters are significantly larger than with $10^{-3}$ M PAF (based on their 95% confidence intervals); ‡diameters are significantly larger with $10^{-7}$ M PAF (based on their 95% confidence intervals).

PAF: platelet-activating factor.
Abrogation of PAF-Induced Arteriolar Constriction by vasoconstrictor response to topically applied PAF Inhibitors That Prevent Formation of Cyclooxygenase the Constrictor Response

PAF-Induced Arteriolar Constriction

Effect of Pharmacological Antagonists on PAF-Induced Arteriolar Constriction

In an effort to elucidate the pathways through which PAF induced arteriolar constriction in the hamster cheek pouch, select enzyme inhibitors and pharmacological receptor antagonists were used in conjunction with PAF. In all experiments, PAF 10^{-7} M was applied, and only vessels with initial luminal diameters ranging between 21 and 40 μm were observed. Data shown as mean ± SD.

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 pressure, ranging between 85 and 110 mm Hg, was also not influenced by topical application of PAF.

Effect of Pharmacological Antagonists on PAF-Induced Arteriolar Constriction

In an effort to elucidate the pathways through which PAF induced arteriolar constriction in the hamster cheek pouch, select enzyme inhibitors and pharmacological receptor antagonists were used in conjunction with PAF. In all experiments, PAF 10^{-7} M was applied, and only vessels with initial luminal diameters ranging between 21 and 40 μm were observed. The dose used was chosen because it produced a reproducible, strong, long-lasting vasoconstrictor effect. The vessel size was also chosen because of the reproducibility of the response.

Abrogation of PAF-Induced Arteriolar Constriction by Inhibitors That Prevent Formation of Cyclooxygenase Products — Evidence That Thromboxane A\textsubscript{2} Mediates the Constrictor Response

The phospholipase A\textsubscript{2} inhibitor, dexamethasone, and BW755C, the equipotent inhibitor of cyclooxygenase and lipoxygenase enzymes, completely blocked the vasoconstrictor response to topically applied PAF (Figure 5). Indomethacin was also found to completely inhibit PAF-induced vasoconstriction (Figure 5), implicating cyclooxygenase products as mediators of this event. The specificity of the indomethacin block was tested in two experiments with norepinephrine. In the first experiment, norepinephrine was applied after pretreatment with indomethacin and challenge with PAF. In the second experiment, norepinephrine was applied immediately after indomethacin pretreatment. In both experiments, norepinephrine induced immediate, intense vasoconstriction of small arterioles (Figure 6). This demonstrated that indomethacin did not interfere with the vessels’ general ability to vasoconstrict but rather was acting through a specific interference.

To test the role of thromboxane in PAF-induced vasoconstriction, OKY-046, a thromboxane synthetase inhibitor, was administered. OKY-046 completely abolished the arteriolar constriction response to topically applied PAF (Figure 5). This finding strongly implicated thromboxane A\textsubscript{2} as the mediator of PAF-induced vasoconstriction.

For purposes of statistical analysis, the curves for PAF plus dexamethasone, BW755C, indomethacin, and OKY-046 were compared with the curve for 10^{-7} M PAF alone. Every curve was divided into three time periods as previously described; regression equations were determined; and the slopes and 95% confidence intervals of the lines for each time group compared for statistical significance. The results of these comparisons are shown in Table 2.

For the time interval between 1 and 4 minutes, both the slopes and 95% confidence intervals of the lines for dexamethasone, BW755C, indomethacin, and OKY-046 were significantly different from the slope obtained with PAF 10^{-7} M alone. Between 4 and 10 minutes, the curves for each inhibitor except OKY-046 were again significantly different from the curve for PAF 10^{-7} M alone. While the slopes of the lines for PAF 10^{-7} M and OKY-046 were similar during this time period, the diameters for OKY-046 were significantly larger than those obtained with 10^{-7} M PAF. Diameters of vessels treated with 10^{-7} M PAF alone were returning toward control values, while those of vessels pretreated with OKY-046 were increasing greatly above control (see Figure 5). Between 10 and 60 minutes, the slopes for both BW755C and kadsurenone were significantly different from the slope for 10^{-7} M PAF alone. The diameters for indomethacin, OKY-046, and dexamethasone were all returning toward control values during this time interval after a period of vasodilation, while the diameters for PAF alone were decreasing away from control, which accounts for the similar slopes (see Figure 5). However, based on their 95% confidence intervals, the diameters obtained with all the inhibitors were significantly greater than those of 10^{-7} M PAF alone over this time interval.

Abrogation of PAF-Induced Arteriolar Constriction by a Specific PAF-Receptor Antagonist, Kadsurenone

To test the hypothesis that PAF activity is receptor mediated, kadsurenone, a synthetic receptor blocker of

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To test the hypothesis that PAF activity is receptor mediated, kadsurenone, a synthetic receptor blocker of
PAF, was administered before PAF application. Kad-  
surenone pretreatment completely inhibited PAF-
induced vasoconstriction (Figure 5). Statistical anal-
ysis showed that the slopes for PAF plus kadsurenone
were significantly different from the curves for
PAF $10^{-7}$ M alone at every time interval tested
(Table 2).

**Effect of Pharmacological Agents on Hematocrits and Systemic Blood Pressure**

The pharmacological agents used in these experi-
ments did not alter hematocrit values or affect systemic
blood pressure. Values for these variables were similar
to those obtained with PAF alone.

**Effect of Pharmacological Agents on Arteriolar Diameters**

To test whether the pharmacological agents used in
these experiments affected arteriolar diameter, vessels
were monitored for 1 hour after administration of the
inhibitors and receptor blockers in separate experi-
ments. BW755C had an adverse effect on flow in all
preparations observed. No other inhibitor decreased
or stopped flow. Dexamethasone, BW755C, indometh-
acin, and OKY-046 had a slight vasodilatory effect.

**Discussion**

In this study, we investigated the direct effects of
PAF on a microvascular bed. The relation between PAF
and arteriolar diameter was characterized, and the
possible pathways through which PAF could be exert-
ing its vasoconstrictor effects were investigated. Our
findings demonstrate that 1) PAF induces a constriction
of arterioles that varies with both the concentration of
PAF applied as well as the size of the arteriole affected;
2) a refractory period for PAF-induced vasoconstriction
exists, lying between 15 and 30 minutes after initial
application; 3) arteriolar constriction is blocked by
enzyme inhibitors that prevent the formation of cyclo-
oxygenase products; more specifically, PAF-stimulated
vasoconstriction appears to be mediated by thrombox-
ane A$_2$; and 4) vasoconstrictor responses are partially
mediated by PAF-receptor interactions.

**Effect of PAF on Vessel Diameter**

Topical application of PAF to the microvascular bed
of the hamster cheek pouch induced constriction of
small arterioles. The times of onset, intensities, and
durations of the vasoconstrictor responses varied with
both vessel size and the concentration of PAF applied.
PAF-induced vasoconstriction has been described qual-
itatively by other investigators. Using the hamster

![Figure 6. Specificity of indomethacin blockade. Panel A, cheek pouch arterioles of a hamster pretreated with indomethacin as indicated in "Materials and Methods." Panel B, same arterioles 2 minutes after topical application of $10^{-7}$ M PAF. Note that the arteriole did not constrict; compare with the effect of PAF $10^{-7}$ M illustrated in Figure 1. Panel C, same arterioles immediately after application of norepinephrine. Note the vasoconstriction; one branch becomes so narrow that it nearly disappears. Panels A through C are shown in the experimental temporal sequence of the blockade and tests.](image-url)
check pouch as a model, Björk and Smedegård reported transient constriction of arterioles after topical application of $10^{-9}$ and $10^{-8}$ M PAF. Vasoconstriction was also observed upon intradermal injection of PAF in humans and guinea pigs. In vitro, PAF constricted smooth muscle strips of guinea pig ileum and lung. To the best of our knowledge, this is the first report to quantify the vasoconstrictor effects of PAF in a microvascular bed and to study the time course. It is important to keep in mind that all vessels in the microvascular bed are exposed to PAF at the same time. The results therefore reflect the combined direct effects of PAF on vessel diameter as well as the indirect effects resulting from alterations in other vascular segments. Results should be interpreted as the relation between PAF concentration and vascular diameter in a microvascular bed rather than as a strict dose-response relation between PAF and individual arteriolar diameter. Perhaps the most significant contribution of this time-course study is the finding that PAF-induced constrictor effects are long lasting. Between 10 and 60 minutes after topical application of $10^{-9}$ and $10^{-7}$ M PAF, arterioles 8-40 μm in diameter remain constricted to 70-80% control size.

The mechanisms through which PAF alters vascular caliber are not known. In contrast with consistent reports of vasoconstriction after topical application or intradermal injection, PAF has been described as a hypotensive agent, a vasoconstrictor, and a material that increases total peripheral resistance when administered intravascularly. Vasodilation or constriction after intravascular administration could be centrally mediated through the stimulation of adrenergic receptors, secondary to cardiac alterations, or result from the production or inhibition of vasoactive compounds. The consistent arteriolar constriction caused by topical application or intradermal administration of PAF is most likely mediated locally. In our experiments, hematocrit and blood pressure were not affected by topical application, indicating that the systemic circulation was not involved. The central nervous system was also likely unaffected.

Since PAF is produced by many blood-borne elements as well as endothelial cells, it is possible that this neutral phospholipid plays an important role in local microcirculatory control mechanisms. In this study, we demonstrate the vasoactive effects of PAF on a microvascular bed, and in a separate report, we show that PAF also increases clearance of macromolecules. Under normal physiological conditions, vasomotor tone and vascular permeability could therefore be modulated by PAF in accordance with the particular needs of the tissue. During inflammation, the regulatory role of PAF in microcirculatory dynamics may be obliterated. PAF could be involved in the initiation and/or mediation of normal physiological inflammatory events, or if excessive or unbalanced amounts of the compound are released, it could play an important role in the pathophysiology of inflammation. The PAF-induced constrictor effect on arterioles shown in our studies may be of significance in the inflammatory process. Immediately upon injury, local arterioles constrict for a few seconds to several minutes, while peripheral arterioles often remain narrowed for much longer periods. It has been suggested that this vasoconstriction serves a protective function. PAF could therefore be involved in the initiation and/or maintenance of this initial protective inflammatory event.

### Pathways of PAF Activity

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

To test whether the arachidonate pathway was involved in PAF activity, the effects of dexamethasone were first assessed. Dexamethasone is a glucocorticoid that binds to a cytosolic receptor and induces the release and de novo synthesis of macrocortin, an intracellular polypeptide. Acting as a second messenger, macrocortin inhibits phospholipase A activity and thereby decreases the amount of arachidonic acid available for conversion to cyclooxygenase and lipoxygenase products. Dexamethasone completely inhibited PAF-induced vasoconstriction. BW755C, an equipotent inhibitor of cyclooxygenase and lipoxygenase activity, similarly prevented arteriolar constriction in response to PAF. These results implicated metabolites of arachidonic acid as mediators of PAF-induced activities. To assess the role of the individual oxygenase pathways, animals were pretreated with indomethacin, a nonsteroidal anti-inflammatory drug that in-
hibits cyclooxygenase activity. \(^{31,32}\) Indomethacin completely abrogated the constriction of arterioles induced by PAF. The specificity of the indomethacin block was verified by subsequent challenge with norepinephrine. These findings indicated that the vasoconstrictor effects of PAF were mediated through the cyclooxygenase pathway. The involvement of lipoxygenase products could not be assessed in this study because of a lack of specific inhibitors.

While the cyclooxygenase pathway was implicated in PAF-induced vasoconstriction, the nature of the cyclooxygenase products responsible for the PAF activity was unknown. Both thromboxane \(A_2\) and prostaglandin \(H_2\) are known vasoconstrictors, but it has also been shown that in high concentrations, vasodilating prostaglandins, such as prostaglandins \(I_1\) and \(E_2\), induce vasoconstriction, presumably by occupying vasoconstrictor receptor sites. \(^{23}\) It is presently impossible to assess the role that individual prostaglandins play in mediating PAF-induced vasoconstriction, because specific inhibitors for most prostaglandins have not yet been developed. However, thromboxane synthetase, the enzyme responsible for conversion of prostaglandin \(H_2\) to thromboxane \(A_2\), can be specifically blocked. To investigate the role of thromboxane \(A_2\) in PAF-induced constriction, animals were pretreated with OKY-046. Pretreatment with the synthetase inhibitor abrogated the arteriolar constriction caused by PAF, a finding that suggested that the vasoconstriction caused by PAF application was mediated through thromboxane \(A_2\) production. Our finding that thromboxane \(A_2\) is responsible for PAF-induced vasoconstriction is supported by Heffner et al. \(^{24}\) who found that imidazole, another thromboxane synthetase inhibitor, reduced the PAF-induced hypertension in isolated lungs.

Further support for the contention that thromboxane \(A_2\) is responsible for PAF-induced arteriolar constriction can be found in the literature. Smooth muscle cells possess receptors for thromboxane \(A_2\), and this substance is known to contract arterial smooth muscle in vitro. \(^{30}\) It has also been shown that thromboxane \(A_2\) can induce pulmonary hypertension in isolated lung preparations. *

Role of receptors. The synthetic PAF receptor blocker, kadsurenone, \(^{22}\) inhibited the vasoconstrictor response to PAF. This finding implies that PAF actions are at least in part receptor mediated.

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References


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Dose-Related Vasoconstrictor Effects of PAF


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