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 (10⁻⁶–10⁻⁴ 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)
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 al.20 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, 10⁻³, and 10⁻² 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 recorder 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 were treated in an appropriate manner, and vessel diameter determined in an appropriate manner, and vessel diameter was determined as described above. PAF 10⁻² M was typically 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, Rahway, New Jersey) was injected 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 was administered at a dose of 1.5 mg/kg i.p. 30 minutes before the PAF challenge. BW755C (3-amino-1-trifluoro methyl phenyl-2-pyrazoline hydrochloride; Burroughs Wellcome, Research Triangle Park, North
Circulation Research Vol 62, No 4, April 1988

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

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 \mu g/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 \mu m. 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 2. Time course of PAF-induced constriction for arterioles between 8 and 15 \mu m in diameter. For PAF 10^{-5} M, 22 vessels were observed in 10 animals; average luminal diameter was 12.7 \pm 2.4 \mu m. For PAF 10^{-7} M, 25 vessels were observed in 6 animals; average luminal diameter was 10.1 \pm 2.3 \mu m. For PAF 10^{-9} M, 20 vessels were observed in 5 animals; average luminal diameter was 11.6 \pm 2.7 \mu m. Data presented as mean \pm 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 $10^{-7}$ 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 μm in diameter also constricted after exposure to PAF (Figure 3). In this size range, the responses to $10^{-8}$ and $10^{-7}$ 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 $10^{-7}$ 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 $10^{-5}$ M PAF. However, between 8 and 20 minutes, vessels constricted to 20–40% control size. After application of $10^{-8}$ M PAF, arterioles 21–40 μm 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 μm in diameter (Figure 4). As with the 21–40-μm group, the response profiles for these two doses were similar, with $10^{-7}$ 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 $10^{-5}$ M PAF, vessels 41–60 μm 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 transitions
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. Within each size group and time interval, the slopes of the curves for PAF 10^{-7} and 10^{-5} 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^{-4} M PAF were applied to the pouch. Both concentrations produced arteriolar constrictions similar to 10^{-7} and 10^{-5} M PAF. Arterioles responded to PAF 10^{-13}-10^{-10} M with slight vasodilation but no notable vasoconstriction.

Effect of Reapplication of PAF on Arteriolar Diameter

Reapplication of PAF 90 minutes after initial application always resulted in vasoconstriction of small arterioles. In further experiments, it was found that there was a refractory period for the vasoconstrictor effects of PAF. Reapplication 10-15 minutes after initial application either did not alter vessel diameter or caused a slight vasodilation. Reapplication 30, 60, and 90 minutes after initial application consistently induced a second vasoconstrictor response. This re-

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**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 + 0.010t‡§</td>
<td>0.952 - 0.004t*§</td>
<td>0.978 + 0.001t‡§</td>
</tr>
<tr>
<td>10^{-9} M</td>
<td>1.498 - 0.262t*§</td>
<td>-0.225 + 0.114t</td>
<td>0.812 + 0.001t</td>
</tr>
<tr>
<td>10^{-7} M</td>
<td>1.168 - 0.328t*</td>
<td>-0.053 + 0.096t</td>
<td>0.837 + 0.001t</td>
</tr>
<tr>
<td>10^{-5} M</td>
<td>0.0t</td>
<td>-1.11 + 0.169t</td>
<td>0.803 - 0.002t</td>
</tr>
<tr>
<td>21-40 μm</td>
<td>1.046 - 0.001t‡§</td>
<td>0.917 + 0.013t§</td>
<td>1.136 - 0.004t‡§</td>
</tr>
<tr>
<td>10^{-9} M</td>
<td>1.078 - 0.066t</td>
<td>0.623 + 0.046t§</td>
<td>0.879 + 0.001t*</td>
</tr>
<tr>
<td>10^{-7} M</td>
<td>0.916 - 0.159t</td>
<td>-0.168 + 0.096t</td>
<td>0.919 - 0.006t</td>
</tr>
<tr>
<td>10^{-5} M</td>
<td>0.423 - 0.081t</td>
<td>0.299 + 0.020t</td>
<td>1.005 - 0.006t</td>
</tr>
<tr>
<td>41-60 μm</td>
<td>1.006 - 0.004t*‡§</td>
<td>1.005 - 0.004t*‡</td>
<td>0.671 - 0.066t*‡</td>
</tr>
<tr>
<td>10^{-9} M</td>
<td>0.965 + 0.013t*‡§</td>
<td>1.104 - 0.016t*‡</td>
<td>0.735 + 0.007t</td>
</tr>
<tr>
<td>10^{-7} M</td>
<td>1.126 - 0.248t</td>
<td>0.120 + 0.063t</td>
<td>0.871 - 0.003t</td>
</tr>
<tr>
<td>10^{-5} M</td>
<td>0.648 - 0.129t</td>
<td>0.121 + 0.100t</td>
<td>0.826 + 0.001t</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 in minutes. The first term is the intercept, and the second term is the slope. The same differences hold true for PAF 10^{-3} M, while PAF 10^{-4} 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^{-5} 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^{-4} M PAF were applied to the pouch. Both concentrations produced arteriolar constrictions similar to 10^{-7} and 10^{-5} M PAF. Arterioles responded to PAF 10^{-13}-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.
Abrogation of PAF-Induced Arteriolar Constriction by Products — Evidence That Thromboxane A2 Mediates vasoconstrictor response to topically applied PAF

Inhibitors That Prevent Formation of Cyclooxygenase the Constrictor Response

Effect of Pharmacological Antagonists on PAF-Induced Arteriolar Constriction

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

Figure 5. Effect of pharmacological antagonists on PAF-induced arteriolar constriction. PAF 10⁻⁷ M was applied and arterioles with luminal diameters between 21 and 40 μm observed. KAD, kadsurenone; OKY, OKY-046; IND, indomethacin; BW, BW755C; DX, dexamethasone. Data shown as mean ± SD.
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.

PAF, was administered before PAF application. Kadsurenone pretreatment completely inhibited PAF-induced vasoconstriction (Figure 5). Statistical analysis 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 experiments 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 experiments. BW755C had an adverse effect on flow in all preparations observed. No other inhibitor decreased or stopped flow. Dexamethasone, BW755C, indomethacin, and OKY-046 had a slight vasodilatatory 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 exerting 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 cyclooxygenase products; more specifically, PAF-stimulated vasoconstriction appears to be mediated by thromboxane A2; 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 qualitatively by other investigators. Using the hamster
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 constriction of 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.

### Table 2. Comparison of Regression Lines for Effects of Inhibitors on PAF-Induced Constriction of Arterioles (21–40 μm)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>1–4 min</th>
<th>4–10 min</th>
<th>10–60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (PAF 10⁻⁷ M)</td>
<td>0.92 ± 0.16t</td>
<td>-0.17 ± 0.10t</td>
<td>0.92 ± 0.01t</td>
</tr>
<tr>
<td>BW755C</td>
<td>0.95 ± 0.02t⁺</td>
<td>0.98 ± 0.01t⁺</td>
<td>1.07 ± 0.01t⁺</td>
</tr>
<tr>
<td>OKY-046</td>
<td>0.97 ± 0.12t⁺</td>
<td>1.03 ± 0.01t⁺</td>
<td>1.32 ± 0.01t⁺</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1.21 ± 0.01t⁺</td>
<td>1.19 ± 0.01t⁺</td>
<td>1.24 ± 0.01t⁺</td>
</tr>
<tr>
<td>Kadsurenone</td>
<td>0.92 ± 0.07t⁺</td>
<td>1.23 ± 0.02t⁺</td>
<td>1.01 ± 0.01t⁺</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.95 ± 0.02t⁺</td>
<td>1.03 ± 0.01t⁺</td>
<td>0.96 ± 0.01t⁺</td>
</tr>
</tbody>
</table>

All diameters normalized to control for each time interval. All table entries are the regression equation for the change in diameter with time during the time interval shown. The first term is the intercept, and the second term is the slope.

* Slope is significantly different from PAF 10⁻⁷ M.
† Diameters are significantly larger than with PAF 10⁻⁷ M (based on their 95% confidence intervals); and ‡ except for the first minute, these diameters were significantly larger than PAF 10⁻⁷ M (based on their 95% confidence intervals).

PAF, platelet-activating factor.

### 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 A₂ 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.\textsuperscript{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 $I_2$ and $E_2$, induce vasoconstriction, presumably by occupying vasoconstrictor receptor sites.\textsuperscript{33} 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.,\textsuperscript{34} 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.\textsuperscript{35} It has also been shown that thromboxane $A_2$ can induce pulmonary hypertension in isolated lung preparations.\textsuperscript{*}

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

Acknowledgments

We acknowledge the following generous gifts: Kadsurenone, Merck Sharp & Dohme; BW755C, Burroughs Wellcome; and OKY-046, Ono Pharmaceuticals.

References

1. Clark PO, Hanahan DJ, Pinckard RN: Physical and chemical properties of platelet-activating factor obtained from human neutrophils and monocytes and rabbit neutrophils and basophils. Biochim Biophys Acta 1980;628:69-75


1988

Key Words • platelet-activating factor • vasoconstriction • hamster cheek pouch • microcirculation • inflammation • arachidonic acid cascade and inhibitors • thromboxane A2 • leukotrienes
Vasoconstrictor effects of platelet-activating factor in the hamster cheek pouch microcirculation: dose-related relations and pathways of action.

P K Dillon, A B Ritter and W N Durán

Circ Res. 1988;62:722-731
doi: 10.1161/01.RES.62.4.722

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