Cyclic blood flow variations induced by platelet-activating factor in stenosed canine coronary arteries despite inhibition of thromboxane synthetase, serotonin receptors, and α-adrenergic receptors

P. APPRILL, M.D., J. M. SCHMITZ, M.D., W. B. CAMPBELL, PH.D., G. TILTON, M.D., J. ASHTON, PH.D., S. RAHEJA, M.D., L. M. BUJA, M.D., AND J. T. WILLERSON, M.D.

ABSTRACT The phospholipid platelet-activating factor (PAF) stimulates platelet aggregation and coronary vasoconstriction. In this study we determined whether PAF alters coronary flow patterns in vivo in a canine preparation with concentric coronary artery stenosis. This preparation is characterized by cyclic flow variations in coronary blood flow associated with transient platelet aggregation at the site of the coronary constriction. Thirty-nine male mongrel dogs were used in three protocols. In protocol 1, PAF (10⁻⁹ or 10⁻⁸ mol/min) was infused into the coronary artery proximal to the stenosis to determine (1) whether PAF induces cyclic flow variations and (2) whether PAF has an effect on systemic hemodynamics. Cyclic flow variations were induced in three of six dogs; in these animals, mean arterial pressure decreased by 5.5% and 42.1% 10 min after infusion of the lower and higher dose of PAF. In protocol 2, cyclic flow variations were abolished with either the thromboxane synthetase inhibitor UK38485 (mean dose 2.2 mg/kg iv), the serotonin antagonist ketanserin (0.5 mg/kg iv), or the α₁-adrenergic antagonist yohimbine (2 mg/kg iv). Subsequent administration of PAF restored the frequency of cyclic flow variations to the preantagonist levels. Thromboxane (Tx) B₂ and 6-keto-PGF₁α, the stable metabolites of TxA₂ and prostacyclin, respectively, were measured in blood obtained distal to the coronary stenosis. TxB₂ levels increased substantially during cyclic flow variations and were returned to control values with the thromboxane synthetase inhibitor UK38485. Infusion of PAF subsequently restored cyclic flow variations without altering coronary arterial TxB₂ levels. Furthermore, although PAF-induced platelet aggregation in vitro was associated with an increase in platelet thromboxane release, thromboxane synthetase inhibition did not alter PAF-induced platelet aggregation. Taken together, these data indicate that PAF may initiate and/or restore cyclic flow variations after their abolition with either a thromboxane synthetase inhibitor or a serotonin or α₁-receptor antagonist. These data support previous studies in vitro demonstrating an action of PAF on platelet aggregation that is independent of thromboxane, serotonin, or α₁-adrenergic agonists.


THE CLINICAL SYNDROME of unstable angina pectoris may occur in association with decreases in myocardial oxygen supply.¹ This may be caused by factors that reduce coronary blood flow. Some of these factors include (1) coronary arterial spasm, (2) hemorrhage into a nonocclusive plaque, (3) platelet aggregation over areas of plaque or endothelial denudation, and (4) natural progression of coronary atherosclerosis. Platelet aggregates may mechanically obstruct an artery or may synthesize and release vasoactive agents such as thromboxane (Tx) A₂, which has vasoconstrictor properties. Hirsh et al.² found elevated coronary sinus-to-aortic ratios of TxB₂, the stable metabolite of TxA₂, in patients with unstable angina pectoris.

Folts et al.³ developed an experimental preparation of a concentrically stenosed canine coronary artery. In this preparation there are episodic, spontaneous decreases in coronary blood flow interrupted by restorations of blood flow. These alterations in coronary blood flow have been called cyclic flow variations, which are associated with microscopic thrombi composed of platelets, erythrocytes, and leukocytes¹ ⁴ and
with endothelial damage at the site of coronary arterial narrowing.\textsuperscript{1,5} We have shown that TxB\textsubscript{2} concentrations increase in the distal portion of the stenosed coronary artery and that an inhibitor of thromboxane synthetase, dazoxiben, abolishes cyclic flow variations and decreases the TxB\textsubscript{2} concentration measured distal to the stenosis.\textsuperscript{6} Similarly, serotonin or \(\alpha\textsuperscript{2}\)-adrenergic antagonists attenuate the frequency of cyclic flow variations.\textsuperscript{7} Taken together, these data suggest that platelet-derived agents that alter platelet aggregation and vascular tone may mediate cyclic flow variations either alone or in concert. It is possible that thromboxane, serotonin, and \(\alpha\textsuperscript{2}\)-adrenergic receptor mechanisms are only some of the mediators of platelet activation in this experimental preparation; other agents may also influence platelet aggregation in the coronary circulation with resulting changes in coronary vascular tone and cyclic flow variations.

In this study we evaluated the recently isolated phospholipid 1-0-hexadecyl-2-acetyl-sn-glyceryl-phosphorylcholine, or platelet-activating factor (PAF). PAF stimulates human platelet aggregation in vitro\textsuperscript{5-9} and is released by human platelets\textsuperscript{10} and leukocytes.\textsuperscript{11} It is a potent peripheral vasodilator\textsuperscript{12} and decreases coronary blood flow when given by intracoronary injection in the pig.\textsuperscript{13} Because of these physiologic effects, we wished to determine (1) whether PAF is capable of inducing cyclic flow variations in a stenosed canine coronary artery and (2) whether its effect is mediated through thromboxane, serotonin, or \(\alpha\textsuperscript{2}\)-adrenergic mechanisms.

Materials and methods

Surgical preparation. Male mongrel dogs weighing 16 to 32 kg were anesthetized with 30 mg/kg pentobarbital and ventilated on room air with a Harvard ventilator. Temperature was controlled with a heating pad and monitored by measuring rectal temperature. Jugular venous and carotid arterial catheters were inserted, and a left thoracotomy was performed. The heart was exposed via a pericardial split, and a left atrial catheter was placed. A Konigsberg pressure transducer (P23) was placed in the left ventricular apex for measurement of left ventricular pressure and dP/dt. Next, a 1 cm segment of the left anterior descending coronary artery (LAD) was cleared and small branches were ligated. A Doppler flow probe\textsuperscript{14} and a small ephedrine plastic constrictor were placed around the artery (figure 1). A branch of the distal LAD below the flow probe was isolated, and a small polyethylene catheter (id 0.034 inch, od 0.050 inch) was placed for monitoring coronary pressure and for blood collection. Next, either a small right ventricular branch of the LAD or a diagonal branch immediately above the Doppler flow probe was isolated and cannulated with a polyethylene catheter of similar size for infusion of the drug. The carotid catheter and both coronary arterial polyethylene catheters were kept heparinized with bovine heparin. Hemodynamic variables were monitored continuously on a Honeywell Electronics for

![FIGURE 1. The experimental preparation includes a Doppler flow probe and plastic constrictor on a 1 cm segment of isolated coronary artery, distal coronary and diagonal branch catheters and left atrial (A) catheter, and a Konigsberg pressure transducer.](http://circ.ahajournals.org/)

Medicine VR12 recorder and recorded on a Hewlett-Packard 7758A eight-channel recorder.

Protocol 1. Six male dogs were used to determine the systemic and coronary hemodynamic effects produced by an intracoronary infusion of PAF. After completion of the above surgical preparation, a small plastic constrictor was placed on the LAD to decrease resting phase coronary blood flow by approximately 50% and to abolish the hyperemic response of a brief coronary occlusion.\textsuperscript{3} Aortic pressure, heart rate, left ventricular pressure, left ventricular dP/dt, distal LAD pressure, and LAD phasic and mean blood flow by Doppler flow probe were measured. PAF, dissolved in 0.9% sodium chloride containing 1% bovine serum albumin, was infused into a catheter placed in the diagonal LAD branch proximal to the constrictor. Initially, \(10^{-9}\) mol/min at 0.1 ml/min was infused with a Harvard infusion pump. The dose was increased to \(10^{-8}\) mol/min after 30 min.

Protocol 2. To determine the mechanism of action of PAF on cyclic flow variations, the same experimental animal preparation was used. Hemodynamics were measured. Simultaneous blood samples were collected on ice in 15 ml Vacutainer tubes (Becton-Dickinson) with 2000 U of bovine heparin sodium and 40 \(\mu\)g of indomethacin from the heparinized carotid arterial and the distal LAD catheters from four animals. These samples were analyzed subsequently as a group for TxB\textsubscript{2} and 6-keto-PGF\textsubscript{1a}, the stable metabolic products of TxA\textsubscript{2} and prostacyclin, respectively. After placement of the plastic constrictor on the LAD, cyclic flow variations were quantitated for at least 1 hr. To decrease coronary arterial thromboxane levels and to abolish cyclic flow variations, the thromboxane synthetase inhibitor UK38485 was administered (2 to 6 mg/kg iv). Hemodynamics were monitored for at least 30 min after cyclic flow variations were abolished and blood samples were obtained again for measurement of prostaglandin levels. Five dogs received an intracoronary infusion of the vehicle only, 1% bovine serum albumin in 0.9% saline at 0.1 ml/min, and 12 dogs received PAF (\(10^{-9}\) or \(10^{-8}\) mol/min). An aortic constrictor was placed above the aortic arch to maintain systolic blood pressure at approximately 100 mm Hg. Hemodynamics were monitored. cyclic flow vari-
ations were quantitated, and blood was collected at the nadir of coronary flow for prostaglandin measurement before and during infusion of PAF in four dogs.

Protocol 3. Ten dogs were used in two variations of protocol 2. Dogs were instrumented as described. Cyclic flow variations were produced in the LAD with the plastic constrictor and were quantitated. After 1 hr, either the serotonin antagonist ketanserin was given in a dose of 0.5 mg/kg iv10 or yohimbine, an α-adrenergic antagonist, was given in a dose of 1 to 3 mg/kg iv. Previous studies6 have shown that ketanserin and yohimbine usually abolish or reduce cyclic flow variations, respectively, in this preparation. Cyclic flow variations were abolished in every dog given ketanserin and in approximately half of the dogs given yohimbine. In those animals in which cyclic flow variations were abolished by ketanserin or yohimbine, PAF was infused into the LAD as described above and cyclic flow variations were quantitated.

In all animals the placement of the catheter was checked with the infusion of 2 ml of monastral blue dye in 10 ml of saline through the diagonal branch catheter. Dogs in which the dye did not stain the area of coronary constriction and the distal LAD bed were excluded from subsequent analysis.

PAF-induced platelet aggregation in vitro. To further evaluate the mechanism of PAF-induced platelet aggregation, studies were performed on blood from seven of the dogs instrumented as described in the above protocols. After instrumentation but before any drugs, 45 ml of blood was obtained from the jugular venous catheter and transferred to a tube containing 5 ml of 3.5% sodium citrate, pH 7.4. The blood samples were centrifuged at 100 g for 20 min, resulting in a plasma suspension of platelets containing 300,000 to 500,000 platelets/mm³. After removing the platelet-rich plasma, the remaining blood was centrifuged at 1500 g for 10 min to obtain platelet-poor plasma. Platelet aggregation was measured in vitro with a Sienco (Model DP 247-E) dual-channel aggregometer by the turbidometric method of Born.16 The rate and extent of aggregation in response to various concentrations of PAF were measured in a reaction volume of 500 μl of platelet-rich plasma. Five minutes after the addition of PAF, a 50 μl sample was removed from the aggregation cuvette and diluted into 450 μl of ice-cold distilled water containing indomethacin 10⁻⁴M. These samples were subsequently assayed for TxB₂. In a separate set of experiments, platelet-rich plasma was preincubated with UK38485 (10⁻⁴M) for 3 min before the addition of PAF.

Prostaglandin measurements. TxB₂ and 6-keto PGF₁α were measured by the method of Dray et al.,17 as modified by Campbell et al.18 and Hirsh et al.2

Statistical methods. All values are expressed as mean ± SEM. A p value of < .05 was considered significant. All hemodynamic variables were analyzed with general linear models procedure and Duncan’s multiple range test developed by SAS Institute, Inc.19 Data involving the frequency of cyclic flow variations and the nadir of coronary arterial pressure during cyclic flow and platelet aggregation were analyzed by analysis of variance and Newman-Keuls multiple comparisons procedure.

Materials. 1-O-Hexadecyl-2-acetyl-sn-glyceryl-phosphorylcholine (PAF) was supplied by Dr. John Pike of Upjohn Co., Kalamazoo, MI. UK38485 was a gift of Dr. Pedro Urquilla of Abbott Laboratories, North Chicago, IL, and bovine heparin from Organon, West Orange, NJ. ³H-TxB₂ and ³H-6-keto PGF₁α were obtained from Amersham and TxB₂ and 6-keto PGF₁α were obtained from the Upjohn Co.

Results

Hemodynamic data. PAF (10⁻⁸ mol/min) produced a significant decrease in aortic systolic, diastolic, and mean blood pressures (all p < .001) (table 1). After 10 min of infusion of PAF 10⁻⁸ mol/min, the systolic, diastolic, and mean pressures were 59.9 ± 8.5, 43.7 ± 5.6, and 49.0 ± 6.5 mm Hg, respectively, compared with control values of 97.7 ± 7.0, 80.1 ± 6.7, and 86.0 ± 6.7 mm Hg (figure 2). Because of the marked changes in systemic blood pressure, no attempt was made to evaluate coronary blood flow. Subsequent studies were performed with aortic pressure controlled by an aortic constrictor (table 2).

There were no major hemodynamic effects of the thromboxane synthetase inhibitor UK38485 (table 2). In the dogs given ketanserin, control blood pressure was reduced (p < .05) compared with the pretreatment value. Treatment with ketanserin had no other major hemodynamic effects (table 3). In the yohimbine-treated dogs there were no significant changes in systolic blood pressure (table 4). There were no other consistent changes in any hemodynamic variable in the yohimbine-treated dogs.

Cyclic flow variations. Cyclic flow variations developed in three of six dogs in protocol 1, in which PAF was infused into a stenosed coronary artery. No cyclic flow variations had occurred in these animals before the infusion. In protocol 2 cyclic flow variations occurred spontaneously in 22 of 23 dogs. In three of these
22 animals, UK38485 in doses up to 6 mg/kg did not abolish the cyclic flow variations; therefore these three dogs were not used in the protocol. One dog died of ventricular fibrillation before infusion of PAF. In another dog the dye through the diagonal branch catheter did not stain the LAD but perfused a branch of the diagonal, and the dog was excluded from analysis. Therefore 17 dogs were available for treatment with PAF or the vehicle. Figure 3 illustrates a typical tracing of the results found in protocol 2. During the initial period, coronary flow declined slowly and then increased only to fall again. Treatment with UK38485 inhibited these cyclic flow variations. The infusion of PAF restored the cyclic declines in coronary flow, and the cyclic flow variations resembled those observed during the initial period. The frequency of cyclic flow variations in dogs in protocol 2 was standardized to the number of cyclic flow variations over 30 min for each of three time periods: (1) the initial period of cyclic flow variations, designated “control”; (2) the 30 min after the thromboxane synthetase inhibitor; and (3) the final treatment time (figures 3 and 4). This procedure

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Control</th>
<th>PAF 10⁻⁶ mol/min</th>
<th>PAF 10⁻⁷ mol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>HR (beats/ min)</td>
<td>Control</td>
<td>143 ± 13.7</td>
<td>140 ± 16</td>
<td>140 ± 22</td>
</tr>
<tr>
<td></td>
<td>AOS (mm Hg)</td>
<td>97.7 ± 7.0</td>
<td>90.8 ± 3.3</td>
<td>87.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>AOD (mm Hg)</td>
<td>80.1 ± 6.7</td>
<td>74.4 ± 5.4</td>
<td>70.8 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>AOM (mm Hg)</td>
<td>86.0 ± 6.7</td>
<td>79.8 ± 4.6</td>
<td>76.3 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>LV dP/dt (mm Hg/sec)</td>
<td>1143 ± 310</td>
<td>1118 ± 272</td>
<td>996 ± 206</td>
</tr>
<tr>
<td></td>
<td>-dP/dt (mm Hg/sec)</td>
<td>-1084 ± 267</td>
<td>-989 ± 185</td>
<td>-1015 ± 135</td>
</tr>
</tbody>
</table>

HR = heart rate; AOS = systolic blood pressure; AOD = diastolic blood pressure; AOM = mean aortic blood pressure; dP/dt = rate of rise of left ventricular pressure; -dP/dt = rate of fall of left ventricular pressure.

²p < .05 vs control value.

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Control</th>
<th>CFV 60 min</th>
<th>UK38485 30 min</th>
<th>Treatment 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats/ min)</td>
<td>BSA (N = 5)</td>
<td>146 ± 12³</td>
<td>135 ± 10⁴</td>
<td>118 ± 10³</td>
<td>110 ± 9³</td>
</tr>
<tr>
<td></td>
<td>PAF (N = 12)</td>
<td>146 ± 7</td>
<td>143 ± 10</td>
<td>142 ± 12</td>
<td>145 ± 12³</td>
</tr>
<tr>
<td>AOS (mm Hg)</td>
<td>BSA</td>
<td>102.8 ± 3⁶</td>
<td>98.1 ± 6.3</td>
<td>87.5 ± 9.0⁶</td>
<td>87.0 ± 4.5⁷</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>119.3 ± 5.0⁶</td>
<td>107.1 ± 7.3</td>
<td>113.6 ± 4.3⁶</td>
<td>113.2 ± 3.8³</td>
</tr>
<tr>
<td>AOD (mm Hg)</td>
<td>BSA</td>
<td>86.5 ± 3.9</td>
<td>84.6 ± 6.7</td>
<td>67.2 ± 9</td>
<td>70.3 ± 5.3³</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>98.9 ± 4.8³</td>
<td>82.8 ± 6.1³</td>
<td>85.7 ± 4.3³</td>
<td>84.5 ± 5.6³</td>
</tr>
<tr>
<td>AOM (mm Hg)</td>
<td>BSA</td>
<td>91.9 ± 3.5</td>
<td>89.1 ± 6.6</td>
<td>73.9 ± 9.0</td>
<td>75.8 ± 4.9⁹</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>105.6 ± 4.7</td>
<td>90.8 ± 6.4</td>
<td>94.9 ± 3.9</td>
<td>93.9 ± 4.7³</td>
</tr>
<tr>
<td>dP/dt (mm Hg/sec)</td>
<td>BSA</td>
<td>1,719 ± 195</td>
<td>1,499 ± 197</td>
<td>1,505 ± 223</td>
<td>1,285 ± 172³</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>1,761 ± 104</td>
<td>1,564 ± 96</td>
<td>1,589 ± 72</td>
<td>1,468 ± 92³</td>
</tr>
<tr>
<td>-dP/dt (mm Hg/sec)</td>
<td>BSA</td>
<td>-1,437 ± 95³</td>
<td>-1,249 ± 177³</td>
<td>-1,063 ± 144³</td>
<td>-977 ± 78³</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>-1,732 ± 78³</td>
<td>-1,402 ± 84³</td>
<td>-1,562 ± 93³</td>
<td>-1,409 ± 62³⁹</td>
</tr>
<tr>
<td>PROD (mm Hg)</td>
<td>BSA</td>
<td>15,120 ± 1,490⁸</td>
<td>13,320 ± 1,430⁸</td>
<td>10,460 ± 2,050³</td>
<td>9,690 ± 1,190³⁹</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>17,440 ± 1,370</td>
<td>15,100 ± 1,350</td>
<td>15,820 ± 1,160</td>
<td>16,200 ± 1,370³⁹</td>
</tr>
</tbody>
</table>

CFV = cyclic flow variations; BSA = bovine serum albumin; PROD = AOS × HR; other abbreviations as in table 1. The units for each of the hemodynamic variables are the same as in table 1.

³p < .05 difference between different time periods within one treatment group.

⁴p < .05 between groups at a given time period.
was used to quantitate the cyclic flow variations for the different times involved in the three phases of the protocol. Dogs were grouped according to the final treatment they received, i.e., the vehicle or PAF. The frequency of cyclic flow variations during control was identical in the two treatment groups (5.6 ± 0.6 vs 5.6 ± 0.4 cyclic flow variations over 30 min) (figure 4). The frequency of cyclic flow variations after treatment with the thromboxane synthetase inhibitor was zero in all groups because only dogs in which cyclic flow variations were abolished were used. As shown in figures 3 and 4, PAF (but not its vehicle) restored cyclic flow variations after the thromboxane synthetase inhibitor from 0 to 5.0 ± 1.0 cyclic flow variations over 30 min. This value was not different from those observed initially.

To evaluate potential hemodynamic differences between the initial and PAF-induced cyclic flow variations, the mean coronary blood flows at the nadir of flows were averaged during the initial cyclic flow variations and after the infusion of UK38485. The nadir of coronary blood flow during the initial cyclic flow variations was similar in the two groups and averaged 30.9 ± 2.6% of control for all dogs (figure 5). The thromboxane synthetase inhibitor increased blood flow to 77.1 ± 6.9% of control values and cyclic flow variations were abolished. The mean nadir of coronary flow decreased in PAF-treated dogs to 28.4 ± 6.0% of control as cyclic flow variations were restored. The mean flow in dogs receiving only the vehicle and in which cyclic flow variations were not restored was 69.9 ± 12.5% (p = .002 difference by analysis of variance).

Cyclic flow variations were produced in 10 dogs in protocol 3 (table 5). In six dogs, there were 6.2 ± 0.6 cyclic flow variations over 30 min in the control period. Ketanserin abolished these cyclic flow variations, but they were restored in all dogs by PAF (table 5). In four dogs, the initial cyclic flow variations were abolished with yohimbine (during the control period), and PAF at least partially restored them to a frequency comparable to that present during the control period.

**Prostaglandin data.** TxB₂ and 6-keto-PGF₁α concentrations in aortic and distal coronary blood were determined in four animals in protocol 2 receiving PAF with control of blood pressure. Distal coronary arterial...
FIGURE 3. Typical tracing from a single dog receiving PAF by intracoronary infusion with an aortic constrictor to control blood pressure. Shown are initial cyclic flow variations (CFVs) (left panel), after UK38485 (middle panels), and during infusion of PAF (right panel) with CFVs restored. DCA = distal coronary artery pressure.

TxB₂ concentrations increased from 51.5 ± 19.3 to 148.5 ± 60.9 during cyclic flow variations (table 6); the thromboxane synthetase inhibitor decreased these elevated values to 55.7 ± 40.2 in association with abolition of the cyclic flow variations. During infusion of PAF the cyclic flow variations were restored. However, the TxB₂ concentrations in the distal coronary artery did not increase. There was no change in aortic TxB₂ concentrations. Both aortic and distal coronary arterial 6-keto-PGF₁₀ concentrations increased over time (table 5). Neither UK38485 nor PAF altered the release of 6-keto-PGF₁₀ into the coronary blood.

Platelet aggregation in vitro. As shown in figure 6,
TABLE 5  
Cyclic flow variations with PAF after ketanserin and yohimbine*  

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control</th>
<th>Nadir of CBF</th>
<th>Drug</th>
<th>Nadir of CBF</th>
<th>PAF</th>
<th>Nadir of CBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketanserin</td>
<td>6.2 ± 0.6 (n = 6)</td>
<td>25.8 ± 7.6%</td>
<td>0</td>
<td>107.3 ± 14.2%</td>
<td>4.4 ± 1.0 (NS)</td>
<td>41.3 ± 12.3%</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>9.0 ± 0.9 (n = 4)</td>
<td>21.8 ± 12.7%</td>
<td>0</td>
<td>82.6 ± 16.5%</td>
<td>4.5 ± 1.2</td>
<td>16.6 ± 6.0%</td>
</tr>
</tbody>
</table>

*Data expressed as number of cyclic flow variations over 30 min.  
*P < .05 compared with control and after PAF.

TABLE 6  
Alterations in TxB₂ and 6-keto PGF₁α levels (mean ± SEM)*  

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>During CFVs</th>
<th>After UK38485</th>
<th>With PAF and return CFVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic TxB₂ (pg/ml)</td>
<td>48.2 ± 15.2</td>
<td>37.5 ± 7.5</td>
<td>46.7 ± 15.7</td>
<td>27.5 ± 9.7</td>
</tr>
<tr>
<td>DCA TxB₂ (pg/ml)</td>
<td>51.2 ± 19.3</td>
<td>148.5 ± 60.9</td>
<td>55.7 ± 40.2</td>
<td>22.7 ± 13.2</td>
</tr>
<tr>
<td>Aortic 6-keto-PGF₁α (pg/ml)</td>
<td>96.7 ± 22.2</td>
<td>255 ± 73.5</td>
<td>310.5 ± 97.8</td>
<td>348.2 ± 81.4</td>
</tr>
<tr>
<td>DCA 6-keto-PGF₁α (pg/ml)</td>
<td>105.2 ± 15.4</td>
<td>320 ± 79.6</td>
<td>278 ± 111.8</td>
<td>492.75 ± 93.9</td>
</tr>
</tbody>
</table>

*CFVs = cyclic flow variations; DCA = distal coronary artery.  
*Protocol 2 (n = 4).

PAF-induced aggregation of canine platelets in vitro at concentrations between 10⁻⁸ and 10⁻⁶M. With the platelet aggregation there was stimulation of the synthesis of TxB₂, which was also concentration related. In the platelet-rich plasma suspensions preincubated with UK38485 at 10⁻⁸M, platelet aggregation still occurred with PAF, but there was no detectable increase in thromboxane synthesis.

Discussion

PAF is a member of a recently described phospholipid family with potent hypotensive and platelet-aggregating properties. PAF causes bronchospasm in the guinea pig and its release is postulated to be a mechanism for inducing asthma. Human leukocytes release PAF when stimulated by immune complexes. Human and rabbit platelets form PAF when stimulated with the calcium ionophore A23187, as do human endothelial cells in culture. Previous histologic studies of canine coronary arteries with concentric stenosis have demonstrated the presence of platelets and polymorphonuclear leukocytes at the site of the constriction. Since PAF may be released from the white blood cells or platelets, the potential for the local generation of PAF is present at the site of an experimental coronary arterial stenosis. This investigation was designed to determine the effect of PAF on coronary blood flow in such a preparation.

FIGURE 6. Effect of increasing the concentration of PAF on percentage platelet aggregation (left) and TxB₂ concentration (right). Complete aggregation was noted at concentrations between 10⁻⁸M and 10⁻⁷M.
The results of our study indicate that PAF, when given by an intracoronary infusion, may cause or restore cyclic flow variations in a stenosed canine coronary artery. Specifically, PAF restores cyclic flow variations in spite of the inhibition of thromboxane synthesis by UK38485 or pretreatment with antagonists for serotonin or α₂-adrenergic receptors. Furthermore, PAF induces aggregation of canine platelets in vitro, and PAF-induced platelet aggregation is not prevented by inhibition of thromboxane synthesis.

Since the initial description of this preparation and of the cyclic declines in coronary blood flow called cyclic flow variations,1 much attention has been directed to defining mechanisms and developing methods for altering or abolishing cyclic flow variations. Folts2,3 initially described the ability of aspirin and indomethacin, both inhibitors of cyclooxygenase, to abolish cyclic flow variations. Aiken et al.4 have used prostacyclin to prevent cyclic flow variations. We have found that TxA₂ levels increase during cyclic flow variations and that a thromboxane synthetase inhibitor, dazoxiben, decreases coronary arterial thromboxane levels and abolishes or markedly attenuates cyclic flow variations.4 Therefore, previous data have implicated thromboxane in the generation of cyclic flow variations in this experimental preparation.

The question arises, however, as to whether increased TxA₂ concentration is the major cause of cyclic flow variations or whether thromboxane is only one of several factors that might potentially cause these variations. In this regard, we wished to determine whether cyclic flow variations could be produced in the absence of an elevated TxA₂ level within the coronary artery. Our data demonstrate that PAF causes platelet aggregation and cyclic flow variations despite thromboxane synthetase inhibition.

The mechanism by which PAF aggregates platelets is controversial. However, most data suggest that PAF induces primary platelet aggregation in spite of inhibitors of cyclooxygenase or thromboxane synthetase5 or removal of ADP with the scavenging system, creatine phosphate/creatine phosphokinase. The lipoxigenase pathway has been implicated by some investigators as the mediator of PAF effects.20 Currently, PAF is postulated to represent a “third pathway” of platelet aggregation.25

We do not know whether release of endogenous PAF plays a significant role in causing cyclic flow variations in this preparation. However, it is interesting that if cyclic flow variations were abolished with various inhibitors, the infusion of PAF usually restored the cyclic flow variations to a value similar to that before the administration of the inhibitors. This lends support to the premise that cyclic flow variations may be due at least in part to platelet aggregates and thrombi.3,4,20,24,26 Others have shown that PAF, when given as an intracoronary bolus in the pig, decreases coronary blood flow.13 Whether PAF causes direct coronary constriction in the dog with a partial coronary stenosis is not clear from our data, but such an effect may have contributed to the restoration of cyclic flow variations in our studies. Cyclic flow variations are associated with an elevated coronary arterial TxB₂ concentration in blood distal to the stenosis, and a thromboxane synthetase inhibitor usually abolishes cyclic flow variations in this preparation.4 Ketanserin and yohimbine abolish or reduce the frequency of cyclic flow variations, respectively.6 PAF induces cyclic flow variations in spite of the presence of each of these drugs. Therefore, with spontaneous cyclic flow variations it appears that thromboxane and serotonin release are important and that inhibition of either agent’s synthesis or agonist effects reestablishes normal coronary blood flow. PAF may not occupy a role as central as that of thromboxane or serotonin, but when given at sufficient concentrations it can cause cyclic flow variations. However, the final determination of the significance of PAF in causing dynamic alterations in coronary blood flow will await the development of a suitable assay for measuring the PAF concentration and production during cyclic flow variations in preparations with partial coronary stenosis and the development of specific antagonists.

In summary, PAF given by intracoronary infusion at 10⁻⁹ and 10⁻⁸ mol/min causes a decrease in systemic blood pressure. PAF can induce cyclic flow variations in a stenosed canine coronary artery. It can also restore cyclic flow variations that have been abolished by a thromboxane synthetase inhibitor (UK38485), a serotonin₂ receptor antagonist (ketanserin), or an α₂-adrenergic receptor antagonist (yohimbine). Therefore, the ability of PAF to restore cyclic flow variations is not dependent on thromboxane, serotonin, or α₂-adrenergic mechanisms.

We thank Janice McNatt, Judy Ober, and Mary Beth Santowski for their technical assistance, Nancy Dickey for her secretarial assistance, and Dr. J. E. Pike of the Upjohn Co. for providing the PAF.

References
2. Hirsh PD, Hillis LD, Campbell WB, Firth BG, Willerson JT: Release of prostaglandins and thromboxane into the coronary circu-


Cyclic blood flow variations induced by platelet-activating factor in stenosed canine coronary arteries despite inhibition of thromboxane synthetase, serotonin receptors, and alpha-adrenergic receptors.

P Apprill, J M Schmitz, W B Campbell, G Tilton, J Ashton, S Raheja, L M Buja and J T Willerson

_Circulation_. 1985;72:397-405
doi: 10.1161/01.CIR.72.2.397

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/72/2/397

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/