Effect of Platelet Activation on Coronary Collateral Blood Flow

James W. Kinn, MD; Robert J. Bache, MD

Background—The platelet products thromboxane $A_2$ and serotonin have been shown to cause constriction of well-developed coronary collateral vessels. This study was performed to determine whether intravascular platelet activation produced with platelet activating factor (PAF) can cause a decrease in coronary collateral blood flow.

Methods and Results—Collateral vessel growth was induced by embolization of a hollow stainless steel plug into the left anterior descending coronary artery (LAD) of adult dogs. The animals were returned to the laboratory 3 to 6 weeks later for surgical instrumentation and measurement of collateral blood flow. Collateral flow was assessed by measuring retrograde blood flow from the cannulated collateral-dependent artery. PAF (10 nmol) was injected into the left main coronary artery to allow products of platelet activation to reach collateral vessels arising from the left coronary system. PAF caused a vasoconstrictor response, which became maximal 3 minutes after injection and resulted in a 40.3±7.4% decrease in retrograde blood flow (32.1±2.1 to 19.6±3.2 mL/min; $P<0.05$). By 15 minutes after the PAF injection, both retrograde blood flow and transcollateral resistance had returned to normal. After pretreatment with the thromboxane $A_2$ receptor antagonist SQ30,741, the vasoconstrictor response to PAF was abolished and, in contrast to the decrease in retrograde blood flow from PAF alone, a weak vasodilator effect was unmasked.

Conclusions—PAF caused a decrease in coronary collateral blood flow. This vasoconstrictor response required the participation of thromboxane $A_2$. (Circulation, 1998;98:1431-1437.)

Key Words: blood flow ■ collateral circulation ■ platelets ■ thromboxane

In response to arterial occlusion, native intercoronary collateral anastomoses undergo remarkable growth and development to provide an alternate blood supply to the dependent myocardium. During this process, the thin walled, veinlike intercoronary anastomoses are transformed into vessels which appear similar to small arteries. However, these collateral vessels demonstrate histological abnormalities including perivascular inflammation, endothelial proliferation, and adherence of monocytes, neutrophils, and platelets to the endothelium. Each of these adherent cell types is capable of producing the biologically active phospholipid, platelet activating factor (PAF). To what extent or by what stimulus these cells produce PAF is unclear, but spontaneous platelet activation can occur in narrowed coronary arteries, and this is associated with PAF accumulation at the site of endothelial injury.

Well-developed canine coronary collateral vessels have been shown to undergo vasoconstriction in response to the thromboxane $A_2$ analogue U46619. Because thromboxane $A_2$ is liberated during platelet degranulation, it is possible that activation of platelets within or upstream from collateral vessels could cause collateral vasoconstriction and decrease blood flow to the dependent myocardium. PAF has the potential to activate platelets adherent to the collateral vessels and to induce aggregation of circulating platelets. No studies of the effect of PAF on coronary collateral vessels are currently available. Infusion of PAF into the normal coronary circulation typically yields a biphasic response, with initial vasodilation followed by vasoconstriction. Houston et al observed that exposure of isolated coronary artery rings to aggregating platelets resulted in relaxation, but after removal of the endothelial layer, exposure to aggregating platelets caused contraction. Inhibition of thromboxane $A_2$ production using a thromboxane synthase blocker (dazoxiben) or a thromboxane $A_2$ receptor antagonist (SQ29,548) attenuated contraction of the denuded coronary artery rings in response to aggregating platelets. These results indicate that thromboxane $A_2$ is an important mediator of platelet-induced coronary artery constriction, whereas an intact endothelium is necessary for the relaxation of coronary artery segments in response to platelet aggregation. The purpose of this study was to determine the effects of intravascular platelet activation on coronary collateral blood flow by measuring the response to PAF in an in vivo canine model. This study also examined the importance of thromboxane $A_2$ in this response using the receptor antagonist SQ30,741. This agent was used because previous studies have demonstrated that SQ30,741 is a potent antagonist of thromboxane $A_2$ mediated vasoconstriction that does not alter the response to serotonin.
Methods

All studies were conducted in accordance with the “Position of the American Heart Association on Research Animal Use” and were approved by the Animal Care Committee of the University of Minnesota.

Induction of Collaterals

Collateral vessel development in adult mongrel dogs was induced by catheter embolization of the left anterior descending coronary artery (LAD) with a hollow plug as previously described. Animals were anesthetized with sodium pentobarbital (25 to 30 mg/kg IV), intubated, and ventilated with a respirator. Under sterile conditions, an 8F Judkins right coronary catheter was introduced into the right carotid artery and advanced in a retrograde direction until the tip could be palpated in the left main coronary artery to allow intracoronal arteriotomy performed between the plug and the ligature. A 0.014-in guidewire was passed through the catheter into the distal LAD. Nitroglycerin (100 μg IC) was given and the coronary artery diameter was assessed with a subsequent contrast injection. The coronary catheter was then removed while the guidewire position was carefully maintained. An appropriately sized (2.3 to 3.0 mm OD, 1.5 cm of the artery was dissected free proximally. The dog was anesthetized with sodium pentobarbital (100 mg/kg SC), anesthetized with α-chloralose (100 mg/kg IV followed by 10 mg/kg per hour), intubated, and ventilated with a respirator. Supplemental oxygen was given to maintain arterial PO2 in the physiological range. Two 6F NIH catheters were introduced into the femoral arteries and positioned in the ascending aorta for blood sampling and pressure monitoring. A similar catheter was introduced into the left carotid artery and advanced into the left ventricle. A left thoracotomy was performed in the fifth intercostal space. The heart was suspended in a pericardial cradle, and a PVC catheter (3.0 mm OD) was inserted into the left atrium through the appendage. The occluding plug was located by palpation of the LAD, and 1.5 cm of the artery was dissected free proximally. The dog was heparinized (5000 U IV), the artery occluded proximally, and a longitudinal arteriotomy performed between the plug and the ligation. The plug was removed and the artery allowed to bleed gradually to remove any residual thrombus. The occluding plug was carefully inspected, and in each case the lumen was found to be totally occluded by white thrombus. The artery was then cannulated with a thin-wall stainless steel cannula (4.0 mm OD). Pressure at the cannula tip was measured with a 23-gauge tube incorporated into the wall of the cannula. A PE50 catheter was inserted into the proximal LAD and advanced in a retrograde direction until the tip could be palpated in the left main coronary artery to allow intracoronary infusions. Aortic, left ventricular, and coronary cannula pressures were measured with Statham P23ID pressure transducers. Left ventricular pressure was recorded at normal and high gain for measurement of end-diastolic pressure. An electronic differentiator was used to obtain left ventricular dp/dt. Data were recorded on an 8-channel direct writing recorder.

Drugs

PAF was purchased from Sigma Chemical Co. PAF was stored at −20°C in chloroform solution. After evaporation, the PAF was suspended in a 0.2% solution of BSA in normal saline. SQ30,741 was obtained from Squibb Pharmaceutical Co, stored at −20°C in 100% ethanol, and diluted in normal saline.

Experimental Protocols

Two groups of animals were studied. Group 1 consisted of 5 animals used to determine the collateral flow response to platelet activation produced by PAF. Group 2 consisted of 7 animals used to determine the role of thromboxane A2 in the collateral response to PAF.

Group 1: Effects of PAF on Collateral Vasomotor Tone

Animals were allowed to stabilize for 20 to 30 minutes after completion of the surgical preparation. Collateral flow was then assessed by collecting retrograde blood from the coronary cannula into a graduated cylinder over 20 seconds while the tip of the cannula was maintained at the level of the heart. Collections were repeated until stable values were achieved. After completion of baseline measurements, the PAF vehicle (3 mL of 0.2% BSA in normal saline) was injected into the left main coronary artery over 5 seconds. Retrograde blood flow was measured at 1, 3, 5, 10, and 15 minutes after vehicle administration. Next, PAF was injected into the left main coronary artery in increasing doses of 0.1, 1.0, and 10 nmol. These doses were chosen because similar doses have produced vasoactive effects in other coronary segments. An intracoronary route of drug administration was used to minimize systemic effects of PAF. Hemodynamic responses to each dose were recorded over a 15-minute interval. Baseline measurements of retrograde blood flow were made 1 minute before injection, and then at 1, 3, 5, 10, and 15 minutes after each PAF injection. In each case, hemodynamic and retrograde flow measurements had returned to control values before the subsequent dose of PAF was administered.

Group 2: Contribution of Thromboxane A2 to the Collateral Response to PAF

Because PAF was observed to cause a decrease in collateral blood flow in Group 1, animals in Group 2 were used to determine the role of thromboxane A2 in this response. Collateral blood flow was assessed by measuring retrograde flow as previously described. The responses of collateral blood flow to vehicle and then to high-dose PAF (10 nmol injected over 5 seconds) were each assessed over a 15-minute interval by measuring retrograde flow 1 minute before and 1, 3, 5, 10, and 15 minutes after intracoronary injection. Next, the selective thromboxane A2 receptor antagonist SQ30,741 (100 μg/kg) was infused into the left main coronary artery over 30 minutes. Retrograde blood flow was measured during the initial 15 minutes after beginning the SQ30,741 infusion using the same time points described above. After 15 minutes of SQ30,741 infusion, PAF (10 nmol) was again injected into the left main coronary artery and the response to PAF was measured over the final 15 minutes of the SQ30,741 infusion. The effects of SQ30,741 were then allowed to subside over a period of 1 hour. After this recovery period, a third

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### Table 1. Hemodynamic Data From 5 Dogs in Group 1 During Control Conditions and 3 Minutes After Administration of PAF

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Heart Rate, beat/min</th>
<th>Aortic Pressure, mm Hg</th>
<th>Rate-Pressure Product, mm Hg · beat⁻¹ · min⁻¹</th>
<th>Distal Coronary Pressure, mm Hg</th>
<th>Aortic-Coronary Gradient, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>115 ± 11</td>
<td>109 ± 9</td>
<td>79 ± 5.0</td>
<td>81 ± 4.4</td>
<td>1100 ± 1400</td>
</tr>
<tr>
<td>PAF 10⁻¹⁰ mol</td>
<td>105 ± 13</td>
<td>111 ± 20</td>
<td>83 ± 6.0</td>
<td>82 ± 5.7</td>
<td>11 500 ± 1200</td>
</tr>
<tr>
<td>PAF 10⁻⁸ mol</td>
<td>110 ± 8</td>
<td>122 ± 13</td>
<td>81 ± 5.9</td>
<td>77 ± 6.4</td>
<td>11 1000 ± 1200</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *P < 0.05 vs control; †P < 0.05 vs control.
dose of 10 nmol of PAF was injected into the left main coronary artery to confirm that the vasoconstrictor response was still intact.

**Data Analysis**

Heart rate, aortic and left ventricular pressures, and left ventricular dP/dt were measured from the strip chart recordings. Hemodynamic data were analyzed using ANOVA for repeated measures. A value of P<0.05 was required for statistical significance. If significant, data were further compared using the Wilcoxon signed rank test.

**Results**

**Group 1: Effects of PAF**

In all animals, the coronary artery was found to be totally occluded. Hemodynamic data and retrograde blood flow measurements were obtained at baseline and 1, 3, 5, 10, and 15 minutes after the start of each intervention. The peak response generally occurred at 3 minutes after PAF administration and subsided by the end of the 15-minute observation period. Measurements obtained 1 minute before and 3 minutes after the start of each intervention are reported in Table 1. At baseline, mean aortic blood pressure was 79±5.0 mm Hg, whereas pressure measured in the LAD with the cannula closed was 62±5.1 mm Hg. The transcollateral pressure gradient, calculated as the difference between mean aortic pressure and mean LAD coronary pressure while the cannula was closed, was 15.8±2.6 mm Hg. When the cannula was opened, baseline retrograde blood flow was 30.4±1.8 mL/min and transcollateral resistance was 2.65±0.29 mm Hg · mL⁻¹ · min⁻¹. PAF vehicle injected into the left main coronary artery produced no significant change in systemic hemodynamics (Table 1). Three minutes after injection of vehicle there was an 8.1±2.3% increase in retrograde blood flow (30.4±1.8 versus 32.8±1.7 mL/min; P<0.05), as seen Figure 1.

The smallest dose of PAF (10⁻¹⁰ mol) administered into the left main coronary artery produced no significant systemic hemodynamic effects (Table 1) but prevented the increase in collateral flow produced by vehicle alone (Figure 1). Injection of PAF at a dose of 10⁻⁸ mol tended to decrease mean aortic pressure and distal coronary pressure with the cannula closed, but the transcollateral pressure gradient was not significantly altered (17.5±3.4 versus 18.2±2.9 mm Hg; P=NS). Heart rate tended to increase with this dose of PAF, but this was not statistically significant. Retrograde blood flow tended to decrease with this dose of PAF, but this did not achieve statistical significance (Figure 1). After injection of the highest dose of PAF (10⁻⁶ mol), inflation of the aortic occluder was generally required to maintain proximal aortic pressure. Peak systemic hemodynamic changes and coronary collateral flow effects occurred approximately 3 minutes after administration of PAF and returned to control values before the end of the 15-minute observation period. This dose of PAF decreased mean aortic pressure and tended to increase heart rate (Table 1). Distal LAD coronary pressure with the cannula closed also decreased (55±6.6 versus 43±3.4 mm Hg; P<0.05), and the transcollateral pressure gradient tended to increase, although this was not statistically significant (21.0±5.8 versus 26.0±6.3 mm Hg). Three minutes after high-dose PAF injection, retrograde flow was decreased 40.2±7.4% (32.1±2.7 versus 19.6±3.2 mL/min; P<0.05). This corresponded to a 70.1±25.0% increase in transcollateral resistance (2.48±0.24 versus 4.40±0.92 mm Hg · mL⁻¹ · min⁻¹; P<0.05). By the end of the 15-minute time interval, retrograde flows had returned to baseline.

**Group 2: Effects of SQ30,741 on the Collateral Response to PAF**

After the vasoconstrictor response to PAF (10⁻⁶ mol intracoronary) had been established (Figure 2), the contribution of thromboxane A₂ was evaluated with the thromboxane A₂ receptor antagonist SQ30,741. Infusion of SQ30,741 into the left main coronary artery produced no systemic hemodynamic effects (Table 2) and no change in retrograde blood flow in comparison with baseline measurements (32.8±5.0 mL/min at baseline versus 34.5±3.2 mL/min at 3 minutes; P=NS), or

![Figure 1](image1.png)

**Figure 1.** Retrograde blood flow from the cannulated collateral-dependent LAD in response to PAF in doses of 0.1, 1.0, and 10 nmol, as well as vehicle control in 5 animals in group 1. *P<0.05 vs baseline. †P<0.05 vs vehicle control at the same time point.

![Figure 2](image2.png)

**Figure 2.** Response of retrograde blood flow from the cannulated collateral dependent LAD to PAF (10⁻⁶ mol IC) during baseline conditions and during thromboxane A₂ receptor blockage produced by infusion of SQ30,741 in 7 animals in group 2. P<0.05 vs vehicle.
in comparison to matched vehicle time points (32.8±1.7 versus 34.5±3.2 mL/min at 3 minutes; *P=NS). Similarly, there were no significant changes in transcollateral resistance.

During the final 15 minutes of the SQ30,741 infusion, PAF (10⁻⁸ mol) was again injected into the left main coronary artery. In comparison to the previous injection, the tendency toward decreased aortic pressure was attenuated (Table 2). As shown in Figure 2, SQ30,741 abolished the collateral vasoconstrictor effect of PAF so that 3 minutes after PAF injection retrograde flow was 37.0±3.0 mL/min as compared with 19.6±3.2 mL/min 3 minutes after PAF injection before SQ30,741 (P<0.05). At this time, the transcollateral resistance was 2.11±0.21 mm Hg · mL⁻¹ · min⁻¹ as compared to 4.40±0.92 mm Hg · mL⁻¹ · min⁻¹ with PAF alone (P<0.05). Furthermore, SQ30,741 unmasked a weak collateral vasodilator response; analysis of variance testing demonstrated increased retrograde flow when PAF was administered during SQ30,741 infusion (P<0.05). However, this response was small so that no significant difference was found between vehicle and PAF after SQ30,741 for any of the individual time points. In comparison with vehicle control measurements, transcollateral resistance was significantly decreased relative to the corresponding 3-minute vehicle value (2.11±0.21 versus 2.51±0.26 mm Hg · mL⁻¹ · min⁻¹; *P<0.05). Figure 2 demonstrates that the vasodilator effect of PAF unmasked by SQ30,741 was somewhat delayed, occurring 3 to 5 minutes after injection of PAF, whereas the vasoconstrictor effect of PAF occurred predominantly 1 to 3 minutes after PAF injection.

After the effects of SQ30,741 had subsided, PAF was again injected to insure that the vasoconstrictor effects of PAF were not attenuated by repeated injection. This final injection of PAF resulted in decreases in retrograde flow (27.7±1.4 versus 18.0±2.0 mL/min; *P<0.05) and increases in transcollateral resistance (2.89±0.30 versus 4.17±0.44 mm Hg · mL⁻¹ · min⁻¹; *P<0.05). As shown in Figure 3, the minimum retrograde blood flow (19.6±3.2 versus 18.0±2.0 mL/min; *P=NS) and peak transcollateral resistance (4.40±0.92 versus 4.17±0.44 mm Hg · mL⁻¹ · min⁻¹; *P=NS) in response to PAF were similar before and 1 hour after SQ30,741 infusion, respectively.

Discussion

This study demonstrates that PAF can produce a marked decrease in coronary collateral flow. This response was dependent on the activity of thromboxane because thromboxane A₂ receptor blockade with SQ30,741 abolished the PAF-induced decrease in collateral blood flow.

TABLE 2. Hemodynamic Data From 7 Dogs in Group 2

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Heart Rate, beats/min</th>
<th>Aortic Pressure, mm Hg</th>
<th>Rate-Pressure Products, mm Hg · beat⁻¹ · min⁻¹</th>
<th>Distal Coronary Pressure, mm Hg</th>
<th>Aortic-Corony Gradient, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Drug</td>
<td>Control</td>
<td>Drug</td>
<td>Control</td>
</tr>
<tr>
<td>SQ30,741</td>
<td>120±12</td>
<td>123±17</td>
<td>79±5.3</td>
<td>76±5.0</td>
<td>53±5.8</td>
</tr>
<tr>
<td>SQ30,741+ PAF 10⁻⁸ mol</td>
<td>120±8</td>
<td>123±6</td>
<td>79±3.9</td>
<td>76±5.1</td>
<td>51±5.6</td>
</tr>
<tr>
<td>PAF 10⁻⁸ mol</td>
<td>120±4</td>
<td>120±3</td>
<td>78±5.0</td>
<td>63±9.8</td>
<td>48±5.0*</td>
</tr>
</tbody>
</table>

*P<0.05 vs vehicle. Values are mean±SE.
manipulation of collaterals arising from the left circumflex, proximal LAD and septal arteries, which are the major source of collaterals to the LAD in the dog. Studies were performed 3 to 6 weeks after coronary occlusion at a time when collateral vessels are not fully developed. However, prominent responses to both vasoconstrictor and vasodilator agonists (including endothelium-dependent vasodilators) are present in developing collateral vessels studied as early as 2 weeks after coronary occlusion. Nevertheless, it is possible that the responses observed in the present study could change with further collateral maturation.

Another limitation of this study is related to the systemic effects of PAF. PAF has powerful hemodynamic effects, the most notable being systemic hypotension. For this reason, PAF was administered by the intracoronary route to allow use of relatively small doses to minimize systemic effects. Nevertheless, there was a trend toward a decrease in aortic blood pressure that was statistically significant during the highest dose of PAF. To account for the decrease in collateral driving pressure, collateral resistance was calculated. This calculation demonstrated a substantial vasoconstrictor response to PAF with a 70 ± 25% increase in collateral resistance in response to the highest dose of PAF. The decrease in arterial pressure following PAF was associated with a trend toward an increase in heart rate, suggesting reflex sympathetic activation. Sympathetic activation would not be expected to cause collateral constriction, however, because both in vitro studies of isolated collateral vessels and in vivo studies of well-developed collateral vessels have failed to demonstrate vasoconstriction in response to α-adrenergic agonists.

**Effects of PAF**

PAF is a potent stimulus for platelet aggregation. Aggregating platelets release multiple vasoactive substances, including thromboxane A_2_, serotonin, ADP, and histamine. Houston et al demonstrated that aggregating platelets caused relaxation of preconstricted coronary artery rings. This was an endothelium-dependent response, because aggregating platelets resulted in vasoconstriction of denuded coronary artery rings that could be blocked with selective antagonists of thromboxane A_2_ and serotonin. Both thromboxane A_2_ and serotonin have been demonstrated to exert vasoconstrictor activity on canine coronary collateral vessels in vivo. Our observation of collateral vasoconstriction in response to PAF is consistent with these previous findings. In contrast to the present results, Leong et al demonstrated that the PAF receptor antagonist WEB 2086 did not improve collateral flow or limit myocardial infarct size in a dog model of acute coronary occlusion. PAF acts on a variety of receptor subtypes, whereas WEB 2086 antagonizes only a subset of these receptors, so the negative results could have been due in part to incomplete receptor blockade. More likely, failure of WEB 2086 to alter collateral flow during acute myocardial infarction was the result of the limited vasomotor capacity of the rudimentary native collateral vasculature present at the time of acute coronary occlusion.

In contrast to the strictly vasoconstrictor response of the collateral system to PAF in the present study, other investigators have observed a biphasic response to PAF in the normal coronary circulation with transient initial vasodilation followed by vasoconstriction. In the normal heart, the initial coronary vasodilation generally lasts 15 to 30 seconds and consequently might not have been detected in the present study, in which the first flow measurement was made 1 minute after the PAF injection. Alternatively, the lack of vasodilation could reflect absence of receptors to platelet-derived substances such as ADP or serotonin on the collateral vessel endothelium. Shimokawa et al demonstrated that 4 weeks after balloon denudation of normal coronary arteries, vessel segments containing newly regenerated endothelium have an impaired vasodilator response to aggregating platelets. It is possible that newly developed endothelium in immature collaterals might have a similarly impaired vasodilator response to platelet aggregation.

Direct effects of PAF on collateral vasomotor tone could have contributed to the modest increase in retrograde flow observed after thromboxane A_2_ blockade in this study. Shimokawa et al reported that PAF caused endothelium-dependent relaxation in isolated porcine coronary arteries at concentrations several orders of magnitude higher (IC_50_ 10^-5 mol) than used in the present study. Hu and Man reported a weak biphasic response after PAF injection in an isolated rat heart preparation perfused with Krebs-Henseleit buffer. Consequently, it is possible that PAF exerted a direct vasodilator effect on the collateral vasculature in the present study. Although a weak PAF-induced collateral vasodilatation cannot be excluded, it did not play an important role in our study because vasoconstriction was the predominant response. Furthermore, vasocostriction was completely abolished by the specific thromboxane receptor antagonist SQ30,741. Similarly, Loots and DeClerck demonstrated that platelet aggregation induced by collagen fibrils caused vasoconstriction in a cat hindlimb collateral model, suggesting that it was aggregation of platelets that led to constriction of the collateral vasculature.

**Role of Thromboxane A_2_ in the Collateral Response to PAF**

Thromboxane A_2_ has previously been demonstrated to cause vasoconstriction in both normal coronary vasculature and in moderately well-developed coronary collateral vessels. SQ30,741 completely abolished the vasoconstrictor response to PAF in the present study, suggesting that thromboxane A_2_ was the predominant agent responsible for coronary collateral vasoconstriction. In accordance with our observations, Forsterman et al found that when isolated coronary artery rings were exposed to aggregating platelets, thromboxane A_2_ lib-
erated from platelets was the predominant vasoconstrictor. In contrast to our findings, Loots and DeClerck24 found that in a cat hindlimb collateral vessel preparation, serotonin had a greater role in collateral vasoconstriction than thromboxane A$_2$. This difference may be due to differences in species or vascular bed. A possible role for serotonin in the collateral vasoconstrictor response to PAF was not examined in the present study. Although thromboxane A$_2$ appears to be the predominant vasoconstrictor, it is possible that any vasoconstrictor effect of serotonin was not powerful enough to override the PAF-induced vasodilator activity which was unmasked during SQ30,741 infusion. Further studies are necessary to determine a potential contribution of serotonin to this response.

Thromboxane A$_2$ receptor blockade with SQ30,741 unmasked a weak vasodilator response. Intracoronary infusion of SQ30,741 did not alter baseline retrograde flow, indicating that the vasodilation from the combination of SQ30,741 and PAF was not due to the effects of SQ30,741 alone. The modest increase in collateral flow could have resulted from vasodilators released by aggregating platelets including ADP, histamine, and prostacyclin or other prostanooids.27 PAF-induced endothelium-dependent vasodilation could also have contributed to the vasodilation from PAF during SQ30,741 infusion.28 A direct vasodilator effect on coronary arterial vessels has been demonstrated at PAF concentrations several orders of magnitude higher than those achieved in the present study.23,27 Although statistically significant, the magnitude of PAF-induced collateral vasodilation during thromboxane A$_2$ receptor blockade was small.

It can be argued that SQ30,741 might have decreased PAF-induced platelet aggregation, thereby attenuating the release of vasoactive platelet derived compounds. However, previous investigators have reported that PAF can induce platelet aggregation independent of thromboxane A$_2$. The mechanisms by which PAF induces platelet aggregation likely include more than 1 pathway. Independent contributions of both the cyclooxygenase and lipoxygenase pathways have been demonstrated in PAF-induced platelet aggregation.29,30 In addition, PAF appears to exert effects via a "third pathway."30,31 Furthermore, although it is unknown whether SQ30,741 directly affects PAF-induced platelet aggregation, the thromboxane A$_2$ receptor antagonist SQ29,548 does not inhibit platelet aggregation in response to ADP in vitro.32 In addition, Aprill et al15 found that pretreatment with the thromboxane synthase inhibitor UK38,485 abolished the increase of measured thromboxane B$_2$ generated in response to PAF but did not alter PAF-induced platelet aggregation. Therefore, it is unlikely that blockade of PAF-induced collateral constriction by SQ30,741 in the present study resulted from inhibition of PAF-induced platelet aggregation.

In designing this study, there was concern that tachyphylaxis might develop during repeated injections of PAF. Thus, PAF-induced bronchial smooth muscle contraction has been found to be attenuated after multiple doses.32 To determine whether the collateral vessel response to PAF remained stable, we injected a final dose of PAF after the effects of SQ30,741 had subsided; the response of collateral flow was not different from the initial injection. Likewise, the absolute magnitude of the response of collateral flow was not altered with serial doses of PAF. Therefore, in this experimental model, serial doses of 10 nmol of PAF produced a reproducible response in the collateral vessels. However, only 3 doses of PAF were used, each separated by 45 to 60 minutes; it is possible that tachyphylaxis would occur with a larger number of doses.

**Clinical Implications**

Ischemia is associated with rapid accumulation of myocardial lyosphospholipids.33,34 Lyso-PAF, the precursor of PAF, can increase by as much as 50% within 20 minutes of myocardial ischemia.35 Developing collateral vessels may have even greater potential to produce PAF because the monocytes, neutrophils, and platelets adherent to the collateral endothelial surfaces are capable of producing PAF.1,2 These findings suggest that collateral dependent myocardial regions could be vulnerable to vasoconstriction produced by PAF. Willerson and associates15,36–38 have demonstrated that cyclic flow variations resulting from platelet aggregation at the site of a coronary artery stenosis are associated with accumulation of PAF in the damaged vessel5 and production of thromboxane A$_2$ and serotonin in concentrations that are sufficient to cause vasoconstriction. Increased thromboxane A$_2$ production has been demonstrated in patients with myocardial ischemia.39 Platelet aggregation in diseased donor arteries from which collateral vessels arise would have potential to decrease blood flow to the dependent myocardial region. In addition, ischemia can cause release of other mediators of vasoconstriction such as neuropeptide Y40 or endothelin41 which could contribute to impaired perfusion of collateral-dependent myocardium.

**Acknowledgments**

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**References**


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