The Mechanism of Coronary Collateral Vasoconstriction in Response to Cyclooxygenase Blockade

John D. Altman, Robert J. Bache

Abstract The present study was performed to examine the mechanism by which cyclooxygenase blockade produces vasoconstriction in well-developed coronary collateral vessels. Eight dogs were studied 4 to 6 months after occlusion of the left anterior descending coronary artery (LAD) had been performed to stimulate collateral vessel growth. At the time of study, the LAD was cannulated at the site of occlusion for measurement of retrograde blood flow as an index of collateral blood flow. Levels of 6-ketoprostaglandin F$_{1a}$ were 32±13% higher in blood diverted from the collateral-dependent LAD than in aortic blood (P<.05); the increase in this stable product of prostacyclin metabolism indicated production of prostacyclin across the coronary collateral system. Administration of arachidonic acid into the left main coronary artery to reach collateral vessels entering the LAD resulted in a 21±6% increase in retrograde flow (P<.01), demonstrating cyclooxygenase activity with production of vasodilator prostaglandins in the collateral system. Ibuprofen (10 mg/kg IV) caused a 55±7% decrease in retrograde flow (P<.03), suggesting that cyclooxygenase blockade inhibited tonic production of vasodilator prostaglandins in the collateral system. In contrast, neither thromboxane synthase inhibition with dazmegrel nor thromboxane receptor blockade with SQ 30741 caused a significant change in collateral flow, thus failing to support thromboxane-induced collateral constriction. After cyclooxygenase blockade, prostacyclin infused into the left main coronary artery was able to restore retrograde flow to the preibuprofen level. In contrast, lipoxigenase blockade with BW A4C (3.4 mg/kg IV) did not increase retrograde flow, indicating that the decreased collateral flow after cyclooxygenase blockade was not the result of increased production of vasoconstrictor leukotrienes. These findings support the concept that collateral vasoconstriction produced by cyclooxygenase blockade is the result of inhibition of the tonic production of vasodilator prostaglandins within the collateral system. (Circ Res. 1994;74:310-317.)

Key Words • coronary blood flow • ibuprofen • thromboxane A$_2$ • lipoxigenase • prostaglandins

We recently reported that indomethacin caused a substantial decrease in blood flow through well-developed canine coronary collateral vessels. Several mechanisms could account for the observed response to indomethacin. First, collateral vasoconstriction could result from interruption of production of vasodilator prostaglandins such as prostacyclin by the collateral vessels. Prostaglandin production could be enhanced as a result of the perivascular inflammatory changes previously described in association with collateral vessel development or from the subintimal and endothelial cell hyperplasia characteristic of collateral vessels. Second, cyclooxygenase blockade could result in diversion of arachidonic acid into the lipoxigenase pathway, thereby causing increased leukotriene production with consequent collateral vessel constriction. Finally, a direct vasoconstrictor action, which appears to be independent of cyclooxygenase inhibition, has been reported for indomethacin and might account for the observed findings.

The present study was designed to examine each of these potential mechanisms. Collateral vessel synthesis of vasodilator prostaglandins was assessed by observing the response to arachidonic acid infusion. To examine whether arachidonic acid may have vasodilating properties independent of prostaglandin production, the infusion was repeated after cyclooxygenase blockade. To determine whether prostacyclin production was enhanced in the collateral vessels, 6-ketoprostaglandin F$_{1a}$, the stable product of prostacyclin metabolism, was measured in blood diverted from the collateral system. We hypothesized that if perivascular inflammation resulted in augmented production of vasodilator prostaglandins, then local production of vasoconstrictor eicosanoids, including thromboxane A$_2$, might also be increased. To test this hypothesis, thromboxane synthase and thromboxane A$_2$ receptor blockade were produced with dazmegrel and SQ 30741, respectively, to determine whether these interventions would increase collateral flow. To determine whether thromboxane synthase inhibition with dazmegrel and SQ 30741 caused a significant change in collateral flow, thus failing to support thromboxane-induced collateral constriction. After cyclooxygenase blockade, prostacyclin infused into the left main coronary artery was able to restore retrograde flow to normal. To determine whether the previously reported collateral vasoconstrictor response is unique to indomethacin, in the present study ibuprofen was used to cause cyclooxygenase blockade. Finally, after cyclooxygenase blockade had been established, a selective inhibitor of lipoxigenase was administered to determine whether the collateral constriction observed in response to ibuprofen could be reversed by blocking leukotriene production.
Materials and Methods

Studies were performed in eight adult mongrel dogs weighing 22 to 28 kg. All studies were performed in accordance with the “Position of the American Heart Association on Research Animal Use,” adopted November 11, 1984, and protocols were approved by the Animal Care Committee of the University of Minnesota.

Induction of Collateral Vessel Growth

Collateral vessel growth was induced by embolization of the left anterior descending coronary artery with a hollow plug.13 Animals were anesthetized with sodium pentobarbital (25 to 30 mg/kg IV), intubated, and ventilated with a respirator. The right carotid artery was isolated under sterile conditions. After administration of heparin sodium (6000 U IV), an 8F Judkins right coronary artery catheter was introduced into the left coronary ostium and directed toward the anterior descending artery. A 0.014-in angioplasty guide wire was advanced through the catheter and into the distal anterior descending coronary artery. After intracoronary administration of nitroglycerin (100 µg), the catheter was removed, leaving the guide wire in place, and a hollow stainless-steel plug (outer diameter, 2.3 to 2.7 mm; inner diameter, 1.1 mm; length, 4 mm) was advanced along the guide wire until the plug was firmly wedged in the coronary artery. The guide wire was then removed. The position and patency of the plug were confirmed fluoroscopically by intracoronary injection of 5 mL of 60% diatrizoate meglumine.

Surgical Preparation

After allowing 4 to 6 months for collateral vessel development, animals were returned to the laboratory, premedicated with morphine sulfate (1 mg/kg SC), anesthetized with α-chloralose (100 mg/kg IV followed by 10 mg · kg⁻¹ · h⁻¹), intubated, and ventilated with a respirator using supplemental oxygen to maintain arterial PO₂ within the physiological range. Two 7F National Institutes of Health catheters were introduced into the femoral arteries and advanced into the ascending aorta for blood sampling and pressure measurement. A similar catheter was introduced into the left carotid artery and advanced into the left ventricle for pressure measurement. A left thoracotomy was performed in the fifth intercostal space. A pulmonary cuff occluder was fitted around the descending thoracic aorta to allow control of proximal aortic pressure. The heart was suspended in a pericardial cradle, and a PVC catheter (outer diameter, 3.0 mm) was introduced into the left atrium. The coronary artery plug was located by palpation, and the anterior descending coronary artery was mobilized for 1.0 to 1.5 cm proximal and distal to the plug. Anticoagulation was produced by administration of sodium heparin (200 U/kg IV followed by 1000 U/h). The coronary artery was occluded proximally, the occluding plug was extracted, and the artery was cannulated with a thin-wall stainless-steel cannula (outer diameter, 4 mm). Coronary cannula resistance determined from the pressure drop produced by passing measured flows of blood through the cannula was 0.097 mm Hg per milliliter per minute of flow. Coronary pressure was measured with a 23-gauge tube incorporated into the wall of the cannula. The proximal occlusion was partially released, and a heparin-filled PE-90 catheter was introduced into the artery and advanced retrograde to the left main coronary artery to allow drug infusion. The position of the catheter tip in the left main coronary artery was confirmed at autopsy after completion of the study.

Experimental Protocol

Before all interventions, blood samples (5 mL each) from five of the animals were collected from the retrograde coronary cannula and from the aortic catheter to assay for 6-ke-toprostagladin F₁α levels. Blood was collected in EDTA tubes containing 10⁻⁵ mol/L indomethacin on ice and separated by centrifugation at 2500 rpm for 15 minutes at 4°C. Plasma was stored at −70°C until assay.

Left ventricular, aortic, and coronary pressures were measured with Statham P23XL pressure transducers. Left ventricular pressure was recorded at normal and high gain for measurement of end-diastolic pressure. Left ventricular dp/dt was obtained by electronic differentiation of the pressure signal. Data were recorded on an eight-channel direct-writing recorder (Coulbourn Instruments Inc, Lehigh Valley, Pa). Measurements of interarterial collateral blood flow were performed by collecting retrograde flow from the coronary cannula into a graduated cylinder for 30 seconds while the cannula tip was maintained at the level of the heart. Control measurements were repeated until consistent collections were obtained; measurements were thereafter performed in duplicate, and the results were averaged. Distal coronary pressure measurements were obtained with the cannula tubing clamped.

After obtaining control measurements, cyclooxygenase activity within the collateral circulation was assayed by infusing arachidonic acid (20 µg · kg⁻¹ · min⁻¹) into the left main coronary artery catheter in eight dogs. Arachidonic acid (Sigma Chemical Co, St Louis, Mo) was prepared by dissolving it in a solution of 10% ethanol in 100 mmol/L Na₂CO₃ under nitrogen. This solution was diluted with isotonic saline to a final concentration of 1 mg/kg and used within 24 hours after preparation. Two minutes after beginning the infusion, hemodynamic measurements were obtained, and retrograde flow collections were performed. After retrograde flow had returned to the baseline level, the effect of basal thromboxane A₂ production on collateral blood flow was evaluated. Retrograde flow measurements were repeated after either thromboxane synthase inhibition with dazmegrel (5 mg/kg IV, n=4) or thromboxane A₂ receptor blockade with SQ 30741 (10 µg · kg⁻¹ · min⁻¹ IC, n=7). Dazmegrel was mixed with 2 mL sterile water to which 1.0N NaOH was added until dissolved. The pH was adjusted to 8.5 with 1.0N HCl and then diluted to 5 mL with normal saline before administration. Farber and Gross14 demonstrated that this dose of dazmegrel causes effective inhibition of thromboxane synthase in dogs, with an 80% decrease in coronary venous thromboxane B₂ levels. SQ 30741 was dissolved in 1.0 mL ethanol and then diluted with normal saline so that an infusion rate of 0.6 mL/min delivered a dose of 10 µg · kg⁻¹ · min⁻¹. Measurements were obtained beginning 10 minutes after the start of the infusion. Schumacher et al10 have reported that a similar dose of SQ 30741 resulted in nearly 100% inhibition of the vasocostrictor response to U46619.

After completion of these measurements, cyclooxygenase blockade was produced by administration of ibuprofen (10 mg/kg IV) in seven dogs. Hemodynamic measurements were recorded continuously, and retrograde flow measurements were obtained 30 minutes after ibuprofen. After completion of these measurements, the adequacy of cyclooxygenase blockade was assessed by repeating the arachidonic acid infusion. Hemodynamic and retrograde flow measurements were performed 2 minutes after beginning the infusion. Since cyclooxygenase blockade decreased collateral blood flow, prostacyclin was infused after ibuprofen to determine whether this agent could restore retrograde blood flow to the level observed before cyclooxygenase blockade. Retrograde flow and hemodynamics were recorded 2 minutes after beginning an intracoronary infusion of prostacyclin (0.5 µg · kg⁻¹ · min⁻¹ IC). Prostacyclin infusion was then discontinued, and hemodynamic variables and retrograde flow were allowed to return to baseline levels.

The contribution of enhanced leukotriene production in mediating collateral vasoconstriction after cyclooxygenase blockade was then examined. In animals previously treated with ibuprofen, retrograde blood flow and hemodynamic measurements were repeated 30 minutes after lipoxigenase blockade with BW A4C (3.4 mg/kg IV).13,15 BW A4C was dissolved...
in 1.0 mL ethanol and then diluted in 5.0 mL normal saline for intravenous administration. This dose of BW A4C was used, since Maxwell et al13 showed that a similar dose of BW A4C caused >80% inhibition of 5-lipoxygenase in canine blood.

6-Ketoprostaglandin F<sub>1α</sub> Assay Method

A volume of 500 µL of plasma was deproteinized with 2 mL cold ethanol and centrifuged at 1500 rpm for 15 minutes. The supernatant was combined with 8 mL of 0.1 mol/L phosphate buffer at pH 7.2 and applied to a 200 mg C18 Sepcolumn (Peninsula Laboratories, Inc, Belmont, Calif), which had been activated with 5 mL ethanol followed by 5 mL distilled water. The plasma was washed from the column with 5 mL distilled water and 5 mL hexane. The adsorbed compounds were eluted with 5 mL of 99% ethyl acetate and 1% methanol, and the samples were dried overnight by vacuum centrifugation. Immunoreactive 6-ketoprostaglandin F<sub>1α</sub> concentrations were determined in reconstituted extracts by enzyme immunoassay (Cayman Chemical Co, Ann Arbor, Mich). The detection limit of the assay is 9 pg/mL. The intra-assay and interassay coefficients of variation were ≤10%.

Data Analysis

Heart rates and pressures were measured from the strip-chart recordings. Hemodynamic and retrograde blood flow data were analyzed using ANOVA for repeated measures. A value of P<.05 was required for statistical significance. When a significant difference was found, the Wilcoxon signed rank test was used to examine differences between individual interventions. Data are expressed as mean±SEM.

Results

6-Ketoprostaglandin F<sub>1α</sub> Measurements

Collateral vessel vessel production of prostacyclin was examined by measuring 6-ketoprostaglandin F<sub>1α</sub>, the stable metabolic product of prostaglandin I<sub>2</sub>, in blood entering and leaving collateral vessels. In five animals, aortic arch and retrograde coronary blood samples were obtained simultaneously and compared. 6-Ketoprostaglandin F<sub>1α</sub> was significantly greater in retrograde blood samples from the collateral-dependent left anterior descending coronary artery (591±122 pg/mL) than in aortic arch blood samples (444±78 pg/mL). This corresponds to a 32±13% increase in 6-ketoprostaglandin F<sub>1α</sub> across the collateral vessels (P<.05).

Effects of Arachidonic Acid

Hemodynamic measurements and retrograde flow responses to arachidonic acid before and after cyclooxygenase blockade are shown in Fig 1 and Table 1. Arachidonic acid infusion did not alter heart rate, aortic or left ventricular pressures, or left ventricular dP/dt. Retrograde flow during control conditions ranged from 19 to 68 mL/min (mean, 34±6 mL/min). Arachidonic acid infusion resulted in a 21±6% mean increase in retrograde flow (P<.01).

Later in the study, cyclooxygenase blockade with ibuprofen (10 mg/kg IV) caused a decrease in retrograde blood flow in all animals (mean decrease, 55±7%, P<.03). In four of these animals, arachidonic acid infusion was repeated after ibuprofen. Retrograde blood flow did not increase significantly during arachidonic acid infusion after ibuprofen (baseline, 14±1 mL/min; arachidonic acid, 15±2 mL/min).

![Figure 1. Bar graph showing response of retrograde blood flow to infusion of arachidonic acid (AA, 20 µg · kg⁻¹ · min⁻¹ IC) and to administration of ibuprofen (IBU, 10 mg/kg IV) in seven dogs. AA infusion was repeated in four dogs after cyclooxygenase blockade with IBU. *P<.05 in comparison with control (CON). †P<.05 in comparison with AA before IBU.](http://circres.ahajournals.org/)

**Table 1. Response to Arachidonic Acid During Control Conditions and After Ibuprofen (10 mg/kg IV)**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Heart Rate, bpm</th>
<th>Aortic Pressure, mm Hg</th>
<th>Left Ventricular dP/dt, mm Hg/s</th>
<th>Left Ventricular End-Diastolic Pressure, mm Hg</th>
<th>Distal Coronary Pressure, mm Hg</th>
<th>Aortocoronary Pressure Gradient, mm Hg</th>
<th>Retrograde Flow, mL/min</th>
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<tr>
<td>Control</td>
<td>126±11</td>
<td>97±8</td>
<td>2400±200</td>
<td>6±1</td>
<td>69±9</td>
<td>27±6</td>
<td>34±6</td>
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<tr>
<td>AA (n=8)</td>
<td>130±12</td>
<td>96±8</td>
<td>2500±300</td>
<td>7±1</td>
<td>67±8</td>
<td>29±6</td>
<td>41±7*</td>
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<tr>
<td>Control</td>
<td>116±6</td>
<td>98±1</td>
<td>2100±100</td>
<td>6±1</td>
<td>63±10</td>
<td>35±10</td>
<td>31±3</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>120±8</td>
<td>104±4</td>
<td>1900±200</td>
<td>9±2</td>
<td>43±6*</td>
<td>62±9*</td>
<td>14±1*</td>
</tr>
<tr>
<td>AA+ibuprofen (n=4)</td>
<td>118±6</td>
<td>111±4</td>
<td>1800±200</td>
<td>9±2</td>
<td>47±11*</td>
<td>64±11*</td>
<td>15±2*†</td>
</tr>
</tbody>
</table>

bpm indicates beats per minute; AA, arachidonic acid. Values are mean±SEM. Distal coronary pressure and the aortocoronary pressure gradient were measured with the cannula closed before the retrograde flow measurement.

*P<.05 vs control.
†P<.05 vs AA before ibuprofen.
Effects of Thromboxane Inhibition

Hemodynamic and retrograde flow responses to thromboxane synthase inhibition with dazmegrel (n=4) and thromboxane A₂ receptor blockade with SQ 30741 (n=6) are shown in Fig 2 and Table 2. Neither dazmegrel nor SQ 30741 caused significant changes in heart rate, aortic or left ventricular pressures, or left ventricular dP/dt. Either agent caused a change in distal coronary pressure or the aortocoronary pressure gradient with the cannula clamped. Neither thromboxane synthase inhibition nor thromboxane A₂ receptor blockade caused a significant change in retrograde blood flow.

Effects of Cyclooxygenase Blockade

Hemodynamic data and retrograde flow measurements during control conditions and after ibuprofen are shown in Table 3 and Fig 3. Heart rate, left ventricular dP/dt, and left ventricular end-diastolic pressure were not altered by ibuprofen. Ibuprofen caused a slight but significant increase in mean aortic pressure (P<.01). Distal coronary pressure with the cannula occluded decreased by 31±7% (P<.01) in response to ibuprofen. The combination of these changes resulted in a 59±28% (P<.02) increase in the aortocoronary artery pressure gradient. Thirty minutes after ibuprofen, retrograde blood flow was decreased in every animal (mean decrease, 56±5%; P<.01).

Effects of Prostacyclin

The ability of prostacyclin to restore retrograde blood flow to control levels after cyclooxygenase blockade was assessed in five dogs. Hemodynamic and retrograde flow measurements during control conditions, after cyclooxygenase blockade, and during prostacyclin infusion are shown in Table 4 and Fig 4. Systemic hemodynamic measurements were not altered by prostacyclin infusion. Retrograde flow during control conditions averaged 28±6 mL/min and was decreased to 12±3 mL/min (P<.01) after ibuprofen (10 mg/kg IV). After inhibition of basal prostaglandin production with ibuprofen, intra-coronary infusion of prostacyclin (0.5 μg · kg⁻¹ · min⁻¹) resulted in a 100±21% (P<.01) increase in retrograde flow, restoring retrograde flow to control levels (control, 28±6 mL/min; prostacyclin after ibuprofen, 24±4 mL/min).

Effects of Lipoxygenase Blockade

Hemodynamic data and retrograde flow measurements during control conditions, after cyclooxygenase blockade with ibuprofen (10 mg/kg IV), and after combined cyclooxygenase and lipoxygenase blockade with BW A4C (3.4 mg/kg IV) in five dogs are shown in Fig 5 and Table 5. After cyclooxygenase blockade, BW A4C did not alter heart rate, left ventricular or aortic pressures, or left ventricular dP/dt. In this group of animals, retrograde flow during control conditions averaged 38±5 mL/min and decreased to 19±6 mL/min after cyclooxygenase blockade (P<.01). BW A4C was administered after ibuprofen, and retrograde flow measurements were repeated 30 minutes later. Lipoxygenase blockade did not change retrograde blood flow (mean, 18±6 mL/min).

Discussion

Six new findings are reported in the present study. First, intracoronary arachidonic acid resulted in collateral vasodilation that was dependent on cyclooxygenase, demonstrating that cyclooxygenase activity is present within the well-developed coronary collateral vascular system. Second, a significant increase in prostaglandin F₂α was found between aortic and retrograde

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**TABLE 2. Responses to Thromboxane Receptor Blockade With SQ 30741 (10 μg · kg⁻¹ · min⁻¹ IC) or Thromboxane Synthetase Inhibition With Dazmegrel (5 mg/kg IV)**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Heart Rate, bpm</th>
<th>Aortic Pressure, mm Hg</th>
<th>Left Ventricular End-Diastolic Pressure, mm Hg</th>
<th>Distal Coronary Pressure, mm Hg</th>
<th>Aortocoronary Pressure Gradient, mm Hg</th>
<th>Retrograde Flow, mL/min</th>
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<td>SQ 30741 studies</td>
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<td>Control</td>
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<td>98±5</td>
<td>2300±200</td>
<td>8±1</td>
<td>65±6</td>
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<td>SQ 30741 (n=6)</td>
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<td>2500±200</td>
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<td>Dazmegrel studies</td>
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<tr>
<td>Control</td>
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<td>96±8</td>
<td>3000±200</td>
<td>9±1</td>
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<td>Dazmegrel (n=4)</td>
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<td>96±8</td>
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<td>33±15</td>
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bpm indicates beats per minute. Values are mean±SEM. Distal coronary pressure and the aortocoronary pressure gradient were measured with the cannula closed before the retrograde flow measurement.
TABLE 3. Response to Cyclooxygenase Blockade With Ibuprofen (10 mg/kg IV)

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Heart Rate, bpm</th>
<th>Aortic Pressure, mm Hg</th>
<th>Left Ventricular End-Diastolic Pressure, mm Hg</th>
<th>Left Ventricular dp/dt, mm Hg/s</th>
<th>Distal Coronary Pressure, mm Hg</th>
<th>Aortocoronary Pressure Gradient, mm Hg</th>
<th>Retrograde Flow, mL/min</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>122±6</td>
<td>101±4</td>
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<td>9±1</td>
<td>62±7</td>
<td>39±9</td>
<td>32±4</td>
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<tr>
<td>Ibuprofen (n=7)</td>
<td>126±5</td>
<td>105±4*</td>
<td>2300±200</td>
<td>8±1</td>
<td>43±5*</td>
<td>62±9*</td>
<td>14±2*</td>
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bpm indicates beats per minute. Values are mean±SEM.
Distal coronary pressure and the aortocoronary pressure gradient were measured with the cannula closed before the retrograde flow measurement.

*P<.05 vs control.

coronary blood, indicating that prostacyclin production occurred across the collateral vasculature during basal conditions. Third, inhibition of basal thromboxane A₂ production or thromboxane receptor blockade did not significantly alter blood flow through well-developed coronary collateral vessels, inferring that the increased basal vasodilator prostaglandin production in collateral vessels is not associated with a parallel increase in thromboxane A₂ production. Fourth, ibuprofen caused a significant reduction in collateral blood flow, demonstrating that collateral constriction previously observed with indomethacin is attributable to inhibition of prostaglandin production and not to a vasoconstrictor effect specific to indomethacin. Fifth, prostacyclin infusion was able to fully reverse collateral vasoconstriction produced by cyclooxygenase blockade. Finally, after ibuprofen, lipoxygenase blockade did not increase collateral flow, indicating that increased leukotriene production was not responsible for the collateral constriction produced by cyclooxygenase blockade. These new findings, as well as the method for determination of collateral blood flow, will be discussed in detail.

Critique of Method

Retrograde flow collection from the cannulated collateral-dependent coronary artery has been commonly used as an index for estimation of collateral flow. This technique slightly underestimates maximum collateral conductance because not all blood is diverted in a retrograde direction when the cannula is opened to atmospheric pressure. Thus, in the well-collateralized heart, opening the coronary cannula decreases but does not abolish blood flow into the collateral-dependent myocardial region. Downey et al. provided evidence that continuing tissue flow in the collateral-dependent region during retrograde collection is derived from microvascular communications that enter the recipient vasculature at a site distal to a significant resistance, so that antegrade flow encounters less resistance than flow back into the arterial cannula. From determinations of mean stem pressures at the origin of the collateral vessels, Harrison et al. concluded that microvascular communications could contribute no more than 25% of total collateral blood flow. This is in agreement with studies in which blood flow measurements with microspheres have demonstrated that when the retrograde cannula is opened to atmospheric pressure, continuing tissue flow generally accounts for <25% of total collateral flow. Thus, although the retrograde flow measurements obtained in the present study would slightly underestimate total collateral flow, they are representative of blood flow through epicardial coronary collateral vessels.

The Leukotriene Shunt

Arachidonic acid flux through the lipoxygenase pathway is enhanced after cyclooxygenase blockade, resulting in increased leukotriene production. Arachidonic acid is converted by 5-lipoxygenase to form 5-hydroxyeicosatetraenoic acid, which is then transformed to numerous leukotrienes including leukotrienes C₄ and D₄, both of which are potent coronary vasoconstrictors. 5-Lipoxygenase activity, first described in leukocytes, has also been reported in both human and canine epicardial coronary arteries. Arachidonic acid shunting has been observed in experimental models of inflammation. This is of significance because coronary artery occlusion is associated with evidence of vascular injury in the native collateral vessels. These developing collateral vessels demonstrate prominent endothelial damage, increased presence of cytosomes, and leukocyte infiltration. In the present study, lipoxygenase blockade did not reverse the collateral vasoconstriction produced by ibuprofen, indicating that increased leukotriene production did not mediate this effect. Animals were studied 4 to 6 months after coronary artery occlusion at a time when perivascular inflammatory changes seen in developing collaterals have largely
TABLE 4. Response to vasoconstrictor effect would result in A Direct Ezra Thus, on collateral blood flow. However, occurred, however, production of leukotriene occurred, thus, Ezranet al reported that the normal canine coronary vasculature rapidly develops tolerance to the vasoconstrictor effect of leukotriene D4, whereas the collateral vasoconstriction produced by cyclooxygenase blockade in the present study persisted for at least 60 minutes.

A Direct Vasoconstrictor Effect

Forman et al have shown that indomethacin can cause coronary vasoconstriction that is independent of its effect on prostaglandin synthesis. In patients undergoing cardiac catheterization, these investigators found that indomethacin resulted in vasoconstriction in the atherosclerotic coronary vasculature with a decrease in coronary sinus blood flow. Pretreatment with high-dose aspirin (2600 mg per os), which would clearly cause cyclooxygenase blockade, did not blunt the coronary vasoconstriction produced by indomethacin. In the present study, cyclooxygenase blockade with ibuprofen resulted in a 56% decrease in retrograde blood flow, which is similar to that previously reported with indomethacin. In addition, we recently observed that aspirin (15 mg/kg) decreased retrograde flow by 43%. These results demonstrate that collateral vasoconstriction is neither unique to indomethacin nor independent of prostaglandin inhibition.

Inhibition of Vasodilator Prostaglandins

Cyclooxygenase blockade produced by indomethacin or ibuprofen is likely mediated by inhibition of synthesis of vasodilator prostaglandins. In ultrastructural studies, Schaper et al found striking histological differences between well-developed canine collateral vessels and normal arterial vessels of similar size. Collateral vessels displayed perivascular inflammation, subintimal proliferation, and endothelial cell hyperplasia and hypertrophy. The present findings suggest that these abnormalities are associated with increased production of vasodilator prostaglandins. In the normal vasculature, prostaglandin production is concentrated in endothelial cells. Prostacyclin is a potent coronary vasodilator that has been shown to cause vasodilation of well-developed coronary collateral vessels in isolated

![Fig 4](https://example.com/fig4.png)

**Fig 4.** Plot showing retrograde blood flow during control conditions (CON), after administration of ibuprofen (IBU, 10 mg/kg IV), and after infusion of prostaglandin I2 (PGI2, 20 μg·kg⁻¹·min⁻¹) in five dogs. Individual animals are indicated by the open circles; the mean response is indicated by the closed circles.

![Fig 5](https://example.com/fig5.png)

**Fig 5.** Bar graph showing retrograde blood flow during control conditions (CON), after administration of ibuprofen (IBU, 10 mg/kg), and after combined cyclooxygenase and lipoxygenase blockade with BW A4C (3.4 mg/kg IV) in five dogs. *P < .05 in comparison with CON.
TABLE 5. Response to Lipoxygenase Blockade With BW A4C (3.4 mg/kg IV) in Five Dogs After Cyclooxygenase Had Been Produced With Ibuprofen (10 mg/kg IV)

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Heart Rate, bpm</th>
<th>Aortic Pressure, mm Hg</th>
<th>Left Ventricular dP/dt, mm Hg/s</th>
<th>Left Ventricular End-Diastolic Pressure, mm Hg</th>
<th>Distal Coronary Pressure, mm Hg</th>
<th>Aortocoronary Gradient Pressure, mm Hg</th>
<th>Retrograde Flow, mL/min</th>
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<tr>
<td>Control</td>
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<td>2200±300</td>
<td>7±1</td>
<td>44±4</td>
<td>46±9</td>
<td>19±6*</td>
</tr>
<tr>
<td>BW A4C+iibuprofen</td>
<td>110±9</td>
<td>91±8</td>
<td>2100±400</td>
<td>8±2</td>
<td>44±5</td>
<td>47±9</td>
<td>18±6*</td>
</tr>
</tbody>
</table>

bp indicates beats per minute. Values are mean±SEM. *P<.05 vs control.

In the present study, prostacyclin infusion was able to completely reverse the vasoconstriction produced by ibuprofen and return retrograde flow to the control level. This confirmed that prostacyclin has sufficient vasodilator potency on collateral vessels to account for the decreased retrograde flow produced by ibuprofen. However, this finding does not imply that prostacyclin has unique activity in the collateral system; it is likely that any vasodilator that is active on collateral vessels would cause an increase in retrograde flow.

In the present study, levels of 6-ketoprostaglandin F₁α were significantly higher in retrograde blood samples obtained from the cannulated collateral-dependent coronary artery than in aortic blood, supporting prostacyclin production across the collateral system. The absolute levels of 6-ketoprostaglandin F₁α in blood collected from the retrograde catheter (59±122 pg/mL) were also higher than levels previously reported by Farber and Gross,14 who used a radioimmunoassay technique on coronary venous blood from open-chest dogs during basal conditions (360±78 pg/mL). We did not measure coronary venous 6-ketoprostaglandin F₁α levels in the present study. However, Imaiizu et al20 found no significant increase in 6-ketoprostaglandin F₁α from aortic to great cardiac vein blood in open-chest dogs during basal conditions, suggesting that the normal coronary circulation does not produce substantial amounts of prostacyclin during basal conditions.

The present findings indicating that products of arachidonic acid metabolism maintain tonic vasodilation of coronary collateral vessels suggest characteristics that are analogous to the fetal ductus arteriosus, in that patency of the ductus arteriosus is dependent on vasodilator prostaglandins.22 Ductus patency can be maintained by the smooth muscle relaxation produced by both prostaglandin E₂ and prostacyclin.26 In isolated ductus arteriosus preparations, prostaglandin E₂ is more potent in causing smooth muscle relaxation than is prostacyclin.26 However, immunohistochemical studies have not demonstrated the presence of prostaglandin E₂ in these vessels, whereas the presence of prostacyclin and prostacyclin synthase has been documented.30 Interestingly, in the ductus arteriosus, the highest concentration of prostacyclin synthase was found in areas of subintimal hyperplasia rather than in the endothelial cells.30 Although similar data are not available for coronary collateral vessels, the prominent subintimal hyperplasia in these vessels suggests a similar source for generation of vasodilator prostaglandins.

Thromboxane A₂, prostaglandin F₂α, prostacyclin, and prostaglandin E are all increased in areas of inflammation.31 We hypothesized that the perivascular inflammation that has been described for collateral vessels would be associated with increased local production of thromboxane A₂, so that inhibition of the action of thromboxane would decrease collateral vessel tone. An effect of thromboxane could be especially prominent because collateral vessels are more sensitive to the vasoconstrictor activity of thromboxane in vitro than are normal arterial vessels of similar size (authors’ unpublished observations). However, in the present study neither thromboxane synthase inhibition with dazmegrel nor thromboxane receptor blockade with SQ 30741 caused an increase in collateral blood flow. Thus, it is unlikely that thromboxane production was substantially increased in the well-developed collateral vessels in the present study. It is possible, however, that earlier during the course of collateral vessel development, when perivascular inflammatory changes are more prominent, production of thromboxane A₂ would have a greater role in regulation of collateral vasomotor tone.

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References


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