Role of Endothelium-Derived Relaxing Factor and Prostaglandins in Responses of Coronary Arteries to Thromboxane In Vivo

Konstantyn Szwarzakun, Kathryn G. Lamping, and William P. Dole

We examined the relative contribution of endothelial and vascular smooth muscle–derived prostaglandins and endothelium-derived relaxing factor in modulating both the large coronary artery and resistance vessel responses to thromboxane in vivo. Vascular responses to the thromboxane analogue U46619 were measured in four separate experimental protocols: 1) The vascular responses were measured in the presence and absence of intact endothelium to examine the role of endothelium-derived vasodilators. 2) Responses were measured in the presence of intact endothelium before and after inhibition of cyclooxygenase with indomethacin to examine the role of endothelial and vascular smooth muscle–derived prostaglandins. 3) Responses were measured after endothelial removal before and after indomethacin to examine the role of vascular smooth muscle–derived prostaglandins. 4) Responses were measured after indomethacin and before and after removal of endothelium to examine the role of endothelium-derived relaxing factor. In anesthetized dogs (n=41) that underwent constant pressure perfusion of the left anterior descending coronary artery (LAD), LAD diameter was measured with sonomicrometer crystals, and coronary flow was measured with an electromagnetic flow probe. Intracoronary infusion of U46619 (0.01–1.0 μg/min) produced a dose-dependent constriction of LAD. Constriction of the LAD was augmented after endothelial removal, after indomethacin treatment in both the presence and absence of endothelium, and after removal of the endothelium in the presence of indomethacin. Inhibition of prostaglandin synthesis had the greatest effect of augmenting constriction of LAD to thromboxane. Coronary flow was decreased by U46619 only in the presence of indomethacin. We conclude that both endothelial and vascular smooth muscle–derived prostaglandins and endothelium-derived relaxing factor can modulate the coronary response to U46619 in vivo; however, prostaglandins appear to play a more prominent role. (Circulation Research 1990;66:1729–1737)

Since the discovery that the endothelium is a major source of vasodilator substance(s) that is distinct from the prostaglandin pathway,1 a great deal of study has focused on the role of endothelium-derived relaxing factor (EDRF) in modulating vascular responses to both humoral and physicochemical stimuli.2,3 Although the release of EDRF has been shown to modulate vascular responses in vivo,3–5 the importance of vasodilator prostaglandins in modulating vascular responses should also be assessed.

Perturbation of the balance of vascular constrictor and dilator influences is thought to be important in the initiation of coronary vasospasm. The opposing effects of platelet-derived thromboxane and vascular prostaglandins may play a role in maintaining coronary artery tone in the normal vessel.6,7 Inhibition of prostaglandin synthesis with indomethacin constricts intact canine coronary arteries both in vitro and in vivo,8 denuded coronary arteries in vivo,9 and the coronary circulation of patients with coronary vascular disease.10 The vasoconstriction to indomethacin is due to inhibition of prostaglandin synthesis and a decrease in the level of vasodilating prostaglandins.8 Inhibition of prostaglandin production also results in augmented constriction to a variety of humoral substances.11,12 Aspirin pretreatment enhanced vasoconstriction to thromboxane in isolated vascular tissue preparations.13 A reduction in either endothelial or vascular smooth muscle–derived vasodilator prostaglandins could result in augmented constriction to humoral agents.
Both endothelium and vascular smooth muscle have been shown to be sources of vasodilator prostaglandins such as prostacyclin. Endothelium can produce vasodilators, such as EDRF, independent of their ability to release prostaglandins. It is valuable to determine which of the vasodilator substances, EDRF or prostaglandins, is the major modulating factor(s) in the response of the coronary artery to vasoconstrictors such as thromboxane.

In the present study, we tested the hypothesis that the vascular wall of the coronary circulation modulates the vasoconstrictor effects of thromboxane in vivo through release of both EDRF and prostaglandins. We determined the source of these vasodilator substances, either endothelium or vascular smooth muscle, by examining large coronary artery and flow responses to the thromboxane analogue U46619 (9,11-dideoxy-9α,11α-epoxymethanoprostaglandin F2α, The Upjohn Company, Kalamazoo, Michigan) in the presence and absence of intact endothelium and before and after inhibition of the cyclooxygenase pathway with indomethacin. By measuring the response of coronary arteries to U46619 before and after indomethacin and before and after removal of endothelium, we assessed the relative importance of EDRF and all vascular (both endothelial and vascular smooth muscle) prostaglandins.

Materials and Methods

Animal Preparation

Adult mongrel dogs of either sex (22–28 kg) were anesthetized with sodium pentothal (25 mg/kg i.v.) followed by α-chloralose (100 mg/kg i.v.). The animals were intubated and ventilated with a mechanical respirator with an end-expiratory pressure of 3–5 cm water. Arterial blood gases were monitored, and the rate and volume of respiration were adjusted to maintain gases in the physiological range (pH 7.35–7.45, PaO2 80–130, PaCO2 35–45 mm Hg).

A left thoracotomy in the fifth intercostal space was used to approach the heart, which was suspended in a pericardial cradle. Heart block was produced by injecting 37% formalin into the atrio-ventricular node. Biventricular epicardial pacer leads were placed, and the heart was paced above the intrinsic rate to maintain constant heart rate throughout the experiment.

One to 2 cm of proximal left anterior descending coronary artery (LAD) was dissected free of surrounding tissue to be used for sonomicrometer crystal placement. Another segment of LAD proximal to the crystal site was dissected free and cannulated for LAD perfusion. Coronary perfusion pressure was monitored through a side port on the cannula. Aortic blood pressure was measured with a catheter placed in the femoral artery and passed to the aortic arch. A femoral vein was cannulated and used for intravenous fluid and drug administration.

Coronary Diameter Measurement

Piezoelectric crystals were used to measure instantaneous coronary diameter with a technique described by Drews et al. Two 20-MHz crystals were mounted on opposing sides of a steel clip. One crystal was embedded in a polyurethane cup that conformed to the artery and ensured constant position of the sonic beam in relation to the artery. The other crystal rested on the adventitial surface on the opposite side of the vessel. Appropriate diameter measurement was verified by noting in-phase diameter and perfusion pressure signals. Stability and resolution of this technique has been previously reported.

Coronary Perfusion

After administration of heparin (500 units/kg followed by 250 units/hr), the LAD was perfused with blood from a pressurized arterial reservoir maintained at 37°C, and the pressure was maintained at 80 mm Hg. The blood leaving the reservoir passed through an in-line electromagnetic flow probe, and coronary flow was measured with a flowmeter. The flowmeter was calibrated with the dog’s blood at the end of each experiment, and mechanical zero was checked repeatedly throughout the experiment.

Assessment of Endothelial Integrity

The presence or absence of endothelium was assessed in two ways. Endothelium-dependent dilator responses were measured to an intracoronary infusion of acetylcholine (40 μg/min) after the coronary diameter and flow had reached steady-state values to the highest dose of intracoronary U46619.

Visual assessment of endothelium was done with scanning electron microscopy after completion of any experiment involving endothelial denudation. Denudation was felt to be adequate if dilation to acetylcholine was reduced to 25% of predened values and if the artery was devoid of endothelium under electron microscopy. Experiments that did not meet these criteria were excluded from analysis.

Experimental Protocol

This investigation is comprised of four separate experimental groups. Each group involves measurement of coronary responses to thromboxane analogue U46619 before and after intervention.

Effect of endothelium on responses to U46619. In the first group of dogs (n=13), the effects of endothelial factors in modulating coronary artery responses to thromboxane were assessed. The thromboxane analogue U46619 was dissolved in 0.9% saline and infused at rates of 0.01, 0.1 and 1.0 μg/min i.c. until flow and coronary diameter were stable, usually within 5–10 minutes. Flow rate of infusion never exceeded 1 ml/min. Endothelial denudation was performed by introducing a 3F balloon-tipped Fogarty embolectomy catheter (Edwards Laboratories, Santa Ana, California) through a side port in the external perfusion system. The catheter was advanced 1 cm
distal to the crystal site, inflated to reduce coronary flow to zero, and then withdrawn to the cannula tip. Total ischemic time was less than 30 seconds. Forty-five minutes later, when coronary diameter and blood flow had returned to baseline, the dose-response curve to U46619 was repeated.

To assess any time-dependent and hemodynamic effects on coronary diameter responses to thromboxane, repeated dose-response curves were carried out in a separate series of 10 dogs without endothelial denudation.

**Effect of endothelial and vascular smooth muscle prostaglandins.** In the second group of dogs (n=10), the effect of vascular prostaglandins both from endothelial and vascular smooth muscle in modulating coronary artery responses to thromboxane was assessed by measuring coronary diameter and flow during a U46619 infusion (0.01, 0.1, 1.0 µg/min) before and after cyclooxygenase inhibition. Cyclooxygenase inhibition was accomplished by indomethacin treatment. Crystalline indomethacin was dissolved in 0.9% saline at a concentration of 1.0 mg/ml with sodium carbonate added 1:3 by weight. Indomethacin (5 mg/kg) was infused intravenously, and 45 minutes was allowed for cyclooxygenase inhibition. Inhibition of cyclooxygenase was verified by loss of flow responses to intracoronary infusion of arachidonic acid (400 µg/min) in three dogs: the percent change in flow was 63±20% before cyclooxygenase inhibition and 4±9% after cyclooxygenase inhibition.

**Effect of vascular smooth muscle prostaglandins.** In the third group of dogs (n=9), the LAD was denuded of endothelium to assess the role of prostaglandins from vascular smooth muscle alone in the response of large coronary arteries to thromboxane. Forty-five minutes later, coronary diameter and flow responses to infusion of U46619 were measured before and after indomethacin treatment (5 mg/kg i.v.). Infusion of U46619 was adjusted for changes in coronary blood flow to keep the concentration similar before and after indomethacin treatment. Control experiments for determining possible hemodynamic and temporal effects on observed differences in denuded coronary artery responses to U46619 consisted of repeated dose-response curves to U46619 after endothelial denudation before and after infusion of saline.

**Effect of EDRF.** In the fourth group of dogs (n=9), the effect of nonprostaglandin EDRF in modulating large coronary artery responses to thromboxane was measured. The dogs were pretreated with indomethacin (5 mg/kg i.v.), and 45 minutes was allowed for stabilization before any other drug infusion. Responses to thromboxane were then measured before and after endothelial removal.

Control experiments consisted of repeated dose-response curves in six dogs to infusion of U46619 in arteries pretreated with indomethacin but without endothelial denudation between the two infusions.

**Statistical Analysis**

All data are represented as mean±SEM. Data from dose-response curves to thromboxane analogue U46619 before (dose-response 1) and after (dose-response 2) an intervention were compared using a two-way analysis of variance, followed by a least-squares mean analysis. The significance level was adjusted for multiple comparisons by the Bonferroni method. Coronary diameters and flows before and after an intervention alone (not in response to U46619) and acetylcholine responses were compared with a two-tailed t test.

**Criteria for an Acceptable Experiment**

Animals were excluded from the study if 1) mean arterial pressure was less than 60 mm Hg, 2) peak reactive hyperemic response was less than 2:1, or 3) blood gases could not be maintained in the physiological range as outlined in the "Animal Preparation" section above.

**Results**

**Effect of Endothelial Removal on Responses to Thromboxane**

Thromboxane analogue U46619 caused a dose-dependent constriction of the proximal LAD artery (Figure 1). Coronary blood flow was decreased from control only at the highest infusion of thromboxane (Table 1).

Endothelial denudation did not significantly affect baseline diameter (before denudation, 2.18±0.12 mm; after denudation, 2.12±0.13 mm) or flow (Table 1). Although the augmented constriction of the LAD to U46619 for the entire group before and after endothelial denudation was statistically significant (p<0.05), comparisons between individual doses before and after endothelial removal were not different (Figure 1). Aortic pressure was lower at each infusion rate when compared with predenudation values, but blood flows were similar (Table 1).

Repeated infusions of U46619 produced similar constriction of the large coronary artery. The maximal change in diameter from control was as follows:
TABLE 1.  Hemodynamics During Intracoronary Infusion of Thromboxane Analogue U46619 Before and After Removal of Endothelium in Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>U46619 0.01 μg/min</th>
<th>U46619 0.1 μg/min</th>
<th>U46619 1.0 μg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelium intact</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>103±5</td>
<td>102±5</td>
<td>102±5</td>
<td>101±5</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>43±4</td>
<td>41±3</td>
<td>42±3</td>
<td>38±3</td>
</tr>
<tr>
<td><strong>Endothelium removed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>84±6*</td>
<td>84±6*</td>
<td>83±5*</td>
<td>85±6*</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>42±4</td>
<td>43±4</td>
<td>40±3</td>
<td>34±3†</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=13 dogs.

*p<0.05 vs. endothelium intact.

†p<0.05 vs. control.

for dose-response 1 (at 1.0 μg/min U46619), −141±46 μm; for dose-response 2 (at 1.0 μg/min U46619), −156±35 μm.

**Effects of Indomethacin Treatment on Responses to Thromboxane**

Indomethacin caused a decrease in control coronary diameter (before indomethacin, 2.50±0.22 mm; after indomethacin, 2.38±0.21 mm; p<0.05). Indomethacin also potentiated the constriction to thromboxane at the 1.0 μg/min infusion rate (Figure 2). Although coronary blood flow was not affected before indomethacin treatment, there was a marked diminution of flow after indomethacin (Figure 3, Table 2).

Because of the decreases in coronary blood flow in response to thromboxane after indomethacin, drug concentrations of thromboxane and flow-dependent factors could have influenced the observed potentiation of coronary artery constriction. To assess possible effects of increased concentrations of thromboxane on this potentiation, in four dogs (included in the original group) infusion rates were decreased in the postindomethacin dose-response curve to achieve the same final concentration of thromboxane. In each experiment, decreases in diameter at the maximal dose were still greater after indomethacin (before indomethacin, −120±40 μm; after indomethacin, −238±56 μm).

Since flow was decreased on the average of 41% at the 1.0 μg/min perfusion rate, a flow-dependent mechanism could explain some of the potentiation of constriction to thromboxane after indomethacin treatment. In two dogs (untreated, endothelium intact), coronary flow was reduced by 50% after coronary diameter responses had stabilized during infusion of thromboxane at 1.0 μg/min with a snare placed just distal to the crystal site. There was no change in diameter over a 5-minute period of time.

**Effect of Indomethacin on Coronary Arteries Without Endothelium in Response to Thromboxane**

To study the effects of indomethacin on denuded arterial segments in response to thromboxane, denudation was carried out before any infusion of U46619 in nine dogs. Coronary artery diameter was 2.29±0.15 mm before and 2.40±0.13 mm after denudation.

Indomethacin treatment of coronary arteries devoid of endothelium reduced control coronary diameter (before indomethacin, 2.40±0.13 mm; after indomethacin...
TABLE 2. Hemodynamics During Intracoronary Infusion of Thromboxane Analogue U46619 Before and After Indomethacin Treatment in Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.01 µg/min</th>
<th>0.1 µg/min</th>
<th>1.0 µg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before indomethacin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>99±8</td>
<td>98±8</td>
<td>95±7</td>
<td>98±7</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>42±4</td>
<td>41±5</td>
<td>42±4</td>
<td>39±4</td>
</tr>
<tr>
<td><strong>After indomethacin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>97±7</td>
<td>97±7</td>
<td>93±7</td>
<td>93±8</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>38±5</td>
<td>35±5</td>
<td>33±5</td>
<td>23±4*†</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=10 dogs.
*p<0.05 vs. before indomethacin.
†p<0.05 vs. control.

There was potentiation of constriction to thromboxane at 1.0 µg/min infusion rate after indomethacin (Figure 4). Coronary blood flow responses were similar to those after indomethacin in the presence of intact endothelium. There was a decrease in coronary blood flow at control and at each infusion rate of thromboxane after indomethacin (Table 3). Aortic blood pressures tended to be higher with postindomethacin infusion of thromboxane, but not significantly higher.

Repeated dose-response curves to U46619 after endothelial denudation constricted the large artery similarly. The maximal change in diameter from control was as follows: for dose-response 1 (at 1.0 µg/min U46619), -136±35 µm; for dose-response 2 (at 1.0 µg/min U46619), -172±52 µm. Coronary blood flows were also similar at each infusion rate of thromboxane, reflecting lack of indomethacin effect, and there was no significant difference in aortic pressure between the two dose-response curves.

**Effects of Denudation on Coronary Artery Responses to Thromboxane Pretreated With Indomethacin**

In nine dogs, the effects of denudation of endothelium on coronary artery response to thromboxane pretreated with indomethacin were determined. Control coronary diameter was increased after denudation in the presence of indomethacin (before denudation, 2.43±0.28 mm; after denudation, 2.55±0.18 mm; p<0.05). Coronary blood flows were similar at each infusion rate but did decrease when compared with control, as was seen previously with indomethacin. Aortic pressure was decreased in the dose-response curve after denudation at infusions of 0.1 and 1.0 µg/min U46619 (Table 4).

Denudation potentiated the constriction of large coronary arteries to U46619 pretreated with indomethacin. The difference was significant at the highest infusion rate (Figure 5).

Repeated dose-response curves to U46619 were carried out in six dogs pretreated with indomethacin, to assess temporal and flow-dependent effects on coronary diameters. In contrast to the U46619-infused group, there were lower coronary blood flows in the second dose-response curve in the control group, significantly lower at the control and 1.0 µg/min U46619 infusion rates (coronary blood flow for dose-response 1: control, 43±7 ml/min; 1.0 µg/min U46619, 20±6 ml/min; coronary blood flow for dose-response 2: control, 27±4 ml/min; 1.0 µg/min U46619, 12±4 ml/min). Nevertheless, coronary diameter responses were similar between the two dose-response curves in the control group, suggesting that the potentiation of vasoconstriction in the U46619-infused group was secondary to denudation. The maximal change in diameter from control was as follows: for dose-response 1 (at 1.0 µg/min U46619), -235±65 µm; for dose-response 2 (at 1.0 µg/min U46619), -262±90 µm.

**Discussion**

The primary finding of this study is that the coronary vascular wall produces factors that modulate the vasoconstriction produced by exogenously administered thromboxane in the intact circulation. In the large coronary artery these factors are derived from both the endothelium and smooth muscle. The factors include, but are not limited to, endothelial nonprostaglandins and smooth muscle prostaglandins. The contribution of endothelial prostaglandins,
alone in this modulation could not be assessed. In addition, vascular wall prostaglandins exert a powerful inhibiting effect on the vasoconstriction produced by thromboxane in the resistance coronary circulation. Mechanical or chemical removal of these factors potentiates the vasoconstriction of coronary vessels to thromboxane. The most important modulating factors in this experimental model were vascular wall prostaglandins.

**Methodological Considerations**

There are several aspects of the experimental design and method that could influence the interpretation of the results. Thromboxane is an unstable molecule; thus, the study of its effects is difficult. In this study, the thromboxane analogue U46619 was used. U46619 mimics natural thromboxane in its vasoconstrictor and platelet-activating properties. It is possible that there are unknown differences between the analogue and the native compound that could alter the conclusions drawn from this study.

Indomethacin was used with the intent to inhibit cyclooxygenase in the vascular wall to assess the modulation of prostaglandins in thromboxane vasoconstriction. Unfortunately, indomethacin probably inhibited cyclooxygenase systemically, including that in platelets. This in turn could have reduced potential thromboxane synthesis in platelets stimulated by external U46619 infusion and may have underestimated the extent of vasoconstriction observed compared with that if vascular wall cyclooxygenase could have been inhibited selectively. The observed results could only have underestimated the potentiation of the constriction to U46619.

Endothelial denudation has been shown to stimulate arachidonic acid release from rabbit aorta with conversion to prostacyclin for up to 45 minutes after denudation. This temporary increase in prostacyclin synthesis could have influenced the amount of constriction seen in response to thromboxane after denudation in this study. For this reason, the second dose-response curve was performed at least 45 minutes after denudation.

**Endothelium-Derived Vasodilating Factors (EDRF and Prostaglandins)**

The removal of endothelium in the proximal coronary artery potentiates the vasoconstrictor responses of large coronary arteries to thromboxane. The potentiation of the constrictor response could have been due to 1) removal of endothelium-derived prostaglandins, 2) removal of endothelium-derived relaxant factor(s), or 3) production of a vasoconstrictor substance. Previous investigations in canine and bovine coronary arteries in vitro have suggested that endothelium has little, if any, effect in modulating constrictor responses to thromboxane analogue U46619. On the other hand, denudation was shown to augment the constrictor responses to another thromboxane analogue, STA2. This observation is

### Table 3. Hemodynamics During Intracoronary Infusion of Thromboxane Analogue U46619 in Arteries With Endothelium Removed Before and After Indomethacin Treatment in Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.01 µg/min</th>
<th>0.1 µg/min</th>
<th>1.0 µg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before indomethacin (endothelium removed)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>89±8</td>
<td>91±7</td>
<td>92±7</td>
<td>95±6</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>31±3</td>
<td>32±4</td>
<td>33±3</td>
<td>31±3</td>
</tr>
<tr>
<td><strong>After indomethacin (endothelium removed)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>100±8</td>
<td>102±8</td>
<td>103±8</td>
<td>102±7</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>25±2*</td>
<td>24±2*</td>
<td>23±2*</td>
<td>17±1*†</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=9 dogs.
* p<0.05 vs. before indomethacin.
† p<0.05 vs. control.

### Table 4. Hemodynamics During Intracoronary Infusion of Thromboxane Analogue U46619 in Indomethacin-Treated Arteries Before and After Endothelial Removal in Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.01 µg/min</th>
<th>0.1 µg/min</th>
<th>1.0 µg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelium intact</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>114±7</td>
<td>109±8</td>
<td>111±7</td>
<td>119±7</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>38±5</td>
<td>38±6</td>
<td>36±7</td>
<td>29±5*</td>
</tr>
<tr>
<td><strong>Endothelium removed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>98±9</td>
<td>93±9</td>
<td>89±9†</td>
<td>92±8†</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>35±6</td>
<td>38±8</td>
<td>32±5</td>
<td>24±4*</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=9 dogs.
* p<0.05 vs. control.
† p<0.05 vs. endothelium intact.
not surprising since U46619 has been shown to stimulate vascular prostacyclin synthesis in rat thoracic aorta in vitro. Furthermore, intravenous infusions of U46619 caused delayed increases in coronary blood flow and decreases in aortic blood pressure, which correlated with enhanced release of systemic prostacyclin. These secondary changes in hemodynamics were abolished by pretreatment with indomethacin. These studies suggest that thromboxane stimulates vascular prostaglandin release, which attenuates thromboxane’s direct constrictor effect. However, the source of these prostaglandins is unknown. Endothelial modulation of constrictor responses has also been shown for other agents, including serotonin and activated platelets.

**Large Coronary Artery Prostaglandins**

The importance of the role of prostaglandins in modulating the vasoconstriction to thromboxane was investigated in the second group of dogs. Indomethacin treatment markedly potentiated the vasoconstriction to thromboxane. The inhibition of prostaglandin synthesis was verified by showing lack of flow responses to intracoronary infusion of arachidonic acid. This potentiation could have been due to 1) inhibition of endothelial or smooth muscle prostaglandin production or 2) shunting of arachidonic acid metabolism to the lipoxygenase pathway, with formation of vasoconstricting leukotrienes.

Importance of vascular wall prostaglandins in the control of vascular tone has been demonstrated previously. Synthesis of prostaglandins has been shown to contribute directly to coronary artery tone in isolated vascular rings of bovine coronary artery. Indomethacin has been shown to potentiate vasoconstriction in large coronary arteries of the dog to U46619 in an in situ preparation. In addition, contraction of isolated rabbit coronary artery strips to thromboxane was greater after endothelial removal. Constriction was not enhanced by indomethacin or aspirin treatment in denuded arteries but was enhanced in intact arteries, suggesting that the endothelial factor responsible for this effect was a prostaglandin. Potentiation of constrictor effects by indomethacin has also been demonstrated for other agents, including noradrenalin and potassium.

By blocking the cyclooxygenase pathway, it is possible that arachidonic acid may be shunted to the lipoxygenase pathway, which could contribute to the enhanced vasoconstriction observed. An increase in leukotriene production and a decrease in prostaglandins were measured after angioplasty in carotid arteries of dogs. It is unlikely that leukotrienes play a prominent role in the augmentation of constriction to thromboxane in the presence of indomethacin since leukotrienes are known to be potent constrictors of coronary vascular resistance vessels and not large coronary vessels. A likely explanation for our findings is that prostaglandin production is stimulated by thromboxane and that removal of these prostaglandins enhances the vasoconstriction to thromboxane. This is in contrast to the observation that indomethacin treatment does not modify the relaxation response to serotonin in endothelial intact arteries, suggesting that serotonin does not stimulate the release of prostaglandins from the arterial wall at least in in vitro preparations.

**Vascular Smooth Muscle Prostaglandins**

The endothelium is a known source of prostaglandin production, although smooth muscle cells have been shown to produce equivalent amounts of prostacyclin as endothelial cells in culture. It has also been demonstrated that denuded rat aorta produces enhanced amounts of prostacyclin, compared with intact aorta, in response to thromboxane analogue U46619 and other vasoconstrictors. The role of smooth muscle prostaglandin production in modulating the constriction to thromboxane was investigated in the third group of dogs. When endothelial factors were removed (prostaglandin and nonprostaglandin) by prior mechanical denudation, indomethacin treatment still markedly potentiated constrictor responses to thromboxane. This suggests that smooth muscle is a large source of prostaglandin production in denuded arteries in limiting the vasoconstriction to thromboxane. Similar enhancement of constriction was observed to serotonin after indomethacin treatment in in situ preparations of denuded canine coronary arteries. Potentiation of constriction to noradrenalin after indomethacin treatment was also observed in denuded canine thoracic aorta segments, which correlated with reduction of prostaglandin production.

**EDRF(s)**

In the fourth group of dogs, the role of EDRF(s) in modulating the constriction to thromboxane was evaluated. Although the potentiation of constriction after denudation in arteries pretreated with indomethacin was significant at the 1.0 μg/min U46619

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**Figure 5.** Bar graph showing effect of thromboxane analogue U46619 on coronary diameter (change from baseline) pretreated with indomethacin before and after endothelial removal in dogs (n=9). *p<0.05 versus endothelium intact.
dose, the degree of potentiation was much less as compared with prostaglandin withdrawal. This suggests that an endothelial nonprostaglandin factor (e.g., EDRF) is released that modulates the constriction produced by thromboxane.

The relative importance of prostaglandins and EDRF in modulating the vasoconstriction to thromboxane in large coronary arteries can be assessed by comparing the degree of constriction seen before and after removal of a specific factor or factors. Overall, prostaglandin synthesis inhibition of the entire vascular wall or smooth muscle had the greatest effect on the constriction to thromboxane. Removal of EDRF had a less pronounced effect on the vasoconstriction.

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Thromboxane infusion alone had little effect on coronary flow at the concentrations used in this study. This contrasts highly with the marked reduction in flow (50%) seen with thromboxane infusion in arteries pretreated with indomethacin. In several experiments, the infusion of thromboxane in dogs pretreated with indomethacin reduced coronary flow to zero with dyskinesis of the anterior left ventricular wall. With cessation of thromboxane infusion, flow would be reestablished, and reperfusion arrhythmias would sometimes ensue. In an investigation that studied the microvascular responses to thromboxane, it was shown that indomethacin augmented the vasoconstrictor response to thromboxane by nine-fold. This augmentation was uniform in all sizes of microvessels studied. It has also been reported that indomethacin by itself has a deleterious effect on coronary flow in patients with coronary artery disease.

It is possible that leukotrienes may play a role in the augmented vasoconstrictor responses seen in the resistance circulation. Leukotrienes are potent constrictors of small coronary arteries. Diversion of arachidonic acid from the cyclooxygenase to the lipoygenase pathway has been suggested as the mechanism of enhanced constriction seen after indomethacin treatment in the canine saphenous vein. This potential mechanism was not addressed in this investigation. As in the large coronary artery, we can conclude similarly that prostaglandins play a prominent role in limiting the vasoconstriction to thromboxane in the resistance circulation.

In summary, we have shown that prostaglandins and nonprostaglandins from the vascular wall modulate vasoconstriction to thromboxane in the large coronary artery of the dog. Vasodilator prostaglandins appear to play a more prominent role in this modulation. Different pathophysiological states may alter the ability of the vascular wall to produce these factors and potentiate the vasoconstriction to thromboxane.

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