Removal of the Endothelium Potentiates Canine Large Coronary Artery Constrictor Responses to 5-Hydroxytryptamine in Vivo

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SUMMARY. Recent studies in isolated epicardial coronary artery rings have shown that the endothelium modulates vasomotor responses to certain endogenous neurohumoral agents. It is not known whether the endothelium plays a role in large coronary vasoregulation in the intact coronary circulation. Accordingly, we examined effects of endothelial removal on vasoconstrictor responses of the proximal coronary artery in anesthetized adult mongrel dogs. The left anterior descending artery was perfused at 100 mm Hg with arterial blood from a pressurized reservoir. Coronary diameter was measured continuously with 7-MHz sonomicrometer crystals attached to the adventitia of the artery. Fifteen minutes after mechanical disruption of the endothelium with a balloon-tipped catheter, baseline diameter was unchanged from a control value of 2.60 ± 0.13 mm (mean ± SE, n = 17). Endothelial denudation resulted in a dose-dependent potentiation of the constrictor response to intracoronary 5-hydroxytryptamine (1-50 μg/min, n = 8). With the endothelium intact, a 5 μg/min infusion of 5-hydroxytryptamine reduced diameter by 24 ± 14 μm, while a 50 μg/min dose reduced diameter by 54 ± 25 μm. After endothelial removal, the decrease in diameter averaged 74 ± 18 μm at 5 μg/min and 132 ± 29 μm at 50 μg/min, indicating a 10-fold increase in the sensitivity to 5-hydroxytryptamine. The constrictor responses to angiotensin II (1 and 5 μg/min, n = 7) and phenylephrine (1-5 μg/min, n = 7) were not altered by endothelial removal. Thus, endothelial removal selectively potentiates the constrictor responses to 5-hydroxytryptamine in the intact coronary circulation of the dog. (Circ Res 57: 46–54, 1985)

RECENT studies in isolated arterial preparations including coronary arteries have demonstrated an obligatory role for endothelial cells in the response of vascular smooth muscle to various neurohumoral agents. Removal of the endothelium abolishes vasodilator responses to acetylcholine, bradykinin, thrombin, and substance P (Furchgott and Zawadzki, 1980; Furchgott, 1983) and potentiates vasoconstrictor responses to 5-hydroxytryptamine (Cohen et al., 1983a; Cocks and Angus, 1983). Recently, Cohen and co-workers (1983b) demonstrated that aggregating autologous platelets cause contraction of isolated coronary artery segments. The contractions were augmented after removal of the endothelium, and were attenuated by the 5-hydroxytryptamine antagonists, ketanserin and cyproheptadine. These investigators have also demonstrated that when coronary artery segments are precontracted with prostaglandin F2α, 5-hydroxytryptamine results in relaxation in some rings with endothelium, but results in contraction in all rings with the endothelium removed. These data suggest that the response of isolated large coronary arteries to 5-hydroxytryptamine is the net result of a direct constrictor effect on the smooth muscle which is opposed by a dilator response mediated by the endothelium (Cohen et al., 1983a). These in vitro observations have suggested that the absence of endothelium may play an important role in the pathophysiology of coronary vasospasm. Although endothelial-dependent vascular responses have been demonstrated in isolated vascular ring preparations, the role of the endothelium in regulating large coronary vasomotor tone in the intact circulation is not well defined. In vivo data in a canine femoral artery preparation indicate that acetylcholine and substance P produce vasodilation only in the presence of an intact endothelium (Angus et al., 1983). In a recent study, Brum and co-workers (1984) demonstrated potentiation of the epicardial coronary constrictor response to 5-hydroxytryptamine after endothelial removal. However, endothelial removal caused a decrease in baseline coronary diameter which could explain the observed change in responsiveness to 5-hydroxytryptamine.

In the present study, we tested the hypothesis that the endothelium modulates vasoconstrictor responses to certain endogenous neurohumoral stimuli in the intact coronary circulation. Specifically, large coronary artery responses to 5-hydroxytryptamine, angiotensin II, and phenylephrine were assessed before and after mechanical disruption of the endothelium in the blood-perfused dog heart.
Methods

Animal Preparation and Hemodynamics

Adult mongrel dogs (20-30 kg) of either sex were anesthetized with sodium pentothal (25 mg/kg, iv) followed by α-chloralose (100 mg/kg, iv). The animals were intubated and ventilated with oxygen-enriched air by a mechanical respirator with an end-expiratory pressure of 5 cm water. Blood gases were maintained within the physiological range (pH 7.35-7.45, PaO₂ 30-40 mm Hg and PaCO₂ 125-150 mm Hg) by varying the rate and volume of respiration.

A left thoracotomy was performed in the 5th intercostal space, the lungs were retracted, and the heart was suspended in a pericardial cradle. Heart block was produced by injecting 37% formalin directly into the atrioventricular (AV) node by a modification of the technique described by Steiner and Kovalik (1968), and the heart was paced at 100 beats/min.

Two segments of left anterior descending coronary artery were isolated approximately 1-2 cm apart. The proximal segment was cannulated to perfuse the myocardium and the distal segment was used to measure coronary diameter. Arterial pressure was measured with a cannula in the right femoral artery connected to a pressure transducer positioned at mid-chest level. A femoral vein was cannulated for administration of drugs and fluid.

Coronary Dimensions

The diameter of the left anterior descending artery was measured continuously with an ultrasonic dimension gauge. Two 7-MHz piezoelectric sonomicrometer crystals (20 mg each) were attached to opposing sides of a stainlesssteel spring (8 mm X 5 mm, 21 mg, spring constant 2.72 mN/mm) to maintain the crystals in focus throughout the experiment. On one side of the spring, a Dacron patch was attached, and 6-0 suture was used to secure the device to the adventitial layer of the left anterior descending artery. The opposing crystal rested on the surface of the vessel. Crystal alignment was confirmed when phasic diameter tracings mirrored phasic coronary pressure. Diameter measurements with spring-mounted crystals were comparable to those obtained with crystals sewn directly to the vessel. The digital electronic dimension gauge measured the time for transmission of an ultrasonic signal by counting a 30-MHz crystal-controlled oscillator between the transmitted pulse and the received pulse. The frequency response of the dimension gauge was 35 Hz. Phasic and mean coronary diameter were monitored continuously by measuring the transit time of the ultrasonic signal between the crystals. Drift of the signal was 8 mm over a 7-hour period. Calibration of the sonomicrometer crystals and approximately 1.5-2 cm distal to the crystals. After fixation in glutaraldehyde, a 1-mm section from the center of each vessel was taken for transmission electron microscopy. The intimal surface of the remaining arterial segments was exposed by opening the vessel with a scalpel blade, and samples were mounted on copper tape attached to aluminum studs for scanning electron microscopy. Samples for scanning and transmission electron microscopy were prepared according to standard histological procedures (Hayat, 1981). A JOEL JSM 35C scanning electron microscope was used to examine the intimal surface of each vessel at 13 kV at a magnification range of 600x. A Hitachi H-600 transmission electron microscope was used at 50 kV over a magnification range of 1000-5000x.

Scanning and Transmission Electron Microscopy

At the end of each experiment, the coronary artery was perfuse-fixed at 100 mm Hg with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. We obtained samples of the artery (1-2 cm long) at the site of attachment of the sonomicrometer crystals and approximately 1.5-2 cm distal to the crystals. After fixation in glutaraldehyde, a 1-mm section from the center of each vessel was taken for transmission electron microscopy. The intimal surface of the remaining arterial segments was exposed by opening the vessel with a scalpel blade, and samples were mounted on copper tape attached to aluminum studs for scanning electron microscopy. Samples for scanning and transmission electron microscopy were prepared according to standard histological procedures (Hayat, 1981). A JOEL JSM 35C scanning electron microscope was used to examine the intimal surface of each vessel at 13 kV at a magnification range of 600x. A Hitachi H-600 transmission electron microscope was used at 50 kV over a magnification range of 1000-5000x.

Scanning and transmission electron microscopy was performed to assess the extent of endothelial removal, the integrity of the basement membrane, and the morphology of the underlying vascular smooth muscle. Scanning electron microscopy in five animals confirmed that attachment of the sonomicrometer crystals did not disrupt the endothelial surface.

Internal cross-sectional area of the left anterior descending artery at the site of crystal attachment was determined by light microscopy (Nordborg and Johansson, 1980). A cross-section of the vessel was planimetered with an electronic digitizer to determine the area of the vessel wall. Based on the assumption that the area of the vessel wall remains constant during changes in vessel diameter, we calculated the internal cross-sectional area for each measurement of outer diameter.

Protocol

The animal preparation was allowed to stabilize 30 minutes following coronary cannulation. In preliminary studies, dose-response curves to intracoronary 5-hydroxytryptamine (0.5-100 μg/min, n = 4), phenylephrine (0.5-10 μg/min, n = 3), and angiotensin II (0.5-5 μg/min, n = 3) were obtained. Based on these studies, the doses of each constrictor agent were chosen to produce near-maximal large vessel constriction without producing systemic effects. Each drug was dissolved in 0.9% saline and infused intracoronary from 0.25-1.0 ml/min until a stable response was recorded (5 minutes). Coronary perfusion pressure was held constant at 100 mm Hg.

A total of 17 animals were studied. Eight animals received 5-hydroxytryptamine (1-50 μg/min), 7 received phenylephrine (1-5 μg/min), and 7 received angiotensin II (1 and 5 μg/min). No animal received more than two different drugs, and a minimum of 30 minutes was allowed between different drugs to allow the preparation to return to baseline. The sequence of drug administration was varied. To minimize tachyphylaxis, 30 minutes were allowed between doses of angiotensin II. Following control dose-response curves, the endothelium was mechanically removed from the proximal segment of the artery with a balloon-tipped Fogarty 4F embolectomy catheter which...
was inserted into the coronary artery approximately 1 cm distal to the sonomicrometer crystals through a diaphragm in the perfusion tubing. The balloon-tipped catheter was inflated to reduce flow to zero and was withdrawn across the intimal surface of the vessel to the cannula tip, two times. About 20 minutes after endothelial removal, the dose-response curve to each drug was repeated.

Animals were excluded from the study if the mean arterial pressure was less than 60 mm Hg or if peak reactive hyperemia flow following a 15-second coronary inflow occlusion (pressure 100 mm Hg) was less than two times greater than baseline flow. One animal was excluded because scanning electron microscopy demonstrated that endothelial removal was incomplete.

Statistical Analysis

All data are expressed as the mean ± SEM. Data before and after endothelial removal were compared by two-way analysis of variance, followed by a least squares means analysis. The significance level was adjusted for multiple comparisons by the Bonferroni method (Wallenstein et al., 1980).

Results

Mechanical Disruption of the Endothelium

A transient constriction of the coronary artery (maximum change in diameter: −77 ± 22 µm) occurred after two passes of the balloon-tipped catheter (Fig. 1). Coronary diameter returned to baseline within 10–15 minutes (before endothelial removal: 2.60 ± 0.13 mm; after: 2.61 ± 0.13 mm). Systemic pressure and coronary flow immediately before and after endothelial removal were not significantly different.

Scanning electron micrographs of the intimal surface of the coronary artery in the region of the sonomicrometer crystals demonstrated endothelial removal in all animals included in the study (Fig. 2, A and C). The underlying basement membrane was intact with numerous platelets adherent to its surface. A segment of vessel approximately 1.5–2 cm distal to the region of the crystals demonstrated an intact endothelial surface (Fig. 2B). Transmission electron microscopy showed no alterations in the underlying vascular smooth muscle of the denuded segment, compared to a distal segment of the same vessel (Fig. 3). There was no evidence of cellular swelling or intracellular edema formation following endothelial removal.

5-Hydroxytryptamine

Intracoronary infusion of 5-hydroxytryptamine (1–50 µg/min, n = 8) caused a dose-dependent increase in coronary blood flow (Table 1) and a decrease in coronary diameter (Fig. 4A). Mean arterial pressure was not altered (Table 1). The percent change in internal cross-sectional area was −2 ± 1% at the 5 µg/min dose and −5 ± 2% at the 50 µg/min dose (Fig. 4B).

Following endothelial removal, 60–90 minutes after the control 5-hydroxytryptamine dose-response curve, arterial pressure was decreased. Although coronary flow tended to increase during this time period, the increase in flow was not statistically significant. Baseline coronary diameter was not affected by endothelial removal (before: 2.66 ± 0.15 mm; after: 2.65 ± 0.15 mm). Coronary blood flow increased in a dose-dependent manner with 5-hydroxytryptamine infusion, with no change in arterial pressure (Table 1). Endothelial denudation potentiated the constrictor response of the proximal coronary artery at both the 5 µg/min dose (before removal: −23 ± 14 µm; after removal: −73 ± 18 µm) and the 50 µg/min dose (before removal: −54 ± 25 µm; after removal: −131 ± 29 µm) (Fig. 4A). The percent change in internal cross-sectional area was significantly greater at both the 5 and the 50

<table>
<thead>
<tr>
<th>Coronary Perfusion Pressure (mm Hg)</th>
<th>250</th>
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<tbody>
<tr>
<td>Phasic Coronary Flow (ml/min)</td>
<td>192</td>
</tr>
<tr>
<td>Mean Coronary Flow (ml/min)</td>
<td>192</td>
</tr>
<tr>
<td>Phasic Coronary Diameter (mm)</td>
<td>3.070</td>
</tr>
<tr>
<td>Mean Coronary Diameter (mm)</td>
<td>3.070</td>
</tr>
</tbody>
</table>

FIGURE 1. Experimental tracing showing hemodynamics and coronary diameter before, during, and after mechanical removal of the endothelium with a balloon-tipped catheter. Endothelial removal caused a transient large coronary artery constriction. The diameter returned to control within 10–15 minutes. Endothelial removal did not affect arterial pressure or coronary blood flow.
FIGURE 2. Panel A: scanning electron micrograph of the intimal surface of a coronary artery after withdrawing an inflated balloon-tipped catheter across the intimal surface two times. The endothelium was completely removed. Numerous platelets are adherent to an intact basement membrane. Panel B: scanning electron micrograph of the intimal surface of a coronary artery approximately 1 cm distal to the denuded segment shown in panel A. An intact endothelial layer was evident demonstrating no apparent endothelial disruption due to LAD cannulation and extended periods of perfusion from a reservoir. Panel C: scanning electron micrograph of the intimal surface of a coronary artery demonstrating the transition zone between a distal segment with intact endothelium (left) and a proximal segment of endothelial disruption (right).

\( \mu g/min \) doses of 5-hydroxytryptamine following endothelial removal (Fig. 4B). The maximum decrease in the percent internal cross-sectional area averaged \(-14 \pm 2\%\).

With the endothelium intact, the change in coronary diameter in response to the high dose of 5-hydroxytryptamine ranged from +10 to \(-60\) \(\mu m\). The change in diameter (\(\Delta CD\)) and percent increase in coronary blood (\(\% \Delta F\)) were inversely correlated: \(-\Delta CD = 0.08\% \Delta F - 50, r = -0.75\). After endothelial removal, however, there was no correlation between the change in flow and diameter (\(r = -0.19\)).

To assess the reproducibility of the 5-hydroxytryptamine dose-response curves and the possible effects of an increase in basal coronary flow on the large coronary artery constrictor response, we performed repeated dose-response curves to 5-hydroxytryptamine separated by a minimum of 90 minutes with the endothelium intact in five different dogs. Despite an increase in basal coronary flow of 119%, the 5-hydroxytryptamine-induced large coronary constriction was not altered (Table 2).

**Angiotensin II**

Since angiotensin II doses were separated by a minimum of 30 minutes, the response to each dose was compared to the control level prior to each infusion of drug. Angiotensin caused an initial decrease in coronary flow at both 1 and 5 \(\mu g/min\); however, mean coronary flow was measured when...
TABLE 1
Hemodynamics during Intracoronary 5-Hydroxytryptamine Infusion in Dogs with Coronary Endothelium Intact and Endothelium Removed

<table>
<thead>
<tr>
<th></th>
<th>Control (5-HT 1 µg/min)</th>
<th>5 µg/min</th>
<th>50 µg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial pressure (mm Hg)</strong></td>
<td>106 ± 5</td>
<td>105 ± 5</td>
<td>102 ± 6</td>
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<tr>
<td><strong>Coronary flow (ml/min)</strong></td>
<td>40 ± 9</td>
<td>51 ± 9</td>
<td>73 ± 10*</td>
</tr>
<tr>
<td></td>
<td>Endothelium removed</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Arterial pressure (mm Hg)</strong></td>
<td>84 ± 7†</td>
<td>83 ± 8‡</td>
<td>8 ± 7‡</td>
</tr>
<tr>
<td><strong>Coronary flow (ml/min)</strong></td>
<td>68 ± 9</td>
<td>71 ± 7</td>
<td>95 ± 9*</td>
</tr>
</tbody>
</table>

\( n = 8; \) results are expressed as mean ± SE.

* \( P < 0.05 \) vs. control.

† \( P < 0.05 \) vs. 5 HT, 5 µg/min.

‡ \( P < 0.05 \) vs. corresponding value for endothelium intact.

the decrease in large vessel diameter was at steady state. Coronary flow had returned to control levels when the large artery constrictor response was maximal (Table 3, \( n = 7 \)). In the presence of an intact endothelium and with the endothelium removed, angiotensin increased mean arterial pressure at 5 µg/min, whereas coronary flow was not different from control at either 1 or 5 µg/min (Table 3). A dose-dependent decrease in large coronary diameter was observed (1 µg/min: —19 ± 5 µm; 5 µg/min: —31 ± 15 µm) (Fig. 5). Coronary diameter was not altered by endothelial removal (before: 2.66 ± 0.25 mm; after: 2.68 ± 0.24 mm). The vasoconstriction of the coronary artery to angiotensin was not altered by endothelial removal (1 µg/min: —14 ± 4 µm; 5 µg/min: —21 ± 7 µm) (Fig. 5).

**Phenylephrine**

Intracoronary infusion of phenylephrine (1–5 µg/min, \( n = 7 \)) had no effect on arterial pressure or coronary flow (Table 4). A dose-dependent decrease in coronary diameter was observed (Fig. 6). After endothelial removal, neither arterial pressure, coronary flow, nor mean coronary diameter (before: 2.34 ± 0.08 mm; after: 2.35 ± 0.12 mm) was altered, compared to control values (Table 4). Phenylephrine resulted in a modest decrease in coronary diameter that was not altered by endothelial removal.

**Discussion**

This study demonstrates that the endothelium can modulate epicardial coronary vasomotor responses to a neurohumoral agent in the intact blood-perfused dog heart. The results indicate that mechanical removal of the endothelium potentiates the vasoconstrictor response to 5-hydroxytryptamine. It is unlikely that the observed potentiation of the constrictor response to 5-hydroxytryptamine following endothelial removal can be attributed to a nonspecific mechanical effect of balloon denudation. First, 10–15 minutes after balloon denudation, coronary artery diameter was not different from control, and, second, constrictor responses to both phenylephrine and angiotensin II were unaltered. Thus, the effects of endothelial removal on coronary constriction were selective for 5-hydroxytryptamine. The fact that the vasoconstrictor responses to angiotensin II
TABLE 2
Hemodynamics and Coronary Diameter (CD) during Repeated Intracoronary 5-Hydroxytryptamine Dose-Response Curves

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 µg/min</th>
<th>5 µg/min</th>
<th>50 µg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose-response curve 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>87 ± 6</td>
<td>88 ± 6</td>
<td>88 ± 5</td>
<td>87 ± 5</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>26 ± 3</td>
<td>33 ± 1</td>
<td>51 ± 1*</td>
<td>130 ± 12*</td>
</tr>
<tr>
<td>CD (mm) and Δ CD from control (µm)</td>
<td>2.31 ± 0.17</td>
<td>−16 ± 10</td>
<td>−25 ± 12</td>
<td>−47 ± 15*</td>
</tr>
<tr>
<td><strong>Dose-response curve 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>83 ± 7</td>
<td>84 ± 6</td>
<td>82 ± 6</td>
<td>80 ± 6</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>57 ± 5†</td>
<td>66 ± 5†</td>
<td>81 ± 4*†</td>
<td>131 ± 12*</td>
</tr>
<tr>
<td>CD (mm) and Δ CD from control (µm)</td>
<td>2.66 ± 0.17</td>
<td>−23 ± 8</td>
<td>−31 ± 14*</td>
<td>−54 ± 15*</td>
</tr>
</tbody>
</table>

n = 5; results are expressed as mean ± se.
* P < 0.5 vs. control value.
† P < 0.05 vs. corresponding value for dose-response curve 1.

and phenylephrine were similar before and after endothelial removal also argues against nonspecific damage to the underlying vascular smooth muscle. In addition, after endothelial removal, the response to 5-hydroxytryptamine was potentiated, making it unlikely that the smooth muscle was damaged. Further, transmission electron microscopy of the vessel showed no alteration in smooth muscle morphology (Fig. 3).

The decrease in arterial pressure over the course of the experimental protocol probably is not an important factor in the altered 5-hydroxytryptamine response, since the left anterior descending coronary artery was perfused at constant pressure. Although coronary blood flow tended to increase, repeated 5-hydroxytryptamine dose-response curves did not differ, despite a similar increase in basal coronary flow. Furthermore, an analysis of covariance showed that baseline flow was not associated with observed changes in diameter following endothelial removal.

We considered the possibility that the enhanced constrictor response following endothelial removal might be related to platelet deposition, rather than to an inhibitory influence of the endothelium. Platelets adherent to the denuded segment of coronary artery could release threshold amounts of 5-hydroxytryptamine, resulting in higher concentrations of 5-hydroxytryptamine during the dose-response curve obtained following endothelial removal. Although this may explain a part of the augmented constrictor response following endothelial denudation, it is unlikely that such a mechanism could completely explain our results. First, in vitro data have shown that threshold amounts of 5-hydroxytryptamine potentiate the constrictor response to

TABLE 3
Hemodynamics during Intracoronary Angiotensin II Infusion in Dogs With Coronary Endothelium Intact and Endothelium Removed

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>1 µg/min</th>
<th>Control 2</th>
<th>5 µg/min</th>
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</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>92 ± 5</td>
<td>103 ± 8</td>
<td>85 ± 7</td>
<td>109 ± 10*</td>
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<tr>
<td>Coronary flow (ml/min)</td>
<td>42 ± 5</td>
<td>33 ± 4</td>
<td>50 ± 6</td>
<td>62 ± 14</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>73 ± 3†</td>
<td>83 ± 4†</td>
<td>73 ± 4</td>
<td>90 ± 8*†</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>76 ± 13†</td>
<td>75 ± 14†</td>
<td>79 ± 17†</td>
<td>76 ± 15</td>
</tr>
</tbody>
</table>

n = 7; results are expressed as mean ± se.
* P < 0.05 vs. corresponding control.
† P < 0.05 vs. corresponding value for endothelium intact.
angiotensin II and norepinephrine (De La Lande et al., 1966; Seabrook and Nolan, 1983; Vanhoutte et al., 1984). However, the responses to angiotensin II and phenylephrine were similar before and after endothelial removal. Second, threshold amounts of 5-hydroxytryptamine would be expected to produce a parallel shift in the dose response curve to 5-hydroxytryptamine. However, the constriction to 5-hydroxytryptamine was potentiated to a greater extent with increasing doses after endothelial removal.

The enhanced proximal coronary vasoconstrictor response to 5-hydroxytryptamine following endothelial removal could be explained by the loss of at least three functions of the endothelium. First, endothelial cells may act as a diffusional barrier to blood-borne neurohumoral agents. Second, the endothelium is a site of 5-hydroxytryptamine degradation via monoamine oxidase. Third, endothelial cells synthesize and release vasodilator substances. Degradation of 5-hydroxytryptamine by monoamine oxidase or release of prostacyclin cannot explain endothelial modulation in vitro, since the difference between contractions of arterial rings with and without endothelium are reportedly not affected by inhibition of monoamine oxidase or cyclooxygenase (Cohen et al., 1983a). Neither does the removal of a diffusional barrier to blood-borne neurohumoral agents appear to be a likely factor in the potentiation of the constrictor response, since neither angiotensin II nor phenylephrine constrictor responses were enhanced following endothelial denudations.

Finally, the possibility remains that the coronary endothelium may release an endothelium-derived relaxing factor (EDRF) in response to 5-hydroxytryptamine, as described for acetylcholine (Furchgott, 1983). 5-Hydroxytryptamine can activate serotonergic receptors and $\alpha_1$-receptors directly, or $\alpha_1$- or $\alpha_2$-receptors indirectly by displacement of stored norepinephrine (Vanhoutte et al., 1984). It is unlikely that 5-hydroxytryptamine is acting at $\alpha_1$-adrenoceptors on the endothelium to release a vasodilator substance, since the response to phenylephrine was not altered by endothelial removal. Data in isolated coronary vascular segments suggest that stimulation of $\alpha_2$- and 5HT$_1$-receptors (Cocks and Angus, 1983; Cohen et al., 1983a) may result in endothelial-dependent relaxation.

The vasomotor response to 5-hydroxytryptamine in the proximal coronary artery may be influenced by a flow-dependent endothelial-mediated dilation. Pohl and co-workers (1983) have reported large coronary artery vasodilation to intravenous infusions of 5-hydroxytryptamine in conscious dogs. This proximal coronary vasodilation was reversed to vasoconstriction when the increase in coronary flow

### Table 4

<table>
<thead>
<tr>
<th>Hemodynamics during Intracoronary Phenylephrine Infusion in Dogs with Coronary Endothelium Intact and Endothelium Removed</th>
<th>Phenylephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 µg/min</td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>75 ± 5</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>63 ± 10</td>
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<table>
<thead>
<tr>
<th>Endothelium intact</th>
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<td>Coronary flow (ml/min)</td>
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<table>
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<tr>
<th>Endothelium removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pressure (mm Hg)</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
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</table>

$\Delta$ CD (µm)

FIGURE 5. Effect of intracoronary angiotensin II (1 and 5 µg/min, n = 7) on mean coronary diameter (change from control, $\Delta$CD) before and after endothelial removal.

FIGURE 6. Effect of intracoronary phenylephrine (1–5 µg/min, n = 7) on mean coronary diameter (change from control, $\Delta$CD) before and after endothelial removal.
during 5-hydroxytryptamine infusion was prevented. Flow-dependent proximal coronary vasodilation has also been described for adenosine and dipyridamole (Holtz et al., 1983), and after reactive hyperemia in the coronary circulation (Hinzte and Vatner, 1984). Holtz and co-workers (1983) have suggested that the increase in coronary flow mediates this response by increasing shear stress upon the endothelial surface, triggering release of a dilator substance that diffuses to the underlying smooth muscle. In the present study, proximal coronary artery dilation to 5-hydroxytryptamine was not observed frequently, suggesting a possible difference in vascular responses in the anesthetized dog preparation. Interestingly, however, the proximal coronary constrictor response was inversely proportional to the change in coronary blood flow with the endothelium intact. Thus, the decrease in diameter was greater with smaller increases in coronary blood flow. This relationship between changes in diameter and flow was not observed when the endothelium was removed. These results are consistent with a flow-dependent endothelial-mediated inhibitory effect on the proximal coronary constrictor response to 5-hydroxytryptamine.

The proximal coronary artery constrictor response to 5-hydroxytryptamine was similar to that measured angiographically by Bove and Dewey (1983) in the dog. In a more recent study from the same laboratory, Brum et al. (1984) examined the effects of endothelial removal on the constrictor response to 5-hydroxytryptamine. Although disruption of the endothelium appeared to potentiate the constrictor response to 5-hydroxytryptamine, the observed decrease in baseline diameter may have accounted for the reported shift in the dose-response curve. The magnitude of the change in baseline cross-sectional area following endothelial removal in their study was comparable to the changes seen in vivo with 5-hydroxytryptamine. Although the authors attempted to correct for this shift in baseline diameter, the normalized data do not establish whether the change in response to 5-hydroxytryptamine was due to the change in baseline diameter or to the absence of the endothelium. In addition, Brum et al. did not examine the specificity of the response to 5-hydroxytryptamine. In the present study, endothelial removal also resulted in a decrease in coronary diameter; however, this effect was transient, lasting only 15 minutes. Vasomotor responses to 5-hydroxytryptamine were obtained only after coronary artery diameter had returned to control values. The transient nature of the constrictor response to balloon denudation indicates that the endothelium is not essential for the maintenance of proximal coronary vasomotor tone.

Scanning electron microscopy of the endothelium-denuded coronary arteries (Fig. 2) suggests that the transient vasoconstriction observed after balloon denudation could be the result of platelet aggregation and release of factors such as 5-hydroxytryptamine or thromboxane A\textsubscript{2}. Human blood contains 0.1-0.3 \( \mu \text{g/ml} \) of 5-hydroxytryptamine, most of which is stored in circulating platelets (Vanhouette and Cohen, 1983). The concentration of 5-hydroxytryptamine in the present study was 0.02-0.3 \( \mu \text{g/ml} \). Although only a small fraction of the circulating platelets adhere to the basement membrane after endothelial removal, the effect on vasomotor tone will depend on the local concentration of platelet-derived vasoactive factors at the vascular smooth muscle.

It has been suggested that damage to endothelium could play a role in the pathophysiology of coronary vasospasm. Although the mechanism of coronary vasospasm is not well understood, the association of atherosclerosis and vasospasm raises the possibility that atherosclerosis could damage endothelium, resulting in altered vasoconstrictor responsiveness to pharmacological and neurohumoral stimuli. Alternatively, the interaction between the endothelium and the vascular smooth muscle may be altered by the presence of the atherosclerotic plaque. Studies in isolated aortic strips from atherosclerotic rabbits have demonstrated increased sensitivity to the constrictor effects of norepinephrine, ergonovine, and serotonin (Henry and Yokoyama, 1980). Augmented in vivo vasconstrictor responses to serotonin in atherosclerotic monkeys have also been demonstrated (Heistad et al., 1984). More recently Kawachi et al. (1984) have demonstrated selective potentiation of the ergonovine-induced coronary vasoconstriction in atherosclerotic canine coronary arteries.

Our findings show that the endothelium can modulate vasomotor responses to humoral stimuli in the intact coronary circulation and that damage to the endothelial lining may result in abnormal vasoconstrictions. Although the decrease in internal cross-sectional area (5-24%) with 5-hydroxytryptamine had no effect on blood flow, such changes in luminal area may reduce flow in the presence of an 80% or greater coronary stenosis when coronary vasodilator reserve is compromised (Klocke and Ellis, 1980).

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