Increased vasoconstrictor activity of proximal coronary arteries with endothelial damage in intact dogs

Jose M. Brum, M.D., Qian Sunan, M.D., Gary Lane, M.D., and Alfred A. Bove, M.D., Ph.D.

ABSTRACT  In this study we examined the hypothesis that endothelial damage increases proximal coronary arterial vasomotor tone and sensitivity to vasoconstrictor stimulation. The response of the left anterior descending coronary artery (LAD) (% area change) to serotonin and nitroglycerin were examined in eight anesthetized (Innovar + nitrous oxide), closed-chest dogs by means of quantitative coronary angiography. Dose-response curves of percent change in arterial cross-sectional area for three doses of intracoronary serotonin were examined before and after endothelial damage produced by a balloon catheter in the LAD. Endothelial damage was verified by postmortem scanning electron microscopic examination. Intracoronary injection of $^{133}$Xe provided coronary flow data. The damaged segment of LAD showed spontaneous vasoconstriction and further constriction in response to serotonin (33 ± 5% before and 52 ± 6% area reduction after damage; p < .05). Nitroglycerin reversed serotonin-induced vasoconstriction in LAD segments without damage but not in the LAD segment with endothelial damage. No significant changes were observed in aortic pressure, and heart rate was kept constant by pacing. Blood flow in the LAD was not affected by endothelial damage itself (control, 2.44 ± 0.09 ml/min/g; damage, 2.53 ± 0.22 ml/min/g). Endothelial damage induced spontaneous proximal coronary constriction and diminished the relaxant response to nitroglycerin in the presence of serotonin. These results suggest that focal coronary narrowing that occurs in some patients after provocation with vasoconstrictor agents may be caused by local areas of damaged endothelium.

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SPONTANEOUS proximal coronary constriction (spasm) is presently considered to contribute significantly to the pathophysiology of coronary disease.1,2 Spasm is thought to occur in some patients with angina pectoris and has been demonstrated to produce myocardial infarction.3 Although the mechanism by which focal coronary spasm occurs is of considerable interest, to date this mechanism remains unknown.

Proximal coronary constriction has been demonstrated in response to the platelet-released mediators serotonin4,5 and thromboxane $A_2$.6 Platelet aggregation in sites of endothelial injury will release these agents locally.7 Intact endothelium not only prevents local platelet aggregation but also acts as a barrier that prevents blood-borne constrictor agents from reaching smooth muscle cells directly.8,9 When endothelium is damaged, platelet and fibrin thrombi that are deposited locally may cause local spasm by release of platelet-borne mediators. Several studies have demonstrated an increased sensitivity to serotonin10 and to ergonovine11 in areas of coronary atherosclerosis, and there is evidence suggesting that ergonovine acts through a serotoninergic mechanism.12 Many of the above-mentioned studies have been done in isolated arterial rings or strips and do not account for interaction between blood and the arterial wall.

These observations lead to the question of whether endothelial damage contributes to spontaneous coronary spasm and to locally increased sensitivity to ergonovine in the intact coronary circulation. To test this hypothesis we examined the vasomotor response of large coronary arteries to endothelial damage and to vasoconstriction induced by the vasoconstrictor serotonin in an intact canine preparation. We also used this preparation to examine the dilating effect of nitroglycer-
erin in the presence of endothelial damage and serotonin-induced vasoconstriction because nitroglycerin is the first-choice drug in a common clinical strategy for treating coronary spasm.

The data suggest that the proximal coronary vasoconstrictor response is increased in areas where the endothelial barrier is damaged. This increased response could explain the focal nature of coronary spasm.

Materials and methods

**Animals and anesthetics.** Mongrel dogs (n = 8) were anesthetized with a combination of intramuscular Innovar-Vet (0.4 mg of fentanyl and 20 mg of droperidol/1 ml, Pitman-Moore; 0.25 ml/kg) and gaseous anesthesia (70% nitrous oxide in oxygen) administered with a Harvard ventilator (Model 807) through an endotracheal tube. This anesthesia was chosen to minimize anesthetic effects on the large coronary arteries. Normal coronary arterial response to constrictors and normal coronary arterial flow are found with this preparation.13, 14

**Experimental procedure.** The left carotid artery, left femoral artery, and external right jugular vein were dissected. The coronary sinus was catheterized via a branch of the right jugular vein with a No. 7F catheter for pressure monitoring and blood sampling. Through another jugular branch vein, a No. 6F bipolar pacing catheter was also placed into the coronary sinus to produce atrial pacing and was connected to a pulse generator set at a proximately 80 beats/min. After a single intravenous bolus of 2% lidocaine HCl (40 mg; Elkins-Sinn) to suppress cardiac arrhythmias during initial coronary cannulation, a specially designed coronary guide catheter was advanced from the left carotid artery to the left coronary ostium. A 2 mm balloon dilatation catheter (USCI) was advanced through the coronary guide catheter and positioned in the proximal portion of the left anterior descending coronary artery (LAD) just proximal to the first large diagonal branch. This intracoronary catheter was used for drug infusions and for 133Xe injection to measure blood flow in the LAD. The large guide catheter was used for measuring proximal coronary arterial pressure and for injection of radioopaque contrast medium. All catheters were placed with fluoroscopic guidance. The pressure transducers (Statham P23Db) were balanced and calibrated with a fluid-filled manometer system at the beginning of each experiment and calibrations were checked periodically thereafter. Pressures were monitored from the proximal coronary artery, the coronary sinus, and the femoral artery and were recorded with the electrocardiogram (lead II) on a physiologic recorder (Honeywell Model 1508 A visi- corder). Serotonin (Sigma Chemical Co.) dissolved in saline was infused (Harvard infusion pump; Model 600-0) into the intracoronary catheter at three infusion rates (0.01, 0.05 and 0.1 mg/min). The infusion volumes were either 0.1 or 0.5 ml/min. Blood samples, pressure records, and coronary angiograms were taken in the control state and 6 to 8 min after the beginning of each infusion. Coronary angiograms were obtained during injection of 4 to 6 ml of meglumine diazirote (Renografin 76; Squibb) into the left main coronary artery. Exposures were made in mid diastole with an R wave–triggered x-ray switch with the delay set to trigger midway in the T-P segment of the electrocardiogram. Images were recorded on x-ray film using a cassette-type film holder. Exposure was 85 kV, 75 to 100 mA for 35 msc. Based on the coronary angiograms, the 5 mm long arterial segment 2 cm proximal to the intracoronary catheter tip was designated segment I, the 5 mm arterial segment at the catheter tip was designated segment II, and the 5 mm segment 2 cm distal to the catheter tip was designated segment III. Coronary sinus and femoral blood oxygen saturation and hemoglobin levels were determined with a co-oximeter (Instrument Laboratories Model 182).

**Blood flow.** Blood flow in the distribution area of the LAD was measured at two times, in the control state and during infusion of the maximum dose of serotonin, with use of 0.2 ml of 133Xe solution (0.25 mCi, Xenieisol; Mallinckrodt) injected selectively into the LAD. The isotope was detected with a single crystal detector placed over the left chest and positioned at midventricular level under fluoroscopic guidance. Data were transferred directly to a PDP 11/34 computer and processed to determine flow by means of the first 60 sec of washout curve and monoexponential log-linear least squares calculation for the slope (k). Flow (ml/min/g) was calculated as

\[ 0.72 k/1.05, \text{ where } 0.72 \text{ is the myocardium-blood partition coefficient and } 1.05 \text{ is the density of myocardium. Flow was also calculated from the washout with the height/area method. The two methods provided similar results.} \]

**Endothelial damage.** After the highest dose of serotonin, nitroglycerin (200 µg; Parke Davis) was injected into the anterior or descending arterial catheter to reverse serotonin-induced proximal coronary vasoconstriction. After 5 to 7 min the balloon catheter was advanced into the middle portion of the LAD, inflated with a solution of 50% meglumine diazirote in saline and gently rubbed against 1 to 2 cm of the arterial wall. We monitored balloon size with fluoroscopic assistance and inflated only enough to fill the balloon. Since the diameter of the LAD was greater than 2 mm, the balloon did not distend the artery. An angiogram was again taken 5 to 7 min after the endothelial denudation.

The infusions of serotonin were repeated according to the same protocol as used before endothelial denudation. After the final measurements, α-azines blue dye was then injected into the intracoronary catheter to identify the region perfused by the LAD. The animals were killed with KCl (10 ml saturated solution; Corning), the heart was removed, and the left main coronary artery was cannulated through the aorta. The left coronary system was cleared of blood with a NaCl 0.9% solution perfused under 100 mm Hg pressure. When the perfusate was clear of blood it was followed with a 1.5% buffered glutaraldehyde solution infused under the same pressure for 40 to 60 min. The coronary arteries were then dissected and separated into LAD and circumflex coronary artery samples for each dog. Those samples were then prepared for histologic and scanning electron microscopic examination of endothelium. Myocardial tissue that was stained blue was weighed separately from the unstained tissue to determine the region of supply of the LAD.

**Quantification of coronary dimensions.** To quantitate the epicardial coronary arterial response to serotonin, angiograms were analyzed for coronary dimensions with a computer-based quantitative angiographic system.2, 15 This method was evaluated for accuracy by measuring dimensions from x-ray images of tubular models with known dimensions. These models included metal wire, contrast-filled polyethylene tubing, and accurately machined hollow plastic cylinders that were inserted into the LAD of intact, anesthetized dogs with a catheter guidewire technique. The regression of calculated vs actual dimensions gave a slope of .986, a correlation coefficient of .997, and a standard error of estimate of 0.062 mm. This small standard error of the estimate allows a high degree of accuracy when measuring coronary dimensions with this technique. This method has also been validated in other laboratories.16 Constrictor response to serotonin was calculated as the percent of control cross-sectional area of the coronary artery. Values are expressed as measured area or percent of change in area relative to the control value.
Resistance. Resistance of the distal coronary bed (mm Hg/ml/min) was determined by calculating the ratio (mean aortic pressure - coronary sinus pressure)/LAD flow.

Oxygen content (O2 saturation ⋅ 1.34 ⋅ hemoglobin concentration) was expressed as milliliters of oxygen per 100 milliliters of blood.

Test of the preparation. Physiologic saline was infused into the LAD in two animals according to the same temporal sequence as with the serotonin infusions to test the possibility that the saline vehicle itself might cause significant coronary dimensional and hemodynamic changes. LAD averaged 5.42 ± 0.35, 5.20 ± 0.15, 5.77 ± 0.80, and 5.58 ± 1.10 mm² with the same infusion protocol, but with serotonin absent from the infusate. LAD flow also remained unchanged over this time, and no change in aortic pressure was noted. The absence of coronary hemodynamic or dimensional alterations with the low infusion volume (0.1 or 0.5 ml/min) of the vehicle demonstrates that saline alone does not produce a change in the coronary circulation. In another two dogs we examined the coronary response to sequential serotonin infusion and nitroglycerin injection without intervening endothelial damage (figure 1). These animals were studied to evaluate the effect of nitroglycerin after serotonin infusion and the possibility of serotonin sensitization due to the persistence of the mediator alone. Nitroglycerin did not affect the profile of the second serotonin dose-response curve.

Statistical evaluation was done with two-way analysis of variance with repeated measures across drug dose, and with paired t tests. Results are expressed as mean values ± SEM.

Results

Pressures. Aortic pressure (table 1) showed no significant changes during the serotonin infusion, and LAD endothelial denudation caused no change in pressure.

Coronary sinus pressure showed no significant variation throughout the study, and the heart rate was between 80 and 95 beats/min with coronary sinus (atrial) pacing (table 1).

Coronary dimensions. The LAD showed evident reduction of cross-sectional area during serotonin infusion (table 2 and figure 5, A through D) with significant changes at 0.05 mg/min infusion in the distal segment (segment III). With 0.1 mg/min infusion there was significant narrowing in segment II (p < .05) and in segment III (p < .0025). Although only minimal constriction was noted in segments I and II after serotonin infusion, nitroglycerin restored their cross sections to initial control values (table 2). After endothelial denudation in segment III, a significant reduction in the LAD area was observed in this denuded portion during 0.05 and 0.1 mg/min serotonin (table 2). Compared with that in the normal state, the LAD area decrease in response to serotonin was greater (p < .05)

![FIGURE 1. Repeated dose-response curves of LAD diameter distal to the infusion site of intracoronary infusion of serotonin (5HT) (segment III). The slight increase in LAD diameter in the control state of the second serotonin infusion was due to injection of nitroglycerin (200 μg) (NTG). The nitroglycerin effect did not change the vasoconstrictor action of serotonin.](http://circ.ahajournals.org/)

**TABLE 1**

<table>
<thead>
<tr>
<th>SHT dose (mg/min)</th>
<th>Heart rate (bpm)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Mean coronary sinus pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86 ± 3</td>
<td>2.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>88 ± 3</td>
<td>2.2 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>93 ± 8</td>
<td>1.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>84 ± 2</td>
<td>1.8 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Before LAD endothelial damage

Data expressed as mean ± SEM.

SHT = serotonin.

**TABLE 2**

<table>
<thead>
<tr>
<th>SHT dose (mg/min)</th>
<th>Segment I (mm²)</th>
<th>Segment II (mm²)</th>
<th>Segment III (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.12 ± 0.39</td>
<td>4.82 ± 0.42</td>
<td>4.15 ± 0.18</td>
</tr>
<tr>
<td>0.01</td>
<td>4.92 ± 0.39</td>
<td>4.37 ± 0.26</td>
<td>3.61 ± 0.29</td>
</tr>
<tr>
<td>0.05</td>
<td>4.57 ± 0.35</td>
<td>4.27 ± 0.40</td>
<td>3.01 ± 0.34</td>
</tr>
<tr>
<td>0.10</td>
<td>4.27 ± 0.42</td>
<td>3.77 ± 0.31²</td>
<td>2.73 ± 0.28²</td>
</tr>
</tbody>
</table>

After LAD endothelial damage

Before endothelial damage

After endothelial damage

5HT = serotonin.

²Difference between before and after damage (p < .05) compared with respective controls (both groups); ³p < .05; ⁴p < .005; ⁵p < .0025.
after endothelial denudation (33.7 ± 5.6% vs 52.2 ± 6.7% of area narrowing). In addition, the denuded segment did not return to control values after nitroglycerin (figure 2). Change in sensitivity of the denuded arterial segment is demonstrated in figure 3. In this figure, the normalized serotonin constrictor response, based on a postdamage control, is shifted downward and suggests that serotonin constriction is augmented in the damaged artery.

Coronary resistances. The small-vessel resistance (pressure/flow) did not show significant changes during serotonin infusion before or after denudation of the LAD (table 3), although a small reduction in average resistance was noted.

LAD blood flow. LAD total blood flow and flow per gram of myocardium did not significantly increase

during the serotonin infusion in the intact artery. After endothelial damage of the LAD, flow showed a significant increase with serotonin (table 3). The change was due to a lower control flow after endothelial damage. The arteriovenous O\textsubscript{2} difference was not affected by serotonin, and there was no difference in values between the intact artery and the denuded artery after infusion of serotonin (table 4).

Histologic studies. The specimens examined by electron microscopy showed intact endothelium with few blood cells in the control segments of LAD. The denuded segments showed large segments of endothelium missing and platelet and blood cell aggregates (figure 4, A and B).

Discussion

The results of this work support our hypothesis that endothelial damage enhances the vasoconstrictor response of intact epicardial coronary arteries.

A previous study done in our laboratory demonstrated that serotonin is a potent constrictor of intact coronary arteries and has a weak dilating effect on coronary resistance vessels. Contractions of isolated rings of canine circumflex coronary arteries produced by serotin.
Serotonin were increased after endothelial damage. The work of Cohen et al. provides the evidence that the effects of serotonin on isolated segments of proximal coronary artery depend on endothelial integrity. Thus vasoactive substances released by platelets in areas of endothelial injury could create a zone of hypersensitivity and cause focal spasm. Our results extend the findings in isolated arteries to intact arteries in the intact circulation and provide a basis for understanding coronary spasm in humans.

In this work, special attention was paid to minimizing surgical stress by using a canine closed-chest preparation and $^{133}$Xe blood flow measurements and to minimizing the effect of serotonin on the systemic circulation by using intracoronary serotonin infusion. The possibility that all measurements obtained after the denudation procedure were affected by the repeated serotonin infusion and nitroglycerin injection was evaluated in two animals. In the undamaged artery the vasoconstrictor response to the second serotonin dose-response curve was not different from the first, and nitroglycerin injection after the first serotonin dose-response curve dilated all the LAD segments but did not change the second serotonin dose-response curve (figure 1).

Special attention was also paid to the dimensions of the balloon catheter that was used for producing the endothelial damage. This catheter should not overdilute the artery wall and cause smooth muscle damage but should produce only endothelial denudation. We used a 2 mm diameter angioplasty balloon and calculated the dimension of the LAD from the initial control angiogram to ensure these conditions were met. This preparation of coronary vasoactivity after endothelial damage is therefore appropriate for studying the response to endothelial injury. Other mediators are also

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**FIGURE 4.** A, Electron micrograph of a perfusion-fixed normal segment of the LAD showing intact endothelium and minimal adherent elements. B, Micrograph of a more proximal segment of same artery showing endothelial damage and adherent blood elements. Bars = 10 μm.

**FIGURE 5.** A, Coronary angiogram of control state showing the LAD and the circumflex coronary artery. The intracoronary catheter tip is visible in the LAD (black arrow). Area of damage lies between the two white lines. B, Same animal during infusion of serotonin (0.1 mg/min). The vasoconstrictor effect is evident in the LAD, middle and distal segments. C, Same animal. Coronary angiogram in the control state after endothelial damage. D, Same animal. Intracoronary infusion of serotonin (0.1 mg/min) after endothelial damage. Vasoconstriction appears more severe when compared with serotonin infusion without endothelial damage (B). E, Same animal. Intracoronary injection of nitroglycerin (200 μg) causes evident coronary vasodilation throughout the coronary tree. However, a small segment corresponding to the denuded area still remains constricted when compared with the control (A).
known to have an augmented response in arterial segments with endothelial damage and could also be studied in this preparation.

An interesting finding in this study and our previous work was the absence of the hypertensive chemoreflex known to occur when serotonin is injected into the left coronary artery. The data of James et al. indicate that serotonin produces a hypertensive reflex when injected

FIGURE 5. For legend see opposite page.
into the left main coronary artery to reach chemoreceptors lying between the aorta and pulmonary artery that are supplied by proximal branches of the left coronary system. In this preparation we infused serotonin into the LAD, distal to the left main bifurcation, and most likely avoided branches to those chemoreceptors. We did not find a hypertensive effect during serotonin infusion into the LAD.

During the first dose-response study with intact endothelium, serotonin infusion diminished the LAD cross-sectional area in the whole artery. The changes were significant in segment II with 0.01 mg/min and in segment III with 0.05 and 0.10 mg/min serotonin.

After endothelial damage in segment III, spontaneous constriction was observed during fluoroscopy. Nitroglycerin injection after the damage procedure restored segments I and II to the control dimension but did not return segment III to its control state. Therefore, despite nitroglycerin action, the denuded segment remained contracted to about 82% of its initial dimension.

The dose-response curve for serotonin observed in segment III after endothelial damage showed a larger vasoconstrictive effect than before the denudation, while the responses in segment I and II were similar to control curves. Figure 5 demonstrates an additive effect of endothelial damage and serotonin on constriction of large coronary arteries. The net constriction from both effects is nearly twice the response to serotonin alone. We also evaluated the sensitivity of the damaged segment to serotonin by comparing the curves of figure 2, using the resting or control value at the start of each curve as the reference dimension. When this comparison is made (figure 3), the slope of the serotonin dose-response curve after endothelial damage is steeper, suggesting that the artery is more sensitive to serotonin after endothelial damage.

Recent studies in isolated arterial segments suggested that smooth muscle responds to direct serotonin stimulation with vasoconstriction, while endothelial stimulation by serotonin produces vasodilation. Consequently, if endothelial damage were to occur the arterial vasodilator response to serotonin would be absent and the smooth muscle vasoconstrictor response would be unopposed. However, this dual effect of serotonin found in isolated arteries was not observed in the current study or in previous studies from our laboratory on the intact coronary artery. The slight nonsignificant decrease in small-vessel resistance produced by serotonin was associated with a slight but nonsignificant increase in myocardial flow before endothelial damage was produced. After damage, the increased flow could be explained by a synergistic vasodilator effect of serotonin in the small vessels plus vasoactive substances released by intact endothelial cells adjacent to the damaged site.

The changes in dimension of the large coronary vessels found in this study occurred in the presence of a constant coronary perfusion pressure and a paced heart rate. Thus the findings cannot be explained by changes in the hemodynamic state of the circulation, but rather by a local constrictor effect of serotonin potentiated by endothelial damage.

Recent studies have shown that serotonin applied topically to canine coronary arteries produced a 22% decrease in mean coronary flow. From our data this observation could be explained by the direct action of serotonin on smooth muscle but not on endothelium. However, proximal coronary constriction must be severe (>60% area reduction) before flow is reduced, and the effect of serotonin on arterioles, if any, is usually vasodilation.

The recent study of Kiwachi et al. indicates that atherosclerotic injury also sensitizes coronary arteries to vasoconstrictors. In their study, ergonovine-induced vasoconstriction was enhanced in coronary segments that demonstrated atherosclerotic injury. The study of Yokoyama et al. also supports this finding. Neither study mentioned whether endothelium in these areas was intact. In addition, several studies have implicated vasoconstriction itself as a cause of endothelial damage.

In agreement with observations in isolated arteries, we find in the intact coronary circulation that serotonin vasoconstrictor activity depends on endothelial integrity and that the absence of endothelium itself is likely to be a factor in initiating localized vasoconstriction. Increased concentration of serotonin and other vasoconstrictors as a result of platelet aggregation at sites of endothelial damage may play an important role in the pathophysiology of coronary spasm. Further experiments with specific serotonin antagonists may be useful to elucidate the role of serotonin in coronary spasm.

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