Vasoactive Effects of Serotonin on Proximal Coronary Arteries in Awake Dogs

Alan Chu and Frederick R. Cobb

This study evaluates the vasoactive effects and mechanisms of action of serotonin on epicardial arteries in awake dogs chronically instrumented with miniature piezoelectric dimension crystals on the proximal circumflex coronary artery. Serotonin (2–16 μg) infused as a bolus in the left atrium effected a dose-related biphasic response, which was characterized by an initial increase in vessel dimension with a peak response at 45–75 seconds, followed by a delayed and more sustained vasoconstriction, with a maximum response at 2–8 minutes. The magnitude of the initial vasodilation was generally greater than the delayed vasoconstriction. The initial vasodilation remained unchanged after selective 5-HT2 blockade with ketanserin (0.3 mg/kg) and was only minimally but insignificantly attenuated when flow was held constant but was reduced after 5-HT1 and 5-HT2 blockade with methysergide (0.25 mg/kg). Selective 5-HT2 blockade with ketanserin (0.3 mg/kg) attenuated the delayed vasoconstriction in most but not all dogs; the effect of ketanserin was, however, not significant when the entire group was considered. Selective endothelial denudation effectively eliminated the initial vasodilation response to serotonin and significantly augmented the delayed vasoconstriction when the dogs were studied 1–2 days after denudation. The data indicate that in the awake dog, serotonin effects a biphasic vasomotor response characterized by an initial vasodilation that is mediated primarily through a direct endothelium-dependent 5-HT mechanism followed by a delayed vasoconstriction that is probably mediated via a direct 5-HT effect on the vascular smooth muscle and is attenuated by the normal endothelium.

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The vasoactive effects of serotonin, a factor released during platelet aggregation, have been extensively evaluated in in vitro vascular ring preparations of proximal canine coronary arteries.1-3 When the vascular rings were relaxed, serotonin effected a dose-dependent constriction that was thought to be mediated via an S1 serotoninergic-receptor mechanism on the smooth muscle cell; constriction was antagonized by ketanserin.1,2,4 When the vascular rings were precontracted with prostaglandin F2α, serotonin effected relaxation that was thought to be mediated by an S2 serotoninergic-receptor mechanism on the smooth muscle cell; constriction was antagonized by ketanserin.2,5 These studies indicate a potential for both serotonin-mediated vasodilation and vasoconstriction of the epicardial vasculature. Acute removal of the endothelium eliminated the serotonin-induced dilation response and potentiated the constriction response, suggesting that the endothelium directly mediated the dilation response and indirectly attenuated the constriction response.2,3

Only a few studies have examined the vasoactive effects of serotonin on proximal coronary arteries in intact animals. Bove et al5 in anesthetized dogs injected serotonin directly into the coronary arteries and demonstrated only a dose-dependent vasoconstriction using quantitative coronary angiography; the constriction was enhanced by acute denudation.4 Lamping et al6 also reported similar findings using sonomicrometer technique in anesthetized dog preparations. In a preliminary report in awake dogs, Pohl et al7 described a vasodilation effect of serotonin that was completely inhibited when flow was held constant and concluded that the primary effect of serotonin on epicardial arteries was constriction.

The present study was designed to evaluate the vasoactive effects of serotonin on proximal coronary arteries of awake dogs using chronically implanted piezoelectric dimension crystals. To address potential mechanisms of serotonin-induced vasoactive effects, measurements were repeated after 5-HT1 and 5-HT2 inhibition, during control coronary blood flow, and after coronary denudation.

Materials and Methods

Mongrel dogs (25–35 kg; n = 21) previously screened for the absence of anemia and infection were anesthetized with intravenous thiopental sodium (60–80 mg/kg). A left thoracotomy was performed at the fourth intercostal space, and the heart was suspended in a pericardial cradle. Heparin-filled polyvinyl catheters were inserted into the left atrium via the left atrial appendage and in the ascending aorta via the left internal thoracic artery. A 0.5–1.0-cm segment of the left circumflex coronary artery just distal to the atrial appendage (3–5 cm from the vessel origin) was dissected free. Care was taken to ensure minimal dissection of the vessel. Miniature 7-MHz piezoelectric crystals (1.5 × 2.5 mm, 15–20 mg) attached to a Dacron backing and insulated with Insul-X (Insul-X Products, Durham, N.C.) were inserted into the left circumflex coronary artery for chronic measurements.
Yonkers, N. Y.) were sutured to the adventitia on opposite surfaces of the dissected coronary segment using 6-0 prolene (Ethicon, Inc., Somerville, N.J.).\(^9\) Correct alignment of the crystals was verified by online sonomicrometry and oscilloscope monitoring (Tektronix 2215A, Tektronix, Inc., Beaverton, Ore.). In 7 dogs, a 10-MHz cuff-type pulsed Doppler flow probe was implanted distal to the crystals. An inflatable balloon snare was also placed adjacent to the flow probe. The catheters and crystal wires were tunneled to a subcutaneous pouch at the base of the neck.

After a recovery period of 7–14 days, the dogs were sedated lightly with intramuscular morphine sulfate (0.3–0.5 mg/kg) and studied while they were loosely restrained and lying awake on their right side in a dimly illuminated laboratory. Catheters and wires were exteriorized under local lidocaine-infiltration anesthesia. Aortic pressures, external coronary dimensions, and electrocardiograms were recorded and monitored continuously throughout the experiment. Any drift in the dimension and pressure measurements were minimized with frequent repeat calibrations during each study period. On a day prior to subjecting the dog to the study protocol, nitroglycerin (0.4 mg) was infused via the left atrial catheter to ensure a responsive vasculature. All animals demonstrating <3% dilation in response to the nitroglycerin infusion were excluded from the study. The same dose of nitroglycerin was infused at the end of each study day to ensure comparable day-to-day vasoreactivity.

Vasoactive effects of serotonin were assessed by infusing increasing doses (2, 4, 8, and 16 μg, expressed as weight of the base; Sigma Chemical Co., St. Louis, Mo.) as a bolus via the left atrial catheter in 16 dogs. A minimum of 15 minutes was allowed between injections to ensure clearance of serotonin and recovery of baseline hemodynamics. Repeat injections were made on the same day and on different days to ensure reproducible vasoresponsiveness.

To assess whether the vasoactive effects of serotonin were mediated via an \(\alpha_1\) or \(\alpha_2\) mechanism, boluses of serotonin (8 μg) were given before and after selective \(\alpha_1\)-receptor blockade with ketanserin (0.3 mg/kg; \(n=7\)) and after nonselective \(\alpha_1\)- and \(\alpha_2\)-receptor blockade with methysergide (0.25 mg/kg; \(n=10\)) (higher doses caused significant hypertension). In the 7 dogs equipped with Doppler flow probe and pneumatic snare, infusions of serotonin (8 μg) were repeated at constant flow controlled by partial occlusion of the balloon snare.

In 6 dogs, selective endothelial denudation was performed after completion of baseline studies. The dogs were anesthetized with 30–40 mg/kg thiamylal sodium. An 8 French right coronary guiding catheter (Advanced Cardiovascular Systems, Temecula, Calif.) was inserted through the right carotid and positioned in the left coronary ostium under fluoroscopic guidance. A 4 French Swan-Ganz catheter (110 mm, Critikon, Inc., Tampa, Fla.) was inserted through the guiding catheter into the left circumflex coronary artery to the area of the piezoelectric crystals. The balloon was inflated with 0.5–0.7 ml air, and the catheter moved back and forth two to three times over the entire length from the crystal area to the left main artery and then deflated and withdrawn. Aortic pressure, electrocardiogram, and coronary dimension were continuously monitored throughout the denudation procedure to ensure that the balloon did not overstretch the vessel. Acetylcholine (4-μg bolus) (Sigma) was infused via the left atrial catheter after denudation. The presence of dilation before and the absence of dilation after the procedure confirmed that denudation was achieved. Serotonin infusion studies were repeated 1–2 days after recovery from anesthesia. Nitroglycerin was also infused at the end of the study to ensure vasoreactivity before and after denudation. The dogs were subsequently fibrillated with an overdose of thiamylal sodium and potassium and their hearts immediately extracted. The left main coronary artery was cannulated, and the left circumflex artery was flushed gently with 20–50 ml 0.1 M cacodylate buffer (pH 7.3) and then fixed in situ by intracoronary perfusion with 2% glutaraldehyde and 0.1 M cacodylate. The denuded coronary segment was excised carefully, washed with buffer, and then fixed in 2% osmium tetroxide and dehydrated with ethanol. The vessel segment was then subjected to critical point drying with carbon dioxide and coated with a 200-Å layer of gold paladum. A control segment distal to the denuded area was carefully excised and processed in a similar fashion. Each segment was examined using a scanning electron microscope. A small transverse ring of the circumflex artery was also excised from the region of the crystals. Duplicate sections were processed for histology after staining with hematoxylin and eosin and Masson’s trichrome and were evaluated by light microscopy for mechanical damage to the media and/or scarring changes in the musculares.

Vasoactive changes were expressed as percent change from baseline dimensions. Vasoactive responses to a dose of serotonin infused after a specific intervention were compared with the responses to the same dose prior to the intervention using Student’s paired \(t\) test.

**Results**

Figure 1A is a representative recording of the phasic and mean aortic pressures and coronary dimensions during injection of serotonin. Serotonin caused a brief transient increase in aortic pressure at the highest dose (16 μg) in some dogs but no change in pressure with lower doses. A biphasic response in vessel dimension was seen in each dog after serotonin infusion; there was an initial transient increase in vessel dimension followed by a delayed vasoconstriction. The peak vasodilatation occurred 45–75 seconds after drug infusion, a time at which pressure either remained or had returned to normal. The delayed vasoconstriction reached a nadir between 2–8 minutes after infusion before gradually returning to baseline. The magnitude of the initial vasodilatation was generally greater than that of the delayed vasoconstriction.
Figure 1. Representative recording of phasic and mean aortic pressures and coronary dimensions before and after injection of 8 μg serotonin into left atrium (Panel A) when flow was allowed to increase and (Panel B) when flow was held constant. Initial transient dilation was followed by delayed vasoconstriction.

Figure 2 demonstrates the mean vasodilation and mean vasoconstriction responses of the intact vessel to increasing doses of serotonin in 16 dogs expressed as a percent of resting dimension. In each case, a dose-related response was seen. The mean vasodilation response was 3.56% (range 0.34 to 13.63%) at the 2-μg dose and 5.09% (range 0.43 to 15.88%) at the 16-μg dose. The mean vasoconstriction response was -0.97% (range 0 to -2.50%) at the 2-μg dose and -2.74% (range -0.16 to -7.25%) at the 16-μg dose.

The responses to serotonin infusions after ketanserin (selective S₂ receptor antagonist) were highly variable. Consequently, the group response did not achieve statistical significance (Figure 3). Ketanserin attenuated the vasoconstriction in 4 of the 7 dogs but had either no or a minimal effect in others, despite doubling the dose to 0.6 mg/kg. The lack of statistical significance when considering the group is probably best explained by the high degree of variability in the responses seen after ketanserin. In contrast, the vasodilation (Figure 3) responses in these same dogs were not affected by ketanserin, which indicates the lack of involvement of an S₂-receptor mechanism in mediating the serotonin-induced vasodilation. Nonselective S₁ and S₂ blockade with methysergide (Figure 3) significantly attenuated both the vasodilation and vasoconstriction responses to serotonin.

In the 7 dogs equipped with flow probes, flow increased transiently by an average of 106 ± 72% (range 31 to 240%) after 8 μg serotonin. When flow was held constant by partial occlusion of the circumflex artery during infusion of serotonin, the vasodilation was only slightly reduced (by 18%) from 3.06 ± 1.12% to 2.51 ± 1.48% (p>0.05), indicating that the serotonin-induced vasodilation was primarily a direct effect and only partially mediated by the increase in flow (Figure 1B).

In the 6 dogs in which selective coronary denudation was performed, serotonin-mediated dilation was severely and significantly reduced, while vasoconstriction was augmented (Figure 4). Successful denudation was verified by the lack of acetylcholine-induced dilation (4 μg) after denudation and electron microscopic evidence of >80% endothelial removal from the vessel segment. Functional integrity of the denuded vessel
musculature was demonstrated by the ability of nitroglycerin (0.4 mg) to induce further vasodilation. Histologic cross sections of the coronary vessel at the level adjacent to the crystals demonstrated normal musculatures and an intact lamina elastica. Scarring within the vessel wall was not seen. Resting coronary flows, aortic pressure, and heart rate were unchanged 1-2 days after denudation. Mean resting coronary dimension was significantly increased from 3.75 (predenudation) to 3.99 mm (1-2 days postdenudation), p<0.05.

Discussion

Phasic Vasomotor Response to Serotonin

In vitro studies using isolated canine coronary rings have demonstrated that serotonin has a potential for inducing both vasodilation and vasoconstriction. When the isolated rings were precontracted with prostaglandin F$_{2\alpha}$, serotonin effected a dose-dependent relaxation; when the rings were relaxed, serotonin effected a dose-dependent constriction. The present study extends these observations to intact, awake animals. The use of chronically implanted piezoelectric crystals on the adventitia of the proximal circumflex coronary artery allowed continuous monitoring of phasic epicardial artery dimensions for several days. Our study demonstrated that bolus injections of serotonin, in dosages that caused minimal or no significant hemodynamic response, effected dose-dependent biphasic vasomotor responses. The initial response was vasodilation that reached a maximum at 45-75 seconds, well after any transient change in arterial pressure. The vasodilation response was then followed by delayed and more sustained vasoconstriction response that reached a nadir between 2-8 minutes after infusion. The magnitude of the vasodilation response exceeded the vasoconstriction response. These studies contrast with previous studies in anesthetized dogs that have reported only dose-dependent vasoconstriction response in the proximal coronary arteries. Different responses may have been influenced by different study conditions. In the anesthetized dog studies, serotonin was infused directly and continuously into the coronary artery; concentrations in the coronary vasculature were likely much higher than those achieved by bolus injection into the left atrium. Anesthesia and acute surgery may also have contributed to the different cardiovascular responses seen.

Effects of $S_1$ and $S_2$ Antagonists on Serotonin-Induced Responses

Studies using isolated ring preparations have reported that the serotonin-induced vasoconstriction but not vasodilation was antagonized by the selective $S_2$ antagonist ketanserin. Vasoconstriction was antagonized by methysergide and methiothepin, nonselective $S_1$ and $S_2$ antagonists, suggesting an $S_2$-related mechanism for the vasodilation response. In the present study, the effects of ketanserin and methysergide on
Effects of Blood Flow

Serotonin infusion effected a variable and transient increase in coronary blood flow. At a dose of 8 μg, serotonin increased flow by 106 ± 72%. Although an increase in blood flow may induce vasodilation, we observed that the vasodilation response was only minimally affected when coronary blood flow was held constant, indicating that the vasodilation responses in the present study were mediated primarily by direct effect on the vasculature. These observations are in contrast with the preliminary study by Pohl et al., who reported that the vasodilation effect was completely blocked by holding flow constant, but are consistent with a direct vasodilation effect as demonstrated in the isolated ring preparations. The discrepancy between our findings and Pohl et al. can also be partially explained by the higher flow changes seen in their studies (up to 460 ± 85%).

Effects of Denudation

Studies in isolated ring preparations have demonstrated that acute removal of endothelium eliminated the serotonin-induced vasodilation response and potentiated the vasoconstriction response. Studies in anesthetized dogs also reported augmentation of serotonin-induced vasoconstriction after denudation. In the present study, coronary denudation eliminated the vasodilation response and increased the vasoconstriction response, supporting the view that the endothelium not only directly mediates the vasodilation response to serotonin but also reduces the sustained vasoconstriction response. The augmentation of the delayed vasoconstriction after denudation is consistent with a direct S₂ vasoconstriction effect on the vascular smooth muscle, which is enhanced by the removal of the opposing endothelium-mediated S₁ vasodilation.

Summary

The present study demonstrates that in the intact awake dog, serotonin effects a dose-dependent vasomotor response that is characterized by an initial vasodilation followed by a relatively weaker but more sustained vasoconstriction. The vasodilation response to serotonin was attenuated by nonselective S₁ and S₂ but not selective S₂ agonists, was only minimally reduced by preventing the increase in blood flow, and was eliminated by denudation. The vasoconstriction response was enhanced by denudation and was attenuated by S₂ receptor blockade in most but not all dogs. The data tend to support the view that the vasodilation effect of serotonin is mediated primarily by an endothelium-dependent S₁-receptor mechanism and that the vasoconstriction response is induced by a direct S₂-receptor mechanism on vascular smooth muscle that is normally attenuated by opposing endothelium-dependent S₁ vasodilation in the intact coronary artery.

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FIGURE 4. Mean (±SD) vasodilation (top panel) and vasoconstriction (bottom panel) responses to serotonin before and 1–2 days after selective coronary endothelial denudation in 6 dogs. *p<0.05.

References


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**Key Words** • serotonin • coronary artery • vasodilation • vasoconstriction • coronary flow • endothelium
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