Cooperative mediation by serotonin S₂ and thromboxane A₂/prostaglandin H₂ receptor activation of cyclic flow variations in dogs with severe coronary artery stenoses

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ABSTRACT We have reported previously that thromboxane A₂/prostaglandin (PG)H₂ and serotonin independently mediate the occurrence of cyclic flow variations (CFVs) in a canine preparation of severe coronary artery narrowing. This may be due to an effect of these substances on platelets and/or the vascular wall. We tested the hypothesis that there is a cooperative effect between thromboxane A₂/PGH₂ and serotonin receptor stimulation in the development of CFVs in this animal preparation. After placement of a hard plastic cylindrical constrictor around the left anterior descending coronary artery, CFVs develop and are characterized by repetitive cycles of declines in coronary blood flow and abrupt increases in flow. In a control group of dogs, CFV frequency (cycles/hour) and severity (lowest coronary blood flow just before its restoration) did not change significantly over a 3 hr interval. In a second group of dogs, CFVs were established after constrictor placement, abolished with the serotonin (5HT₂) receptor antagonist ketanserin, and reestablished by the continuous infusion of serotonin into the left atrium. Serotonin-induced CFVs were then abolished with a thromboxane A₂/PGH₂ receptor antagonist, SQ29,548, or a thromboxane synthetase inhibitor, dazoxiben (UK37,248). The relative specificity of the respective antagonists, SQ29,548 and ketanserin, was determined in canine platelets and rat aortic vascular strips. No significant cross-reactivity between ketanserin and SQ29,548 was found. Thus, the data obtained in these studies demonstrate a cooperative interaction between thromboxane A₂/PGH₂ and serotonin S₂ receptors that contributes to the development of CFVs in this experimental preparation. Circulation 76, No. 4, 952–959, 1987.

THE NATURE OF the interaction between platelets and the vascular wall is altered by the presence of atherosclerosis and coronary artery narrowing.¹ Transient platelet aggregation and subsequent release of platelet contents in damaged and atherosclerotic coronary arteries may be important in the pathogenesis of selected coronary artery disease syndromes such as unstable angina and acute myocardial infarction.²,³

Data reported from our laboratory have demonstrated that thromboxane A₂ and occupation of the platelet and/or vascular thromboxane A₂/prostaglandin (PG)H₂ receptors are important in mediating transient blood flow reductions in narrowed canine coronary arteries.⁴–⁶ Both thromboxane⁶ and serotonin⁷ concentrations are increased at the site of severe canine coronary artery narrowing. We reported that thromboxane synthetase inhibition with dazoxiben (UK-37,248, Pfizer)⁴ or selective thromboxane A₂/PGH₂ receptor antagonism with SQ29,548 or SQ28,668 (Squibb)⁸ eliminates the transient flow reductions in narrowed canine coronary arteries in approximately 70% of the animals. Moreover, a serotonin S₂ (5HT₂) receptor antagonist, ketanserin (R41,468), abolishes transient blood flow reductions in stenosed coronary arteries in over 90% of dogs tested.⁸

Once platelets are activated by subendothelial collagen and undergo the release action, multiple medi-
atars are available to other platelets in vivo. Many of these mediators are known to act synergistically, at least in vitro, to augment the overall platelet response and ultimately result in irreversible aggregation of the activated platelets. The present study was designed to test the hypothesis that stimulation of both thromboxane A$_2$/PGH$_2$ and 5HT$_2$ receptors on platelets and/or within the coronary vascular wall interact cooperatively in leading to the development of transient flow reductions in a canine preparation of severe coronary artery stenosis.

Methods

Surgical preparation. The experimental preparation used was one originally described by Folts et al. Fifty-one male mongrel dogs (18 to 33 kg) were anesthetized with sodium pentobarbital (30 mg/kg) and ventilated with room air. Catheters were placed in the common carotid artery and jugular vein for arterial pressure measurement and fluid administration, respectively. A thoracotomy was performed in the fifth left intercostal space of each dog and the heart was suspended in a pericardial cradle. A segment of left anterior descending coronary artery (LAD) was gently dissected free from surrounding tissue and a pulsed Doppler flow probe (Dr. C. J. Hartley, Houston) was positioned around the LAD proximally but adjacent to where the coronary artery constrictor would be placed. Arterial blood gases and body temperatures were maintained within normal physiologic limits.

Experimental protocols. At the conclusion of the surgical instrumentation, dogs were allowed to stabilize for 30 min. Control hemodynamics, including systolic and diastolic blood pressures, heart rates, and mean and phasic coronary blood flow velocities, were recorded continuously on an eight-channel recorder (Hewlett-Packard, model 7758A). A plastic cylindrical constrictor was placed around the LAD, resulting in a luminal diameter that reduced control phasic blood flow velocity by approximately 50%; this degree of coronary artery constriction was required to attenuate reactive hyperemia after a 10 sec coronary artery occlusion. Cyclic flow variations (CFVs) normally began within 15 min of constrictor placement. Animals were divided into two groups for this study.

Group I — control animals. CFV frequency (number of CFVs per hour), severity (nadir of coronary blood flow velocity), and hemodynamics were monitored continuously for 3 hr in 28 dogs. No drug intervention was used in these animals.

Group II. CFVs were established in 23 dogs. Once CFV frequency and severity were reproducible, CFVs were recorded for a 30 min period. Ketanserin, a 5HT$_1$ receptor antagonist (Janssen Pharmaceutical, Beerse, Belgium), was dissolved in warm saline to make a 1 mg/ml solution and was administered into the left atrium at doses beginning at 0.1 mg/kg and increasing at 15 min intervals to 0.25, 0.5, 0.75, 1.0, 1.5, and 2 mg/kg until CFVs disappeared.

Serotonin (5-hydroxytryptamine ergotamine sulfate, 5-HT, Sigma Chemical Co., St. Louis), was dissolved in normal saline, and was then infused continuously into the left atrium of dogs in which CFVs had been abolished for 60 min (Harvard infusion/withdrawal pump, model 600, Harvard Apparatus Co., Natick, MA). Serotonin infusion was begun at 0.1 mg/min and increased to 0.25, 0.5, and 1 mg/min at 30 min intervals until an infusion rate was identified that caused CFVs to return spontaneously. An adjustable constrictor was placed around the descending aorta in one dog receiving 1 mg/min serotonin to maintain mean arterial pressure. When CFVs returned with a continuous serotonin infusion, CFVs were documented for a 30 min period. Blood was collected for plasma catecholamine analysis immediately preceding serotonin infusion and 30 min after serotonin restored CFVs in five dogs and was processed as described below. Dogs in which ketanserin was effective in eliminating CFVs and serotonin was effective in restoring CFVs were treated subsequently in one of two ways:

(1) Nine group II-A dogs (group IIA-1) were given 5 to 40 mg/kg of the thromboxane synthetase inhibitor dazoxiben (disolved in saline) (UK37,248, Pfizer Pharmaceutical, Groton, CT) in 5 mg/kg increments at 5 min intervals simultaneously during serotonin infusion until CFVs were eliminated.

(2) Eight group II-A dogs (group IIA-2) received the thromboxane receptor antagonist SQ29,548 (Squibb Institute for Medical Research, Princeton, NJ) simultaneously during the serotonin infusion. SQ29,548 was dissolved in 95% ethanol to make a 10 mg/ml solution and added to 2 mM sodium carbonate solution to dilute the solution to 1 mg/ml. SQ29,548 was administered initially intravenously in a 0.1 mg/kg bolus and increased at 0.1 mg/kg increments up to a total of 1.2 mg/kg or abolition of CFVs.

Ketanserin did not abolish CFVs in five of 23 group II dogs in which abolition was attempted at doses of up to 2 mg/kg. In these five dogs (group II-B), dazoxiben was administered in two dogs and SQ29,548 was given to three dogs to eliminate CFVs. Serotonin was not infused into these animals.

Isolated rat aorta contractility studies. Strips of rat thoracic aorta were prepared as described by Hemker and Aiken and suspended in Krebs-Henseleit solution containing indomethacin (2.8 μM) bubbled with 5% CO$_2$ in O$_2$ and maintained at 37°C. After equilibration for 1 hr at 1 g of tension, isometric tension development was measured after adding epinephrine (5.5 μM) as a reference compound. The concentration-response relationships for the thromboxane mimetic U46619 in the absence (n = 10) or presence of ketanserin (0.1 to 10 μM, n = 10) were determined. The influence of ketanserin on serotonin-induced contractions of rat aortic strips was also studied. Concentration-response relationships were established for serotonin in the absence (n = 12) and presence of various concentrations of ketanserin (1 to 100 nM, n = 12).

Canine platelet studies. Canine blood was collected in 3.8% sodium citrate (9 volumes blood : 1 volume citrate). Platelet-rich plasma (PRP) was prepared by centrifuging the citrated blood at 150 g for 20 min at 25°C. Platelet-poor plasma (PPP) was obtained by centrifuging the PRP at 2500 g for 30 min at 25°C. Platelet aggregation was studied photometrically14 with a Born MKIII aggregometer or a Chronolog aggregometer (Model 300-1) connected to a linear recorder (Linear Instruments Corp., Model 285). Platelets in dog PRP failed to aggregate in response to serotonin alone. Platelets were sensitized to serotonin by incubation with a subthreshold (10 μM) concentration of epinephrine for 2.5 min. A dose-response relationship was established for serotonin in the presence of ketanserin, and for serotonin in the presence of SQ29,548 (10 μM serotonin).

Plasma catecholamines. Blood was collected in green top (heparinized) test tubes and centrifuged at 1000 g for 20 min at 2° to 4°C and the plasma was separated and frozen for later analysis by high-pressure liquid chromatography (Smith Kline Bio-Science Laboratories, St. Louis).

Statistical analyses. All values are expressed as the mean ± SEM. Comparisons between values obtained at different times within each group of dogs and between treatment groups were made by a two-way analysis of variance and a Duncan’s multiple-range test. Comparison with control unconstricted coronary blood flow, designated as 100%, was done with a Dunnett’s test. A p value < .05 was used to define a significant difference between populations.
Results
Placement of the constrictor around the LAD reduced mean and phasic blood flow velocity in group I and group II dogs to 60.1 ± 3.7% and 43.1 ± 2.6% of control values, respectively.

Hemodynamic changes. There were several significant hemodynamic changes observed during the 3 hr of cyclic flows in the control (group I) dogs. Heart rates declined significantly immediately after CFVs were initiated (table 1). Heart rates declined still further during the second and third hours of CFVs in this group, an effect likely due to the length of time under anesthesia. A slight but significant elevation in mean arterial pressures was observed during the first hour of CFVs. Heart rates also declined before ketanserin treatment in group II dogs, which was similar to the changes observed in group I control dogs.

Mean arterial pressures and heart rates declined significantly with the administration of ketanserin (group II-A and II-B animals), an effect that has been reported. Mean arterial pressure was not significantly changed during serotonin infusion after ketanserin, but heart rates were significantly lower than those after ketanserin alone (group II-A dogs). Mean arterial blood pressure was unaltered during treatment with dazoxiben (group II-A1), but heart rates were lower than those observed during the serotonin infusions. Heart rates rose during administration of SQ29,548 (group II-A2), but systemic blood pressures declined. These hemodynamic effects were not as pronounced in group II-B1 and B2 dogs, probably due to the absence of serotonin.

TABLE 1
Hemodynamics in control and treated dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>HR (beats/min)</th>
<th>AOM (mm Hg)</th>
<th>PHF Peak (mm Hg/min)</th>
<th>Nadir (mm Hg/min)</th>
<th>MNF Peak (mm Hg/min)</th>
<th>Nadir (mm Hg/min)</th>
<th>CFV frequency (cycles/hr)</th>
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<tr>
<td>I (n = 28; no intervention)</td>
<td></td>
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<tr>
<td>Control</td>
<td>142 ± 4</td>
<td>113 ± 2</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>0</td>
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<tr>
<td>Constricted</td>
<td>140 ± 4</td>
<td>112 ± 2</td>
<td>45 ± 4</td>
<td>—</td>
<td>62 ± 5</td>
<td>—</td>
<td>0</td>
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<tr>
<td>Hour 1</td>
<td>130 ± 4</td>
<td>117 ± 2</td>
<td>75 ± 5</td>
<td>5 ± 1</td>
<td>107 ± 9</td>
<td>7 ± 2</td>
<td>5.2 ± 0.3</td>
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<tr>
<td>Hour 2</td>
<td>121 ± 4</td>
<td>115 ± 2</td>
<td>74 ± 5</td>
<td>5 ± 1</td>
<td>102 ± 9</td>
<td>8 ± 2</td>
<td>4.9 ± 0.3</td>
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<tr>
<td>Hour 3</td>
<td>114 ± 4</td>
<td>112 ± 2</td>
<td>69 ± 5</td>
<td>4 ± 1</td>
<td>95 ± 8</td>
<td>8 ± 2</td>
<td>5.2 ± 0.3</td>
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<tr>
<td>II (n = 23; ketanserin treated)</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>146 ± 5</td>
<td>120 ± 3</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Constricted</td>
<td>146 ± 4</td>
<td>119 ± 3</td>
<td>42 ± 3</td>
<td>—</td>
<td>58 ± 5</td>
<td>—</td>
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<tr>
<td>Initial CFVs</td>
<td>140 ± 5</td>
<td>118 ± 3</td>
<td>68 ± 5</td>
<td>8 ± 2</td>
<td>91 ± 8</td>
<td>10 ± 2</td>
<td>4.9 ± 0.3</td>
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<tr>
<td>II-A — ketanserin abolished CFVs (n = 18)</td>
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<tr>
<td>Ketanserin</td>
<td>128 ± 6</td>
<td>93 ± 3</td>
<td>54 ± 4</td>
<td>—</td>
<td>63 ± 6</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>5HT-restored CFVs (n = 17)</td>
<td>108 ± 6</td>
<td>87 ± 4</td>
<td>74 ± 8</td>
<td>19 ± 3</td>
<td>95 ± 8</td>
<td>28 ± 4</td>
<td>4.2 ± 0.4</td>
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<td>5HT-restored CFVs abolished with:</td>
<td></td>
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<tr>
<td>Group IIA-1 Dazoxiben (8/9)</td>
<td>97 ± 8</td>
<td>97 ± 5</td>
<td>62 ± 10</td>
<td>—</td>
<td>84 ± 12</td>
<td>—</td>
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<tr>
<td>Group IIA-2 SQ29,548 (7/8)</td>
<td>129 ± 9</td>
<td>82 ± 7</td>
<td>83 ± 10</td>
<td>—</td>
<td>103 ± 10</td>
<td>—</td>
<td>0</td>
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<tr>
<td>II-B — Ketanserin did not abolish CFVs (n = 5)</td>
<td></td>
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<tr>
<td>After ketanserin</td>
<td>119 ± 10</td>
<td>108 ± 9</td>
<td>61 ± 15</td>
<td>18 ± 2</td>
<td>77 ± 19</td>
<td>24 ± 13</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>Group IIB-1 Dazoxiben (n = 2)</td>
<td>112 ± 36</td>
<td>120 ± 26</td>
<td>53 ± 4</td>
<td>—</td>
<td>64 ± 7</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Group IIB-2 SQ29,548 (n = 3)</td>
<td>122 ± 13</td>
<td>99 ± 7</td>
<td>47 ± 25</td>
<td>—</td>
<td>53 ± 21</td>
<td>—</td>
<td>0</td>
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</table>

HR = heart rate (beats/min); AOM = mean aortic pressure (mm Hg); PHF = peak phasic (diastolic) flow velocity as a percent of control, unconstituted peak phasic blood flow velocity; MNF = mean blood flow velocity as a percent of control, unconstituted mean blood flow velocity; CFV frequency = frequency of CFVs per 30 min observation period.

Significantly different from value measured during control, unconstituted coronary blood flow.

Significantly different from value of nadir in initial CFVs, p < .05.

Significantly different from value after ketanserin treatment.
velocity nadir of 8.2 ± 2% of control flow velocity. Ketanserin was effective in eliminating CFVs in 18 of 23 group II dogs (78.2%) (group IIA-1) at an average dose of 0.56 ± 0.13 mg/kg (range 0.125 to 2 mg/kg: 0.125 mg/kg, n = 3; 0.25 mg/kg, n = 6; 0.5 mg/kg, n = 5; 0.75 mg/kg, n = 1; 1 mg/kg, n = 1; 2 mg/kg, n = 2), and it reduced CFV frequency for the entire group to 1.22 ± 0.52 cycles/30 min. Left atrial infusion of serotonin restored CFVs in 17 or 18 dogs (94.4%) at an average infusion rate of 7.5 ± 2.0 μg/kg/min (range 0.9 to 35 μg/kg/min). CFV frequency was not significantly different than CFVs during the control period in these 17 dogs (4.84 ± 0.34 vs 4.24 ± 0.39 cycles/30 min). The severity of CFVs was also not significantly different than that measured during the initial period (nadir phasic flow velocity was 19 ± 3% with 5HT-restored CFVs versus 8 ± 2% of unconstricted blood flow velocity during initial CFVs).

Nine of the 17 dogs (IIA-1) in which CFVs were reestablished with serotonin were treated with dazoxiben intravenously during a continuous serotonin infusion. CFVs were abolished quickly (average dose = 15.0 ± 3.9 mg/kg, range 5 to 40 mg/kg: 5 mg/kg, n = 1; 10 mg/kg, n = 5; 20 mg/kg, n = 2; 40 mg/kg, n = 1) in eight of nine dogs. The ninth dog received 10 mg/kg dazoxiben and CFVs were not abolished.

Eight of the 17 group II-A dogs in whom CFVs were reestablished with serotonin were given SQ29,548 while the serotonin infusion continued. SQ29,548 abolished CFVs in seven of eight dogs (0.14 ± 0.02 mg/kg) within 3.6 ± 1.1 min of intravenous injection. Thus, the thromboxane receptor antagonist was effective in abolishing serotonin-induced CFVs in 88% of the dogs. A recording from an animal that completed this protocol is shown in figure 1.

Ketanserin was ineffective in abolishing CFVs in five of the original 23 group II dogs (2 mg/kg total dose administered to all five). A significant bradycardia was observed during ketanserin treatment in this group, but there was no significant change in systemic arterial pressure when compared with pressure in the initial group of 23 animals. Initial CFV frequency in this group was 5.2 ± 0.7 cycles/30 min; this frequency was not significantly different after 2 mg/kg of ketanserin (5.6 ± 0.9 cycles/30 min). The severity of the cyclic flows was not significantly different in these ketanserin-resistant dogs (phasic flow velocity at nadir = 18.4 ± 12% after ketanserin compared with 7.5 ± 1.5% of

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**FIGURE 1.** A, Control. B, A representative recording from a dog with severe coronary artery stenosis and CFVs. C, Abolition of CFVs after administration of ketanserin (0.25 mg/kg). D, The CFV pattern returned after administration of 0.25 mg/min serotonin. E, Abolition of serotonin-induced CFVs after administration of SQ29,548, while serotonin is infused simultaneously.
control phasic flow velocity during initial CFVs). CFVs in this group were abolished rapidly after administration of 5 to 10 mg/kg dazoxiben in two dogs and after 0.2 to 0.5 mg/kg SQ29,548 in three dogs (groups II B-1, II B-2, table 1).

**Isolated rat aortic contraction studies.** The control 50% effective concentration (EC50) values for serotonin-induced contractions of rat aortic strips in the absence of ketanserin was 239 ± 19 nM (mean ± SEM) (n = 12). Ketanserin antagonized serotonin-induced aortic smooth muscle contractions with a Kp of 1.83 ± 0.30 nM and a PA2 of 9.35 ± 0.18, but the slope of the Schild plot was significantly different from unity (−0.68 ± 0.07, 95% confidence interval −0.83 to −0.53), with a correlation coefficient of −.94, suggesting that the observed antagonism might not be strictly competitive at a single receptor type.

The dose-response relationship for U46619-induced contractions of rat aortic strips in the absence and presence of ketanserin is shown in figure 2. Control EC50 values for U46619-induced contractions in the absence of ketanserin averaged 1.8 ± 0.4 nM. In the presence of ketanserin at 0.1, 1.0, and 10 μM, EC50 values for U46619-induced contractions were 1.8 ± 0.2, 1.4, and 2.0 nM, respectively.

**Receptor antagonist cross-reactivity in canine platelets.** Canine platelets failed to aggregate in response to either U46619 or serotonin alone. However, subaggregatory concentrations of epinephrine (10 μM) sensitized canine platelets to U46619 and serotonin. Serotonin, at 10 μM concentration, caused maximal aggregation responses in epinephrine-primed platelets. The slope, or initial rate of aggregation, was 44% per minute.

SQ29,548 inhibited U46619-induced platelet aggregation in epinephrine-primed canine platelets with a 50% inhibitory concentration (IC50) of 3.2 nM (figure 3, A). SQ29,548 inhibited serotonin-induced platelet aggregation by 50% only at the highest concentration tested, which was 1000 μM (IC50 = 1000 μM) (figure 3, B). Therefore, SQ29,548 is approximately 300,000 times more active as a thromboxane-receptor antagonist than as a serotonin antagonist in canine platelets.

Ketanserin inhibited serotonin-induced aggregation of epinephrine-primed canine platelets with an IC50 of 14 nM (figure 3, B). However, the IC50 for ketanserin inhibition of U46619-induced platelet aggregation was 100 μM (figure 3, A). Thus, ketanserin is approximately 7000 times more active as a serotonin antagonist than as a thromboxane antagonist in canine platelets.

**Plasma epinephrine.** Plasma epinephrine concentrations were extremely low (<10 pg/ml) immediately before serotonin infusion and 30 min after serotonin restoration of CFVs in all five animals tested.

**Discussion**

The present study was designed to test the hypothesis that activation of both 5HT2 and thromboxane A2/PAH receptors on platelets and/or in the vascular wall occurs when cyclic flow variations develop in a
canine preparation with a severe coronary artery stenosis and endothelial damage. We have reported previously that the concentration of serotonin is elevated at the site of a severe coronary artery stenosis, probably due to the large platelet mass adherent to the vessel wall, and that pharmacologic blockade of 5HT2 receptors with ketanserin eliminates CFVs in most animals. We also reported in another group of animals that thromboxane A2 synthesis is increased and prostacyclin synthesis is decreased in the vascular tissue at the site of the coronary artery stenosis. Furthermore, we found that both a thromboxane synthetase inhibitor, dazoxiben (UK37,248) and thromboxane-receptor antagonists (SQ29,548 or SQ28,688) were effective in eliminating CFVs in a majority of animals. The data obtained in the present study demonstrate that a continuous serotonin infusion restores CFVs after ketanserin blockade of CFVs. Arterial hypotension after administration of ketanserin may have been expected to reflexly elevate circulating epinephrine levels, potentiating the effect of serotonin on platelet aggregation. Plasma epinephrine concentrations were very low during an initial period of CFVs and remained extremely low after administration of ketanserin and subsequent CFV restoration with serotonin. The observation has been made in this study and others that hypotension caused by ketanserin administration does not result in an expected reflex tachycardia, indicating that normal baroreflex activity is not functioning. There is evidence to suggest, however, that ketanserin decreases preganglionic nerve activity and blocks reflexly induced changes in blood pressure and heart rate, probably due to 5HT2 receptor blockade in the vasomotor center. Since the adrenal medulla is anatomically and functionally a sympathetic ganglia, this may explain the lack of reflexly mediated release of epinephrine.

Serotonin-restored CFVs were abolished by the inhibition of thromboxane synthesis or by the blockade of thromboxane A2/PGH2 receptors. These data confirm and extend our initial observations that blockade of either the 5HT2 or thromboxane A2/PGH2 receptor abolishes CFVs in this preparation in most cases. These data suggest that pharmacologic blockade of either 5HT2 or thromboxane A2/PGH2 receptors or diminishing the synthesis of thromboxane A2 prevents the necessary platelet and vascular wall interaction for CFVs to occur.

The range in the doses of antagonists used to abolish CFVs in group II dogs varied considerably from animal to animal. This may be due to variability in the extent of stenosis and extent of vascular damage inflicted on the artery before initiation of CFVs. In addition, variation in the amount of released mediators during platelet activation and receptor sensitivity to released mediators may also be factors. Potentially, the intensity of the response of the vascular wall to the released mediators also contributes to the severity of platelet/vessel wall interaction and, therefore, to the dose of antagonist required to abolish this response. The availability of other mediators and the platelet sensitivity to these potentially synergistically acting mediators may also play a role in the concentrations of antagonist necessary to eliminate CFVs. In those dogs in which the initial thromboxane A2/PGH2 or 5HT2 receptor blockade did not abolish CFVs, addition of the thromboxane A2 synthetase inhibitor or the receptor-blocking agent abolished CFVs in all cases. These data suggest that pharmacologic blockade of both thromboxane A2/PGH2 and serotonin S2 receptor types is an effective way of restoring a uniform flow pattern in stenosed arteries.

An alternate explanation for these data is a lack of specificity of the drugs used. This point was investigated in a series of studies. SQ29,548 is a very selective and potent thromboxane A2/PGH2 antagonist in the family of compounds with the 7-oxabicyclo[2.2.1]heptane nucleus identified thus far. It does not alter the activities of thromboxane synthetase, cyclooxygenase, or adenylyl cyclase, and it does not possess thromboxane A2 agonist properties. SQ29,548 was more than 300,000 times more effective in inhibiting U46619-induced canine platelet aggregation than serotonin-induced aggregation. Ogletree et al. reported previously that SQ29,548 has no detectable influence on serotonin-induced contraction of rat aorta, but competitively inhibits U46619-induced contractions in aortic strips obtained from the rat.

The specificity of ketanserin in blocking both vascular and platelet 5HT2 receptors has been well documented. Although it also is known to possess α1-receptor antagonist properties, previous data from our laboratory have demonstrated that the administration of the selective α1-adrenergic antagonist prazosin does not diminish the frequency of CFVs in this canine preparation. This suggests that it is ketanserin’s SHT2 antagonistic properties and not α1-blockade that is responsible for eliminating CFVs. In the present study, we verified that ketanserin is a poor antagonist of the vascular or platelet thromboxane A2/PGH2 receptor. Ketanserin was more than 7000 times more effective in antagonizing serotonin-induced than U46619-induced aggregation of canine platelets. Furthermore, ketanserin is an excellent inhibitor of sero-
agonist binding to both 5HT₂ and thromboxane A₂/PGH₂ receptors to restore a normal flow pattern through the narrowed artery. The relative contributions of platelet and/or vascular receptor stimulation to the development of CFVs is unclear and will require further study.

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References
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ton-induced aortic smooth muscle contraction, but the results from this study show that is does not influence contractions of isolated rat aortic muscle induced by the thromboxane mimetic U46619.

Platelet aggregation and adherence of platelets to a vascular wall in vivo probably occur most commonly as the result of the presence of more than one aggregating agent. The amplying effect of serotonin on other mediators of platelet aggregation was first described by T. O’Brien and later by Baumgartner and Born. Serotonin potentiates ADP-, collagen-, and epinephrine-induced platelet aggregation in a synergistic manner, and this effect is blocked by ketanserin. The secretion of contents of the α-granules and arachidonic acid liberation from membrane phospholipids induced by collagen are also potentiated by serotonin, and this effect is inhibited by ketanserin. Serotonin also amplifies contractions induced by thromboxane A₂ in canine coronary arteries, and this potentiation may be blocked by ketanserin.

Agonist-receptor interaction on platelet membranes results in release and elevation of cytoplasmic Ca²⁺, which in turn initiates a series of reactions, including further release of Ca²⁺ from intracellular stores. A critical concentration of cytoplasmic Ca²⁺ may be necessary to initiate secretion from the α- and dense granules and activation of phospholipase A₂, which liberates arachidonic acid from the membrane. Serotonin can mobilize both intracellular and extracellular Ca²⁺ stores in vascular smooth muscle.

In summary, the results from this study show that occupation of both thromboxane A₂/PGH₂ and 5HT₂ receptors on the platelet and/or in the vascular wall may be necessary to provide sufficient platelet/vessel wall interaction to lead to CFVs in severely narrowed and endothelially injured canine coronary arteries. CFVs can be eliminated in 70% to 75% of dogs by pharmacologic blockade of either the thromboxane A₂/PGH₂ or the 5HT₂ receptor. Even though thromboxane A₂/PGH₂ receptor antagonists did not inhibit serotonin-induced platelet aggregation and the 5HT₂ antagonists did not inhibit U46619-induced aggregation in vitro, serotonin-induced CFVs were eliminated with a thromboxane A₂/PGH₂ receptor antagonist or thromboxane A₂ synthesis inhibitor. These results suggest that in a majority of dogs, stimulation of both thromboxane A₂/PGH₂ and 5HT₂ receptors is necessary to induce sufficient interaction to lead to CFVs and that receptor antagonism for either agonist is sufficient to interrupt CFVs. In a minority of dogs, stimulation of both receptor types causes CFVs and requires antagonism of
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