Local Platelet Activation Causes Vasoconstriction of Large Epicardial Canine Coronary Arteries In Vivo
Thromboxane A₂ and Serotonin are Possible Mediators

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The goal of the present study was to demonstrate that intracoronary platelet deposition may trigger intense vasoconstriction of large epicardial coronary arteries in vivo and that this is largely mediated by thromboxane A₂ and serotonin released by activated platelets. Cyclic flow variations (progressive declines in blood flow followed by sudden restorations of flow) due to recurrent intracoronary platelet activation and thrombus formation were induced by damaging the endothelium and placing a cylindrical constrictor on the left anterior descending coronary artery (LAD) in open-chest, anesthetized dogs. Coronary diameters were measured in vivo by means of ultrasonic crystals sutured on the LAD immediately distal to the constrictor (LAD1) and 1 cm below (LAD2) and on the circumflex coronary artery (Cx). Coronary artery diastolic diameters were measured continuously before and during cyclic flow variations and after they were abolished by administration of LY5387, a serotonin-receptor antagonist (group 1, n=7), or SQ29548, a thromboxane-receptor antagonist (group 2, n=7). During cyclic flow variations, at the nadir of coronary flow, LAD1 (a site of maximal platelet accumulation) cross-sectional area decreased by 52±10% and 38±6% in group 1 and 2 animals, respectively (p <0.001 compared with values recorded during a brief LAD occlusion obtained by a suture snare), whereas LAD2 (a site of minimal or no platelet accumulation) cross-sectional area did not differ from that recorded during the brief LAD occlusion. SQ29548 abolished cyclic flow variations in seven of seven dogs and LY5387 in six of seven, but they affected the increased coronary vasoconstriction differently: LAD1 cross-sectional area increased by 32±6% of the control value in SQ29548-treated animals, whereas it returned to baseline dimension values in the LY5387-treated group as these interventions also abolished the cyclic flow variations. We conclude that a marked coronary vasoconstriction may be triggered by local platelet deposition and that thromboxane A₂ and serotonin are mediators of this vasoconstriction. (Circulation 1989;79:154-166)

It is currently believed that the pathophysiology of unstable angina is a primary reduction in coronary blood flow. In particular, there is a growing body of evidence indicating that in most patients, unstable angina is the consequence of platelet aggregation or in situ thrombosis at sites of coronary artery narrowing and endothelial injury. Platelet deposition and aggregation may lead to intermittent coronary obstruction and contribute to the development of unstable angina. Studies by our group and more recently by others have demonstrated a temporal relation between increases in plasma transcardiac thromboxane concentration and the presence of active unstable angina, thus suggesting an association between intracoronary platelet activation and this syndrome. Folts et al, in an experimental canine model of concentric coronary artery stenosis and endothelial injury, have described the occurrence of spontaneous declines in coronary blood flow, which are interrupted by...
sudden restorations of flow. These alterations in coronary blood flow have been referred to as cyclic flow variations and are caused by transient formation of platelet thrombi at the site of the coronary stenosis and endothelial injury.\textsuperscript{14-17} Subsequent studies from our laboratory have demonstrated that serotonin (5HT) and thromboxane A\textsubscript{2} (TXA\textsubscript{2}) released from activated platelets are two important mediators of cyclic flow variations in this canine model\textsuperscript{15,17,18} and that the tissue concentration of 5HT and TXA\textsubscript{2} is markedly elevated in the coronary artery at the site of the stenosis.\textsuperscript{18,19}

The primary goal of this investigation was to test the hypothesis that platelet activation and deposition with the consequent release of vasoactive substances, such as 5HT and TXA\textsubscript{2} at sites of endothelial injury and coronary artery stenosis cause a marked vasoconstriction of the large epicardial coronary artery. A second goal of the present study was to demonstrate that 5HT and TXA\textsubscript{2} are the mediators primarily responsible for this coronary vasoconstriction. Accordingly, measurements of the left anterior descending coronary artery diastolic diameter were obtained in vivo by means of an ultrasonic dimension gauge before and during intracoronary platelet activation and after administration of 5HT\textsubscript{2} and TXA\textsubscript{2}-prostaglandin H\textsubscript{2} receptor antagonists.

**Materials and Methods**

**Surgical Preparation**

Nineteen mongrel dogs (30–45 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and ventilated with room air by a Harvard respirator. Heating pads were placed under the animal to maintain rectal temperature between 37° and 39° C. Aortic and venous catheters were inserted via the common carotid artery and external jugular vein, respectively. A left thoracotomy was performed at the fifth intercostal space, and the heart was suspended in a pericardial cradle. A segment of the left anterior descending (LAD) coronary artery was gently dissected from the surrounding tissue, and a pulsed Doppler flow probe was placed on it proximal to where a constrictor would subsequently be positioned. A 1-cm LAD segment immediately distal to the flow probe was then endothelially injured by gently squeezing the artery between a pair of rubber-covered forceps. One pair of miniature 7-MHz ultrasonic crystals (2×1 mm, 12 mg) (Triton Technology, San Diego, California) attached to a Dacron backing was sutured to the adventitia of opposing sides of the LAD (LAD1) with a 6-0 silk (Ethicon, Somerville, New Jersey). A second pair of ultrasonic crystals was sutured more distally (1–1.5 cm beyond the first one) on the LAD (LAD2), taking care not to damage the endothelium. A third pair of crystals was implanted on the circumflex coronary artery (Cx). Finally, a small polyethylene heparin-filled catheter was positioned in the distal end of a diagonal branch of the LAD below the site of constriction, enabling the pressure gradient across the stenosis to be measured (Figure 1).

**Experimental Protocol**

After all surgical procedures were completed, animals were allowed to stabilize for at least 30 minutes. Control hemodynamic measurements, including heart rate, systemic and coronary arterial pressure, and mean and phasic coronary blood flow velocity as well as external diameters of LAD1, LAD2, and Cx, were recorded continuously on a Hewlett-Packard (Model 7758) eight-channel recorder. A plastic cylindrical constrictor was placed around the LAD between the Doppler flow probe and the first pair of crystals (Figure 1) as previously described.\textsuperscript{15,17} This usually results in intracoronary platelet activation with cyclic formation of thrombi and occurrence of cyclic flow variations.\textsuperscript{14} Once cyclic flow variations occurred, they were observed for 30 minutes. During this period, cyclic flow variation frequency, phasic and mean coronary blood flow velocity, heart rate, and systemic and distal coronary blood pressures as well as LAD1, LAD2, and Cx diastolic diameters were recorded. Coronary arterial flow velocity and coronary diameters were compared with values for the same variables before constricting the LAD. To estimate the extent and the localization of platelet deposition in the coronary arteries with respect to the ultrasonic crystals, six SQ29548-treated dogs received an intravenous injection of autologous platelets labeled ex vivo with \textsuperscript{111}In (as described below) before cyclic flow variations developed.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A schematic representation of the instrumentation used in this study. A pair of ultrasonic crystals was sutured on the left anterior descending coronary artery (LAD) immediately distal to the plastic constrictor (LAD1) where platelet accumulation is known to be maximal. A second pair of crystals was sutured on the LAD 1–1.5 cm below the first one (LAD2), where little or no platelet accumulation occurs. Finally, a third pair of ultrasonic crystals was sutured on the circumflex coronary artery (CX) and served as a control. See text for details.}
\end{figure}
After cyclic flow variations were observed for 30 minutes, all of the animals received either LY53857 [(6-methyl-1-(1-methylethyl)ergolino-8-carboxylic acid 2-hydroxy-1-methyl-propylester-(Z)-2-butenedioate (1:1)], a selective 5HT3 receptor antagonist20,21 (Eli Lilly, Indianapolis, Indiana) (n = 7), or SQ29548 ([1S-[1α,2β(5Z),3β,4a]]-7-[3]-[2-[(phenylamino)carbonyl]hydrazinomethyl]-7-oxabicyclo[2.2.1]hept-2-yl]-heptenoic acid), a TXA2 receptor antagonist22 (E.R. Squibb & Son, Princeton, New Jersey) (group 2) (n = 7) to abolish them.23,24 The dose of SQ29548 and LY53857 required to abolish cyclic flow variations averaged 0.23±0.07 and 0.12±0.04 mg/kg, respectively. As described in “Results,” SQ29548 and LY53857 administration completely reversed the marked vasocostriction associated with cyclic flow variations.

To demonstrate that serotonin and TXA2 are specific mediators of the observed vasoconstriction, cyclic flow variations were evaluated in three additional dogs instrumented as described above. After 30 minutes of cyclic flow variations, dogs received an infusion of nitroglycerin (5 μg/kg/min) for 30 minutes. After discontinuing nitroglycerin and when hemodynamics returned to prenitroglycerin values, an infusion of diltiazem (15 μg/kg/min) for 30 minutes was started. After the diltiazem was discontinued, these animals received an intravenous bolus of SQ29548 to abolish cyclic flow variations as described above. Thirty minutes after abolition of cyclic flow variations, all of the animals were killed by an overdose of sodium pentobarbital, and the hearts were promptly excised. Thereafter, the LAD was dissected free from the surrounding tissue and divided into a segment proximal to the stenosis, a stenotic segment (portion of the LAD included in the plastic constrictor), and those two segments where the proximal (LAD1) and the distal pair of crystals (LAD2) were sutured. Similarly, the portion of the CX where the ultrasonic crystals were positioned as well as two other CX segments that were not manipulated during the experiment were obtained. All arterial segments were rinsed rapidly in saline and subsequently fixed in 10% buffered formalin for later histologic assessment of endothelial damage and platelet deposition. For comparative purposes, histologic sections were obtained also from two dogs that received no treatment and that were killed at the nadir of coronary blood flow. Arterial segments isolated from dogs in whom radiolabeled platelets were injected were also placed in a gamma counter to measure 111In activity.

**Measurement of Coronary Diameter In Vivo**

Coronary artery diameters were measured continuously with an ultrasonic dimension gauge (Model 120.2, Triton Technology, San Diego, California). The instrument generates a voltage linearly proportional to the transit time of acoustic impulses traveling at the sonic velocity of −1.5×106 mm/sec between the 7-MHz piezoelectric crystals, thus giving a record of instantaneous external coronary arterial diameter. The transit time technique has been applied to measurement of coronary artery dimensions by Vatner et al.,25 and it has been demonstrated to be accurate and reliable.25,31

While external diameter was measured continuously, the internal radius and the cross-sectional areas of the vessels at the site of crystal implantation were calculated as previously described.25,26

Changes in LAD and CX internal diameters and cross-sectional areas were related to values obtained before cyclic flow variations (control) but with the constrictor in place on the LAD. During cyclic flow variations, a marked decrease in distal LAT distending pressure occurs at the nadir of coronary blood flow (Figure 2). To distinguish the passive decrease in LAD diameter consequent to a reduction of coronary distending pressure from actual vasoconstriction due to activation of arterial smooth muscle cells, we compared coronary artery dimensions obtained during cyclic flow variations with those recorded during a 10-second occlusion of the LAD produced by a suture snare placed proximally to the Doppler flow probe. The latter measurement was made early in the experiments before the onset of cyclic flow variations but with the constrictor in place.

**Preparation of 111In-Labeled Platelets**

In six SQ29548-treated dogs, autologous platelets were isolated and labeled with 111In according to the method of Thakur et al32 before cyclic flow variations were established. After labeling, platelets were gently washed, resuspended in 5 ml autologous platelet poor plasma, and re injected into the dog.32 Labeling efficiency defined as

\[
\frac{111\text{In bound to platelets}}{111\text{In bound to platelets} + \text{unbound } 111\text{In activity}} \times 100
\]

was 80.5% (range, 61–90%). The viability of the platelets after radiolabeling was assessed in vivo by calculating the percentage of administered radioactivity bound to circulating platelets at different time intervals. Two, 4, 10, 30, and 60 minutes after the radiolabeled platelets were administered, a 1-ml blood sample was obtained from a peripheral vein. Platelets were isolated,32 and radioactivity was counted both in the platelets and in the platelet poor plasma. Only a small amount of radioactivity was found in platelet poor plasma (range, 3–7%). The percent recovery of radiolabel was calculated by standard methods.33 In addition, the responsiveness of radiolabeled platelets to various aggregating agents was tested in vitro according to the turbidimetric method of Born34 and compared with the values obtained before labeling. Aggregation was obtained by adding adenosine diphosphate to platelet rich plasma and 5HT to epinephrine-primed platelet rich plasma (10 μM).
**Figure 2.** Representative tracing of hemodynamic data and coronary artery diameters obtained from a single dog. Between the first and second panels, a constrictor was placed on the LAD. Please note that cyclic flow variations (CFVs) are accompanied by a marked decrease in LAD1 diameter, whereas changes in LAD2 diameter are less pronounced. Administration of SQ29548 completely abolished CFVs, eliminated the marked vasoconstriction at LAD1 occurring with CFVs, and dilated the LAD.

**Measurements of Vascular \( ^{111} \text{In} \) Platelet Accumulation**

The arterial segments were weighed and placed in a gamma spectrometer (Packard Autogamma) to determine the amount of \( ^{111} \text{In} \) radioactivity present in each piece of tissue. The accumulation of \( ^{111} \text{In} \)-labeled platelets is expressed as the increase in \( ^{111} \text{In} \) radioactivity in the LAD segments over \( ^{111} \text{In} \) radioactivity in the Cx segments, subsequently referred to as the intracoronary platelet accumulation ratio. The raw radioactivity counts of the LAD and Cx arterial segments were divided by the tissue wet weight to yield counts per minute per gram (cpm/g) tissue. By dividing the activity of the LAD segments (cpm/g) by the average activity in the unmanipulated Cx segments (cpm/g), the increase in \( ^{111} \text{In} \) radioactivity in the LAD segments was calculated, yielding the intracoronary platelet accumulation ratio.

**Histologic Methods**

All arterial segments were microscopically examined for vessel wall injury, leucocyte accumulation, and platelet aggregation. After fixation in 10% buffered formalin, tissues were embedded in methacrylate and sections were obtained and stained with hematoxylin-eosin. Platelet accumulation was semi-quantitatively evaluated by means of score from 0 to 4+ as follows: 0 = no platelets attached to the luminal surfaces; 1+ = few platelets adjacent to or attached to luminal surfaces; 2–3+ = larger platelet
Table 1. Hemodynamic Effects of LY53857 and SQ29548 in Dogs With Cyclic Flow Variations

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>AoM (mm Hg)</th>
<th>DCMP (mm Hg)</th>
<th>PHF (% control)</th>
<th>MNF (% control)</th>
<th>CFVs (cycles/hr)</th>
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<tr>
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<td>111±4</td>
<td>82±4†</td>
<td>94±12</td>
<td>98±13</td>
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</tbody>
</table>

Values are expressed as mean±SEM.

HR, heart rate; AoM, aortic mean pressure; DCMP, distal coronary mean pressure; PHF, phasic diastolic coronary blood velocity; MNF, mean diastolic coronary blood velocity; CFVs/hour, frequency of cyclic flow variations; CAO, brief mechanical occlusion of left anterior descending coronary artery.

* Nadir is defined as the lowest flow velocity recorded just before flow restoration.
† Value is significantly different from original control value, p<0.05.

aggregates; 4+=occlusive or nearly occlusive, platelet-rich thrombus. The extent of leukocyte accumulation in and on the vessel wall was also graded on a 1–4 scale. Vessel wall injury was evaluated as follows: 0=no endothelial damage; 1+=loss of endothelial cells, damage to internal elastic lamella, media intac; 2–4+=intimal damage plus degenerative changes in the media. Histologic examination and grading were performed without knowledge of the treatment group.

Platelet Aggregation Studies

Although LY53857 has been shown to possess no antagonist activity with respect to a variety of substances in vitro, no data are available regarding the interaction of LY53857 with TXA2 receptors. To exclude the possibility that LY53857 has important thromboxane receptor antagonistic properties, in vitro platelet aggregation was studied before and after the administration of LY53857 in three additional dogs at a dose of 0.1 mg/kg. Aggregation was induced in epinephrine-primed platelet rich plasma (10 μM) by a dose range (10, 25, 50, and 100 mg) of U46619, a TXA2 mimetic.

Statistical Analysis

Values are expressed as mean±SEM. Comparison between two means were made with Student’s t test for group observations. One-way analysis of variance was used for multiple comparisons between groups. For comparisons of hemodynamics and coronary artery dimensions between groups, a two-way analysis of variance for a design with repeated measures was used.

Results

Placement of the LAD constrictor reduced mean coronary blood velocity 78±4% of control and eliminated the hyperemic response to a total brief coronary occlusion. Among 20 dogs, cyclic flow variations were successfully produced in 19 animals. LY53857 abolished cyclic flow variations in six of seven group 1 and SQ29548 in seven of seven group 2 dogs. After 30 minutes of cyclic flow variations, five additional dogs received an intravenous infusion of nitroglycerin followed by an infusion of diltiazem before abolition of cyclic flow variations with SQ29548.

Hemodynamic Changes

Heart rates and aortic pressures did not change significantly during the study in both group 1 and 2 dogs (Table 1). More importantly, no changes in these two variables were observed after the administration of SQ29548 or LY53857. Distal coronary arterial pressures measured before the placement of the constrictors were consistently lower than aortic pressures in both groups. This difference can probably be attributed to the small size of the catheter used to cannulate the small side branch of the LADs. Table 2 summarizes the hemodynamic changes in the five dogs receiving nitroglycerin and diltiazem during cyclic flow variations.

Coronary Artery Dimension Changes

Representative waveforms for simultaneous coronary pressures, aortic pressures, phasic coronary blood velocity, mean coronary blood velocity, and coronary diameters (Cx, LAD1, and LAD2) before, during, and after abolition of cyclic flow variations are shown in Figure 2.
The brief mechanical coronary artery occlusion caused a decrease in LAD1 cross-sectional area of 24±7% and 14±3% of the control values in group 1 and 2 dogs, respectively (p=NS). Likewise, LAD2 cross-sectional areas decreased by 18±6% and 16±3% as compared with control values in the same groups of animals. During cyclic flow variations at nadir flow, LAD1 cross-sectional area decreased by 52±10% and 38±6% from control values in group 1 and 2 animals, respectively. These values were both significantly different from those obtained during the brief mechanical occlusion (Figure 3). LAD2 cross-sectional area at the nadir of coronary flow during cyclic flow variations was not different from the values recorded during the brief mechanical occlusion (Figure 3). Both LY53857 and SQ29548 abolished cyclic flow variations. However, they affected LAD dimensions differently: LAD1 cross-sectional area increased by 32±6% of the control value in the SQ29548-treated group as cyclic flow variations were abolished, whereas in the LY53857-treated dogs, it returned to control dimensions as the cyclic flow variations were abolished (Figure 3). The same pattern was shown by LAD2 in both groups (Figure 3). Cx dimensions did not change during the study in animals in both groups (Figure 3).

In five additional dogs, cyclic flow variations were observed for 30 minutes. Again, a marked vasoconstriction was observed in the LAD at the site of placement of the first pair of crystals (LAD1) at the nadir of coronary blood flow as compared with a brief

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**Table 2. Hemodynamic Effects of Nitroglycerin and Diltiazem in Dogs With Cyclic Flow Variations**

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>AoM (mm Hg)</th>
<th>DCMP (mm Hg)</th>
<th>PHF (% control)</th>
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Values are expressed as mean±SEM.

HR, heart rate; AoM, aortic mean pressure; DCMP, distal coronary mean pressure; PHF, phasic diastolic coronary blood velocity; MNF, mean diastolic coronary blood velocity; CFVs/hour, frequency of cyclic flow variations; CAO, brief mechanical occlusion of left anterior descending coronary artery.

*Nadir is defined as the lowest flow velocity recorded just before flow restoration.

†Value is significantly different from original control value, p<0.05.

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**Figure 3. Plots of changes in coronary diastolic cross-sectional area in dogs with cyclic flow variations (CFVs).** A marked reduction in cross-sectional area was observed in the left anterior descending coronary artery immediately distal to the plastic constrictor (LAD1). This value was significantly different from that obtained during a brief mechanical occlusion (CAO) of the LAD. However, at a more distal site (LAD2), no significant vasoconstriction was observed. The circumflex coronary artery (CX) did not show significant changes throughout the study. Left panel: LY53857-treated dogs. Right panel: SQ29548-treated dogs. Bars represented ±1 SEM; *p<0.05 vs. control; #p<0.05 vs. CAO.
mechanical LAD occlusion (LAD1 cross-sectional area decreased by 38±4% during cyclic flow variations as compared with 15±4% during the brief mechanical coronary occlusion, p<0.001). Intravenous infusion of 5 µg/kg/min nitroglycerin decreased mean blood pressure by about 20%, indicating that the drug exerted significant hemodynamic effects. Nitroglycerin tended to reduce LAD1 vasoconstriction during cyclic flow variations (LAD1 cross-sectional area decreased by 33±4% during nitroglycerin as compared with 38±4% during the control cyclic flow variations), but this difference was not statistically significant. LAD1 cross-sectional area during diltiazem infusion decreased by 35±4%, a value not different from that recorded during the first 30 minutes of cyclic flow variations. LAD2 cross-sectional area during cyclic flow variations did not differ significantly from the values obtained during the brief mechanical LAD occlusion (14±6% compared with 13±4%). Nitroglycerin and diltiazem infusion did not change LAD2 cross-sectional area during cyclic flow variations. Cx cross-sectional area did not change throughout the study. SQ29548 administered at the end of the diltiazem infusion was effective in each of the five dogs in abolishing cyclic flow variations and again caused a dilatation of the LAD (18±6%). Figure 4 shows a representative tracing from one of the dogs in this protocol.

**III-In-Labeled Platelet and Histologic Findings**

To verify that platelet viability was not altered during labeling, two tests were performed. The in vivo method assumes that III-In-labeled platelets injured during the labeling process are sequestered by the spleen and liver within 30 minutes of injection into the circulation system. In these experiments, an average of 69% of total administered III-In activity was present in the circulation after 30 minutes. Furthermore, in vitro platelet aggregation was not substantially modified by III-In labeling: the responsiveness of radiolabeled platelets to adenosine diphosphate and 5HT (in epinephrine-primed platelets) was similar to that recorded before labeling. These data indicate that the ex vivo labeling did not substantially alter the normal platelet function. Quantitative assessment of intracoronary platelet accumulation was obtained by calculating the increase in radioactivity present in the various LAD segments compared with the mean radioactivity present in the Cx segments, which were not surgically manipulated (i.e., the intracoronary platelet accumulation ratio).

In the present study, intracoronary platelet accumulation ratio after cyclic flow variations were abolished with SQ29548 averaged 57±9 in LAD segments immediately distal to the stenosis (LAD1), indicating that a marked platelet deposition occurred in those LAD arterial segments during cyclic flow alterations (Figure 6). On the other hand, only trivial platelet accumulation was observed in the circumflex artery at the site of placement of the ultrasonic crystals and in the LAD2, where the second pair of crystals was sutured (Figure 5), suggesting that important platelet deposition did not occur in those coronary arterial segments during cyclic flow variations as a result of vessel manipulation.

Microscopic analyses confirmed these findings: the highest histologic score for platelet deposition was associated with the highest III-In activity (Table 3).

**Figure 4.** Plot of changes in coronary diastolic cross-sectional area in a representative dog receiving nitroglycerin (5 µg/kg/min) and diltiazem (15 µg/kg/min) during cyclic flow variations (CFVs). Note that nitroglycerin and diltiazem, despite a significant hemodynamic effect, only slightly reduced the marked LAD1 vasoconstriction associated with CFVs (nitroglycerin, nadir) as compared with control CFVs (nadir). CAO, brief occlusion of left anterior descending coronary artery. See text for details.

**Figure 5.** Plot of systemic blood III-In counts as a function of time after injection of labeled platelets in dogs with cyclic flow variations. III-In counts are normalized with respect to their values at 2 minutes. Bars represent ±1 SEM.
Stenotic segments and segments just distal to those sites were characterized by loss of endothelium, degenerative changes in the media with focal infiltration by leucocytes (predominantly neutrophils) and mild-to-moderate deposition of platelets on the luminal surface without luminal obstruction (Figure 7). Conversely, in the distal LAD (LAD2) and Cx segments, no or mild endothelial damage and no or very few platelets were evident by histologic analysis (Figure 8). In the two dogs that did not receive pharmacologic intervention and were killed at the nadir of coronary blood flow, stenotic and immediately distal segments showed marked platelet and leucocyte accumulation and luminal obstruction (Figure 9).

Table 3 correlates the histologic findings with the $^{111}$In-labeled platelet accumulation in six dogs receiving SQ29548.

**Platelet Aggregation Studies**

To determine whether LY53857 has also a TXA$_2$ receptor antagonist activity, U46619-induced aggregation was allowed in epinephrine-primed canine platelets obtained before and after administration of LY53857 ($n=3$) in vivo. Epinephrine was added to platelet rich plasma to prime platelets in all experiments because U46619 alone had a modest effect on aggregation (i.e., approximately 3%). Epinephrine (10 $\mu$M) alone had no effect on aggregation. There was no difference in U46619-induced aggregation (measured as percentage of maximal transmission) before and after administration of LY53857 for concentrations of U46619 up to 100 mg.

**Discussion**

In this experimental preparation, endothelial damage associated with coronary arterial constriction usually results in a cyclic pattern of reduction and restoration of flow that is caused by alternating platelet aggregation and subsequent dislodgement.$^{14-18}$ It has been demonstrated that when platelets aggregate they release several substances, including serotonin and TXA$_2$, which are known to cause vasoconstriction and to further promote aggregation.$^{35-38}$ Ashton et al.$^{18}$ in the same animal preparation used in this study, have demonstrated that serotonin concentrations measured in the area of the LAD stenosis are 18-fold to 27-fold higher than those found in the LAD proximal to the stenosis and in the unmanipulated circumflex artery. Similarly, Schmitz et al.$^{19}$ reported that, at the site of stenosis, TXA$_2$ concentration is elevated as compared with the normal Cx. Furthermore, it has been demonstrated that the endothelium plays an important role in modulating the vessel response to various vasoactive substances.$^{30,40,41}$ These experimental observations led us to formulate and test the hypothesis that during intracoronary platelet activation, the increased concentration of vasoactive substances, such as serotonin and TXA$_2$ at the site of coronary stenosis, may cause increased vasoconstriction, especially in those vascular segments where endothelial injury has occurred.

The results of the present study clearly demonstrate that in this experimental model, intracoronary platelet deposition may trigger a marked local vasoconstriction of large epicardial coronary arteries. This vasoconstriction is maximal immediately distal to the stenosis, where we have shown platelet accumulation to be increased.$^{35}$ Preliminary results from our laboratory indicate that intracoronary platelet deposition in the same animal preparation used in the present study is maximal at the site of and immediately distal to the stenosis. In dogs that

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LAD1, left anterior descending coronary artery segments immediately distal to the stenosis, site of placement of the first pair of ultrasonic crystals; LAD2, left anterior descending coronary artery segments, 1-1.5 cm distal to the stenosis, site of placement of the second pair of ultrasonic crystals. (See text for details.)
received no treatment and whose coronary arterial segments were taken at the nadir of the coronary flow, intracoronary platelet accumulation ratio was $556 \pm 131$ at the site of the stenosis and $1,486 \pm 169$ in the segment immediately distal to it.\textsuperscript{35} Administration of a TXA$_2$ receptor antagonist resulted in ratios of $40 \pm 12$ and $89 \pm 23$ in the stenotic and distal segments, respectively, indicating a significant reduction but not a complete elimination of intracoronary platelet accumulation where previously maximal deposition occurred.\textsuperscript{35} The reduction in LAD cross-sectional area at the site of placement of the first pair of crystals (LAD1) was significantly more pronounced at the nadir of coronary flow than that observed during a brief, mechanical occlusion of the LAD, thus excluding the possibility that the decreased cross-sectional area during cyclic flow variations was merely the consequence of a reduction in coronary perfusion pressure.

In this study, substantial platelet deposition on the arterial wall appeared to be necessary to elicit significant vasoconstriction. A significant vasoconstriction was observed only at LAD1, where platelet accumulation was marked during cyclic flow variations. Nitroglycerin significantly reduced blood pressure and tended to decrease the LAD1 vasoconstriction associated with cyclic flow variations. This change, however, was not statistically significant. Diltiazem did not seem to affect LAD tone during cyclic flow variations. It is possible that
failure of diltiazem to reduce the vasoconstrictor responses occurring during cyclic flow variations may be due to administration of too low a dose. In the same dogs, however, the TXA\textsubscript{2} receptor antagonist, SQ29548, induced a marked increase in LAD cross-sectional area, thus supporting the hypothesis that TXA\textsubscript{2} is a specific mediator of the observed increase in coronary tone. Our additional experiments using the serotonin S\textsubscript{2} receptor antagonist, LY53857, and the TXA\textsubscript{2} receptor antagonist, SQ29548, also provide support for the concept that serotonin and thromboxane are mediators of the increased coronary vasoconstriction in this experimental model. Furthermore, the possibility that the two antagonists used in the present study are not specific for their respective receptors can be excluded. In fact, in human platelets and in guinea pig trachea and rat aorta, SQ29548 antagonized responses to U46619 (a TXA\textsubscript{2} mimetic) but not to a wide range of other agonists (including serotonin).\textsuperscript{22} In another study, SQ29548 has been shown to inhibit U46619-induced aggregation in canine platelets with an IC\textsubscript{50} of 3.2 mM, whereas, it inhibited serotonin-induced aggregation in epinephrine-primed canine platelets with an IC\textsubscript{50} of 1,000 \textmu M. Thus, SQ29548 is approximately 300,000 times more active as a TXA\textsubscript{2} receptor antagonist than as a serotonin antagonist. Similarly, LY53857 has been shown to possess no antagonist activity with respect to a variety of substances in vitro.\textsuperscript{21} The additional experiments performed in the present study on U46619-induced aggregation of canine platelets obtained before and after administration LY53857 in vivo demonstrate that this agent does not block the TXA\textsubscript{2} receptor, and it can be considered a potent and specific 5HT\textsubscript{2} receptor antagonist.

To our knowledge, the present study is the first to describe the occurrence of a marked, focal vasoconstriction of a large epicardial coronary artery related to a platelet deposition in an in vivo preparation. Previous studies have demonstrated the importance of serotonin and TXA\textsubscript{2} in inducing vasoconstriction in isolated coronary arteries\textsuperscript{37,41,42} or in vivo preparations.\textsuperscript{30} Other studies have demonstrated that aggregating platelets cause vasoconstriction of isolated canine pulmonary arteries\textsuperscript{43} or isolated canine coronary arteries.\textsuperscript{44} Furthermore, Lam et al.\textsuperscript{45} in a recent report, have described the occurrence of vasoconstriction at sites of platelet deposition in damaged porcine carotid arteries after balloon angioplasty. In that study, the extent of vasoconstriction was roughly correlated with the intensity of platelet deposition.\textsuperscript{45} An additional original contribution provided by the present study is that serotonin and TXA\textsubscript{2}, besides their importance...
in activating and sustaining cyclic flow variations, also appear to be important mediators of the focal coronary vasoconstriction in vivo in this experimental model.

In the present study, both LY53857 and SQ29548 were shown to be very effective in abolishing cyclic flow variations. This is in agreement with preliminary results\(^{46,47}\) and a recent study from our laboratory\(^{23}\) in which we have shown that both LY53857, a specific and potent 5HT\(_2\) receptor antagonist, and SQ29548, a potent TXA\(_2\)-prostaglandin H\(_2\) receptor antagonist, are effective in abolishing cyclic flow variations. However, they differently affected LAD dimensions: abolition of cyclic flow variations by SQ29548 administration was associated with a marked and prolonged dilatation of the LAD at both sites of placement of the ultrasonic crystals. The reason why SQ29548 dilated LAD\(_1\) and LAD\(_2\) is not clear, but it could be related to a relative predominance of an endogenous vasodilator after the blockade of TXA\(_2\) receptors. LY53857, in contrast, did not have any vasodilator effect on coronary vascular dimensions in stenosed LAD and normal circumflex arteries, but it did eliminate the increased vasoconstriction occurring at LAD1 during the cyclic flow variations.

It is currently believed that the primary mechanism responsible for the development of unstable angina is a reduction of myocardial oxygen supply rather than an increase in myocardial oxygen demand.\(^{1-10}\) In particular, angiographic and angioscopic evidence suggest that plaque ulceration and coronary thrombosis are frequent findings in patients with unstable angina.\(^{7-9}\) In addition, metabolic studies have suggested that periodic platelet activation occurs in patients with unstable angina and that this may eventually lead to coronary occlusion by either formation of a thrombus or the release of platelet-derived vasoactive substances or both, which may in turn cause marked coronary vasoconstriction.\(^{7-10}\) In so far as data obtained in experimental preparations can be extrapolated to humans, the present observations suggest that intracoronary platelet deposition at a site of plaque ulceration may, indeed, cause vasoconstriction of a large epicardial coronary artery. Such vasoconstriction might be extremely pronounced in patients with unstable angina because atherosclerotic coronary arteries have been shown to be hypersensi-
tive to different vasoconstrictor substances. Additionally, from a therapeutic point of view, the administration of a TXA2—prostaglandin H2 receptor antagonist seems to possess the additional and very useful property of dilating the stenosed vessel. This effect could be potentially very desirable in the clinical setting.

In conclusion, our results demonstrate that activated platelets may cause increased coronary vasoconstriction in vivo at sites of coronary stenosis and endothelial injury as the result of their release of serotonin and TXA2.

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References


KEY WORDS • coronary artery vasoconstriction • serotonin • thromboxane A2
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