ADP Plays an Important Role in Mediating Platelet Aggregation and Cyclic Flow Variations In Vivo in Stenosed and Endothelium-Injured Canine Coronary Arteries

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The goal of this study was to test the hypotheses that endogenous ADP plays an important role in vivo in mediating platelet aggregation and cyclic coronary artery blood flow variations (CFVs) in stenosed and endothelium-injured coronary arteries in an experimental canine model. Anesthetized animals were studied and coronary blood flow velocities monitored by a pulsed Doppler flow probe positioned around the left anterior descending coronary artery. CFVs were established by an external constrictor positioned at sites with injured endothelium. Apyrase, an ADP-removing enzyme, was infused into the left anterior descending coronary artery (0.3–1.8 units/min) 30 minutes or 2 hours after the establishment of CFVs. Complete abolition of CFVs was achieved in 81% (13/16) of dogs with 30-minute CFVs and in 83% (five of six) of dogs with 2-hour CFVs. In other dogs, a potent inhibitor of ADP-induced platelet aggregation, clopidogrel, was administered as a 10 mg/kg i.v. bolus and a 2.5 mg/kg/hr infusion 30 minutes and 3 hours after the establishment of CFVs. This treatment resulted in complete abolition of CFVs in 14 dogs (100%) with either 30-minute or 3-hour CFVs. Epinephrine was infused into some dogs after CFVs had ceased as a result of either apyrase or clopidogrel administration and into some dogs in whom SQ29548, a thromboxane A₂ receptor antagonist, had been given when apyrase failed to abolish CFVs. Epinephrine restored CFVs in all dogs treated with apyrase alone, 67% (four of six) of dogs treated with the combination of apyrase and SQ29548, and 29% (two of seven) of dogs treated with clopidogrel. The plasma epinephrine levels required for CFV restoration were 20 times higher than baseline values in dogs receiving apyrase alone, 100 times higher when a combination of apyrase and SQ29548 had been given, and more than 5,000 times higher in dogs receiving clopidogrel. In vitro studies showed that apyrase only inhibited ADP-induced platelet aggregation, whereas clopidogrel not only inhibited ADP-induced platelet aggregation, but also reduced platelet aggregation induced by the thromboxane mimetic U46619 and serotonin. These data suggest that 1) ADP is an important mediator of platelet aggregation and CFVs in vivo and 2) combined inhibition of thromboxane A₂ and ADP’s effects provides marked protection against CFVs in experimentally stenosed and endothelium-injured canine coronary arteries. These data and our previous observations are consistent with the possibility that specific antagonists of thromboxane A₂, serotonin, and ADP, alone and together, may provide substantial protection against platelet aggregation leading to CFVs at sites of endothelial injury and coronary artery stenosis. (Circulation Research 1992;70:39–48)
Platelet aggregation in atherosclerotic coronary arteries and the release of platelet-derived factors may play an important role in the development of acute coronary artery disease syndromes. \(^1\) Blockade of the synthesis of thromboxane and inhibition of platelet aggregation by aspirin have been shown to be effective in preventing acute myocardial infarction and death in some patients with unstable angina and also in some healthy persons. \(^6,7\) Experimental studies from this laboratory and others have also shown that receptor antagonists for thromboxane \(A_2\) and/or serotonin have potential usefulness in preventing the conversion from chronic stable to unstable coronary disease syndromes. \(^8\)

ADP is present in the dense granules of platelets, and it has been shown to be important in mediating platelet aggregation in previous in vivo studies. \(^9\) \(^14\) Some studies have shown ADP’s effect in mediating platelet aggregation in vitro. \(^13\)

This study was designed to test the hypothesis that ADP is an in vivo mediator of cyclic coronary artery blood flow variations (CFVs) in a canine model with coronary artery stenosis and endothelial injury and should be added to a list of such mediators that already include thromboxane \(A_2\) and serotonin. \(^1\) \(2\) \(3\) An animal model, initially described by Fols et al. \(^15\) \(16\) and characterized by the development of CFVs in stenosed and endothelium-injured arteries, was used to test our hypothesis. An ADP-removing enzyme, apyrase, acting through dephosphorylation of ADP, \(^17\) was administered to deplete ADP in the coronary arterial circulation. Another potent inhibitor of ADP-induced platelet aggregation, clopidogrel, \(^18\) \(21\) was also given to some of the animals.

**Materials and Methods**

**Surgical Preparation**

Thirty mongrel dogs of either sex (25–31 kg) were anesthetized with sodium pentobarbital (30 mg/kg) and ventilated with a mechanical respirator (model 613, Harvard Apparatus, South Natick, Mass.). Plastic catheters were placed into a carotid artery for aortic pressure monitoring and jugular veins for drug and fluid administration. A left fifth intercostal space thoracotomy was performed, and the heart was suspended in a pericardial cradle. A 1–2-cm segment of left anterior descending coronary artery (LAD) was carefully isolated and nearby branches ligated. An ultrasonic Doppler flow probe (Hartley Instruments, Houston, Tex.) was placed around the proximal portion of the isolated segment of the LAD to measure coronary blood flow. In some experiments, a plastic catheter was inserted retrograde into a small branch proximal to the isolated LAD segment for apyrase administration. Another plastic catheter was inserted into a small branch distal to the exposed LAD segment for blood sample collection. Hemodynamics, including heart rates, systolic and diastolic aortic pressures, and phasic and mean coronary blood flow velocities, were continuously recorded on an eight-channel recorder (model 3000, Gould Inc., Cleveland, Ohio).

CFVs were induced by gently squeezing the LAD with cushioned forceps to damage the endothelium and placing a plastic constrictor around the injured portion of the artery. Then, dogs were divided into two groups.

**Group 1.** Sixteen dogs were treated with apyrase (grade V, ATPase 4.1 units/mg, ADPase 3.8 units/mg; Sigma Chemical Co., St. Louis, Mo.). The drug was delivered into the coronary circulation directly through a catheter positioned in the LAD proximal to the stenosed and endothelium-injured area. The initial dose of apyrase was 0.3 units/min, and it was step-increased to 0.6, 1.2, and 1.8 units/min if CFVs were not completely abolished. After complete abolition of CFVs, the infusion of apyrase was discontinued in some of the dogs at 30 minutes, and saline was infused to determine whether CFVs might recur with time. If CFVs recurred, they were followed for 2 hours and apyrase was infused again in the same manner to determine the correlation between the duration of CFVs and the dosage of apyrase required to abolish them. For some of the dogs (group 1b), apyrase infusion was continued and epinephrine was infused intravenously at concentrations of 1.2–17 \(\mu\)g/min, attempting to restore CFVs. Thirty minutes after the restoration of CFVs by epinephrine, apyrase infusion was increased to test its effect on epinephrine-restored CFVs. In some studies, SQ29548, a thromboxane \(A_2\) receptor antagonist (E.R. Squibb & Sons, Inc., Princeton, N.J.), was given as a bolus of 0.2–0.6 mg/kg i.v. and as a sustained infusion of 0.1 mg/kg/hr to dogs in whom apyrase failed to abolish either the initial or the epinephrine-restored CFVs. After SQ29548 abolished CFVs, epinephrine infusion was given attempting to restore CFVs. The dosage of epinephrine required to restore apyrase-abolished or apyrase and SQ29548–abolished CFVs was recorded.

**Group 2.** Fourteen dogs were treated with clopidogrel (Sanofi Recherche, Toulouse, France) intravenously. Clopidogrel is a methyl-5-(2-chlorophenol)-(4,5,6,7-tetrahydrothieno[3,2-C]pyrid-5-yl)-acetate hydrogenosulfate. Seven dogs (group 2a) were treated 30 minutes after CFVs were established. Clopidogrel was given as a 10 mg/kg i.v. bolus and 2.5 mg/kg/hr continuous infusion. If CFVs were abolished, dogs were monitored for 30 additional minutes to ensure sustained protection. Then, a continuous intravenous infusion of epinephrine was started and step-increased to restore CFVs as described above. Coronary arterial tissues were removed at the end of each study from LAD segments with stenoses, LAD segments proximal and distal to the stenoses, and the left circumflex coronary arteries for serotonin measurements. Tissues were frozen in liquid nitrogen and stored at −85°C until analyzed. An immunoenzymatic assay developed by Benedict et al. \(^25\) \(24\) was used for serotonin measurements.
Seven additional dogs (group 2b) were treated with intravenous clopidogrel 3 hours after CFVs were established at the same concentrations used in the group 2a dogs. When CFVs were abolished, animals were monitored for 30 more minutes to ensure their abolition. Then, coronary arterial tissues were removed for serotonin measurements as described above. Blood samples for the measurement of thromboxane B₂ (the stable metabolite of thromboxane A₂) were collected from catheters positioned in the LAD distal to the stenoses before the establishment of CFVs, after 3 hours of CFVs, and 15 minutes after their abolition. A modified radioimmunoassay developed by Campbell et al.⁵ was used for the measurement of thromboxane B₂.

**Plasma Epinephrine Measurements**

Measurements of plasma epinephrine concentrations were performed before induction of CFVs and 30 minutes after CFVs were restored by epinephrine infusion or 30 minutes after the highest dosage of epinephrine was given if CFVs were not restored in group 1b and 2a dogs. Blood samples were obtained from a catheter placed in a carotid artery and collected in iced and heparinized tubes. Plasma was obtained after the samples were centrifuged at 2,000g for 20 minutes at 4°C and stored at −85°C until analyzed. All measurements were performed by high-pressure liquid chromatography at Smith-Kline Biotechnology Laboratories, Van Nuys, Calif.

**Platelet Aggregation Studies**

The effects of apyrase and clopidogrel on platelet aggregation were evaluated. Blood samples were collected into plastic tubes containing 3.8% sodium citrate (9 vol blood:1 vol sodium citrate). Platelet-rich plasma was obtained by centrifuging whole blood at 200g for 20 minutes at 37°C. Platelet-poor plasma was obtained after centrifugation of platelet-rich plasma at 2,000g for 10 minutes. The platelet counts in the platelet-rich plasma were adjusted to 300,000/ml. A modified method of Born for the platelet aggregation studies and a dual-channel aggregometer (Chrono-Log Instruments, Haverton, Pa.) were used. The agonists used in this study were ADP (Sigma) at a final concentration of 5–40 μM; U46619, a thromboxane A₂ mimetic (Cayman Chemical Co., Ann Arbor, Mich.) at 0.1–1.0 μg/ml; serotonin (Sigma) at 0.5–4 μg/ml; and collagen (Horman-Chemie, FRG) at 1–10 μg/ml. Platelet aggregation was reported as a percent of maximal increase of light transmission as compared with values in the platelet-poor plasma samples.

**Measurements of ADP Before and After Apyrase**

ADP concentration in the coronary circulation was estimated in six dogs by a modified luminescence method originally described by Holmsen et al.²⁶ The principle of the method is 1) using phosphoenolpyruvate–pyruvate kinase to convert ADP to ATP-activating firefly luciferase and 2) detecting the luminescence with light-measuring equipment (Chrono-Log dual-channel platelet aggregometer and luminescence detector). Blood samples were collected from a plastic catheter positioned in a branch of the LAD distal to the site of stenosis into ice-cooled vacuum tubes containing an anticoagulant (EDTA) at a final concentration of 5 mM. A 1.5-ml sample of plasma was obtained by centrifugation of the blood at 10,000g for 10 minutes immediately after the collection. Plasma was extracted with equal amounts of 96% ethanol immediately and then stored at 4°C. ADP standards were prepared in the same manner. Each 1 ml extracted plasma or ADP standard was mixed with 3 ml phosphoenolpyruvate–pyruvate kinase mixture or inactivated phosphoenolpyruvate–pyruvate kinase mixture (by heating at 80°C for 6 minutes) and kept at 4°C for 10 minutes. The initial light intensity was measured after injection of 50 μl chronolum reagent into 450 μl extracted plasma or ADP standard and phosphoenolpyruvate–pyruvate kinase or inactivated phosphoenolpyruvate–pyruvate kinase mixture. An ADP standard curve was obtained by detecting four or five different concentrations of ADP standards. The minimal amount of ADP detectable was 10 mM. ADP concentration in each sample was obtained by subtracting the initial light intensity of the plasma with inactivated phosphoenolpyruvate–pyruvate kinase solution from that of the same plasma with phosphoenolpyruvate–pyruvate kinase solution and comparing it with the ADP standard curve.

**Coagulation Studies**

Activated whole-blood coagulation times and partial thromboplastin times were measured before and 15 minutes after the administration of clopidogrel in group 2 dogs. An automated blood coagulation timing device (Hemochron 801, International Technidyne Corp., Edison, N.J.) was used for the measurements.

**Statistical Analyses**

All values were expressed as mean±SEM. A one-way analysis of variance with repeated measures was used for the comparisons of plasma epinephrine concentrations and hemodynamic values obtained at different time periods in these studies. Thromboxane A₂ and serotonin accumulation within the coronary arteries, platelet aggregation, and values for activated whole-blood coagulation and partial thromboplastin times measured before and after treatments were compared by paired Student’s t tests. Restoration rates of CFVs in dogs receiving different treatments were compared by Fisher’s exact test. A value of p<0.05 was considered significant.

**Results**

After the endothelial injury and placement of constrictors around the coronary arteries, CFVs developed in 30 dogs. Average phasic coronary flow velocity reductions to 58–70% of baseline and mean coronary flow velocity reductions to 59–86% of baseline were observed (Table 1). The frequency of CFVs was approximately 5 cycles/30 minutes. The severity
of CFVs, as indicated by the nadir flow of CFVs, was similar for the two groups of animals (Table 1).

**ADP Concentrations in the Canine LAD**

Plasma ADP concentrations increased in the canine LAD with 30 minutes of CFVs and increased further with 2 hours of CFVs (Figure 1). Apyrase administration reduced ADP concentrations to values comparable to those found before the development of CFVs (Figure 1).

**Effects of Apyrase and Clopidogrel on Cyclic Coronary Blood Flow Variations**

The direct administration of apyrase into LADs abolished CFVs in 13 of 16 dogs (81%) with 30 minutes of CFVs. The average dosage of apyrase required to abolish the 30-minute CFVs was 0.4±0.04 units/min (range, 0.3–0.6 units/min). A four times higher dose of apyrase (1.8 units/min) was given to each of the remaining three dogs in whom CFVs continued despite apyrase treatment. Addition of SQ29548, a thromboxane A₂ receptor antagonist, abolished CFVs in these three dogs. In six of the dogs (group 1a) with CFVs abolished by apyrase, saline (1.2–2.4 ml/hr) was infused into the LAD instead of apyrase 30 minutes after CFVs were abolished. Six of the dogs had CFVs restored at an average time of 30 minutes after discontinuation of the apyrase infusion. After 2 hours of consistent CFVs occurring during saline infusion, apyrase was given again by intracoronary infusion. Five of the six dogs (83%) had CFVs abolished once again. However, the dosage of apyrase required to abolish CFVs of 2-hour durations was significantly higher than required for the CFVs of 30-minute duration (1.32±0.12 units/min, p=0.001, Figure 2).

Systemic administration of clopidogrel eliminated CFVs in another 14 dogs (100%) with both 30-minute (group 2a) and 3-hour CFVs (group 2b).

**Epinephrine- Restored Cyclic Coronary Blood Flow Variations**

Seven of the apyrase-treated dogs (group 1b) received continuous intravenous infusions of epineph-
and abolished all of their CFVs at an average dose of 0.4±0.05 mg/kg bolus and 0.2 mg/kg/hr infusion.

The epinephrine infusion speed was increased again in three dogs after SQ29548 abolished the epinephrine-restored CFVs. In the other three dogs that combined administration of apyrase and SQ29548 was needed for the elimination of CFVs, an epinephrine infusion was also given. Four of these six dogs had their CFVs restored by the infusion of epinephrine. The epinephrine dosage required to restore CFVs in these four dogs was 0.6±0.05 μg/kg/min, and it was significantly higher than needed for the restoration of CFVs abolished by apyrase alone (p=0.02, Figure 3). These data indicate that combined use of apyrase with SQ29548 may provide more protection against the development of CFVs than apyrase alone.

Seven dogs treated with clopidogrel in group 2a received continuous infusions of epinephrine after the abolition of CFVs. Five dogs (71%) did not have CFVs restored by epinephrine infusions. The average dose of epinephrine given to these dogs was 1.3±0.04 μg/kg/min. The two dogs with CFVs restored received epinephrine at 1.3 and 1.4 μg/kg/min, which were greater concentrations of epinephrine than were required to restore CFVs abolished by the combination of apyrase and SQ29548.

**Plasma Epinephrine Concentrations**

The administration of epinephrine significantly increased plasma epinephrine levels. In dogs treated with apyrase, plasma epinephrine concentrations increased from 0.1±0.02 to 2.3±0.5 ng/ml after the restoration of apyrase-abolished CFVs and to 12±3.7 ng/ml after the restoration of CFVs abolished by apyrase and SQ29548 (p<0.05, Figure 4). In dogs treated with clopidogrel, plasma epinephrine increased from 0.1±0.02 to 47 and 54 ng/ml in the two dogs with CFVs restored and to 41±5.6 ng/ml in the dogs in whom CFVs were not restored. These data suggest strong protection against epinephrine-
restored CFVs provided by clopidogrel and the combination of apyrase with SQ29548.

**Thromboxane A$_2$ Production**

Plasma thromboxane B$_2$ concentrations in the LADs distal to the stenoses increased to three times baseline values after 3 hours of CFVs (205±109 compared with 66±25 pg/ml, p>0.05). Fifteen minutes after the abolition of CFVs by clopidogrel, thromboxane B$_2$ concentrations were still increased at 240±132 pg/ml (compared with baseline, p>0.05). These data demonstrate that thromboxane A$_2$ production during CFVs was not importantly reduced by clopidogrel.

**Accumulation of Serotonin**

Serotonin concentrations in the coronary arteries were measured after clopidogrel and epinephrine in group 2a dogs and clopidogrel alone in group 2b dogs. There were no significant differences in serotonin concentrations in coronary arteries between dogs treated with clopidogrel and epinephrine and clopidogrel alone. However, the LAD segments with stenoses and endothelial injury had 40-fold higher serotonin concentrations in group 2a animals and 58-fold higher in those in group 2b than values in the left circumflex coronary arteries (198±95 versus 5±1 ng/g and 231±114 versus 4±1 ng/g, respectively). The LAD segments proximal and distal to the stenoses also had higher serotonin concentrations than the circumflex coronary arteries, but lower than values in LAD segments with stenoses and endothelial injury.

**Platelet Aggregation Studies**

The in vitro effect of apyrase on platelet aggregation was evaluated. The IC$_{50}$ for 10 μM ADP-induced platelet aggregation was 0.01 units/ml. At the concentration of 0.1 units/ml, apyrase completely inhibited 20 μM ADP-induced platelet aggregation. However, neither 100 ng/ml U46619 nor 1 μM serotonin-induced platelet aggregation was significantly affected by 0.1 units/ml apyrase (Figure 5).

In clopidogrel-treated dogs, ADP platelet aggregation was completely inhibited after in vivo administration of clopidogrel. Serotonin and U46619-induced platelet aggregation were also inhibited approximately 70% and 40%, respectively (Figure 5B). These data suggest that apyrase is relatively
specific in inhibiting ADP-induced platelet aggregation, while clopidogrel's antiplatelet effects in dogs are complex and include an ability to prevent ADP-induced platelet aggregation as well as partial inhibitory effect on thromboxane A₂ and serotonin-induced platelet aggregation.

Blood Coagulation Studies

Activated whole-blood clotting times and partial thromboplastin times were measured before and 15 minutes after clopidogrel. Activated whole-blood clotting times were prolonged from 125±1 to 148±9 seconds and partial thromboplastin times from 38±3 to 48±5 seconds (p<0.05 for both), thereby indicating a mild anticoagulant effect of clopidogrel.

Discussion

The data from the present study suggest that ADP is a mediator of platelet aggregation and CFVs in stenosed and endothelium-injured canine coronary arteries.

Cyclic Coronary Blood Flow Variations

At sites of arterial stenosis and endothelial injury, recurrent platelet aggregation and dislodgment leading to CFVs may represent the pathophysiological phenomenon that occurs in at least some patients with acute coronary artery disease syndromes.¹⁻³ Morphological studies reveal that thrombus initiated by platelet aggregates may exist in the endothelium-injured and stenosed coronary arteries and may cause intermittent and persistent severe reductions in coronary blood flow.¹⁵⁺⁻³⁶ Dynamic coronary artery vasoconstriction also occurs during CFVs in experimental canine models.³³

Studies from our laboratories and others have shown that thromboxane A₂ and serotonin are two important mediators of CFVs in open-chest, anesthetized dogs and in closed-chest, fully awake canine models.¹⁵⁺⁻³⁶ Inhibition of thromboxane A₂ synthesis and/or blockade of thromboxane A₂ and serotonin S₂ receptors are usually effective in eliminating CFVs.¹⁵⁺⁻³⁶

ADP has been identified as a mediator of platelet aggregation in vitro.⁹⁻¹³ It is released by activated platelets from their dense granules, and it may also be derived from the dephosphorylation of ATP that occurs at sites of arterial injury.¹¹ At very low concentrations, ADP induces platelet aggregation and

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**FIGURE 5.** Effect of apyrase and clopidogrel on platelet aggregation in platelet-rich plasma. The final concentrations of agonists were ADP at 20 μM, U46619 (a thromboxane A₂ mimetic) at 100 ng/ml, and serotonin at 1 μM. Panel A: Samples were treated with saline (control) or apyrase at a final concentration of 0.1 unit/ml 5 minutes before the addition of agonists. Values are averaged from three different experiments. *p<0.01 compared with control. Panel B: Samples were obtained before (control) and 5–10 minutes after the administration of clopidogrel at 10 mg/kg i.v. bolus and 2.5 mg/kg/hr i.v. infusion (n=10). *p<0.01 compared with control.
this results in further platelet activation as more ADP is released.\textsuperscript{11–13}

By dephosphorylation of ADP, apyrase acts as an ADP scavenger and has been used in numerous in vitro studies. McClure et al.\textsuperscript{14} reported that apyrase increases bleeding time from punctures of rat mesenteric arteries after in vivo administration. In the present study, direct administration of apyrase into coronary arteries eliminated platelet aggregation–associated CFVs in approximately 80\% of animals. In vitro studies revealed apyrase’s inhibitory effect on ADP-induced platelet aggregation but not on platelet aggregation mediated by the thromboxane A\textsubscript{2} mimic U46619 or serotonin, which indicates that apyrase’s antiplatelet effect is relatively specific for ADP. These data suggest that ADP plays an important role in mediating in vivo platelet aggregation and the platelet aggregation–associated CFVs in this experimental model.

Clopidogrel is a novel antiplatelet agent. It has been shown to be a potent inhibitor of ADP-induced platelet aggregation.\textsuperscript{18–21} It has also been suggested to be effective in inhibiting smooth muscle cell proliferation after endothelial injury in rabbits.\textsuperscript{20} In the present study, the administration of clopidogrel abolished CFVs that had been present for 30 minutes to 3 hours. Ex vivo platelet aggregation induced by high-dose ADP was inhibited by clopidogrel. Furthermore, the thromboxane A\textsubscript{2} mimetic, U46619, and serotonin-induced platelet aggregation were also reduced 40\% and 70\%, respectively, by clopidogrel. It appears that clopidogrel’s action on platelet inhibition, while caused by ADP antagonism, in part, is complex and broader than simple ADP antagonism alone, apparently including an inhibiting effect on thromboxane A\textsubscript{2} and serotonin-induced platelet aggregation.

**Epinephrine-Restored Cyclic Coronary Blood Flow Variations**

Epinephrine is present in the circulation at low concentrations normally and does not stimulate platelet aggregation importantly.\textsuperscript{37,38} However, plasma epinephrine concentrations may increase two to 10 times with severe stress or exercise\textsuperscript{39,40} and may stimulate platelet aggregation through synergistic effect with other platelet activators.\textsuperscript{41} In experimental studies, CFVs that have been abolished by inhibitors of thromboxane A\textsubscript{2} synthesis or antagonists of thromboxane A\textsubscript{2} and serotonin S\textsubscript{2} receptors may be restored by infusing epinephrine.\textsuperscript{34,43} Simultaneously inhibiting the synthesis of thromboxane A\textsubscript{2} and blocking the activation of thromboxane A\textsubscript{2} and serotonin receptors appears to provide more complete protection against epinephrine-restored CFVs in experimental canine models.\textsuperscript{34,43}

In the present study, epinephrine infusion restored CFVs that had been abolished by apyrase alone in each of seven dogs. The plasma epinephrine concentrations required to restore CFVs were more than 20 times baseline levels. This suggests that removal of ADP from the coronary circulation and inhibiting ADP-induced platelet aggregation alone may provide protection against epinephrine-enhanced platelet aggregation, but it does not completely prevent it. As SQ29548 was added to apyrase, the incidence of CFV restoration was reduced and the plasma epinephrine concentrations required to restore CFVs were higher than for apyrase treatment alone. Therefore, the combination of a thromboxane A\textsubscript{2} receptor antagonist and reductions in ADP concentrations in the coronary artery circulation may provide stronger protection than just reducing ADP concentrations alone. Clopidogrel prevented CFV restoration by epinephrine in five of seven dogs. However, it appears to have a broad spectrum of antagonism, including serving as an antagonist of ADP, thromboxane A\textsubscript{2}, and serotonin-induced platelet aggregation, thereby possibly accounting for its pronounced protective effect.

**Thromboxane A\textsubscript{2} and Serotonin Production**

Thromboxane A\textsubscript{2} and serotonin are two important mediators of canine coronary CFVs.\textsuperscript{1–3,29–36} Thromboxane A\textsubscript{2} and serotonin accumulation occurs at arterial sites with stenosis and endothelial injury during cyclic reductions of coronary blood flow.\textsuperscript{1–3,16,26,30} In patients with unstable angina and coronary artery atherosclerosis, transcardiac thromboxane B\textsubscript{2} and serotonin levels are also increased.\textsuperscript{44,45} Inhibition of cyclooxygenase by aspirin reduces the production of thromboxane A\textsubscript{2} in the stenosed and endothelium-injured canine coronary arteries.\textsuperscript{46} However, inhibition of cyclooxygenase or blockade of serotonin receptors does not prevent the accumulation of serotonin in severely stenosed and endothelium-injured canine coronary arteries.\textsuperscript{30,46} Because clopidogrel did not markedly reduce thromboxane A\textsubscript{2} and serotonin concentrations, one may assume it does not affect the synthesis or release of thromboxane A\textsubscript{2} and serotonin importantly.

In conclusion, the data obtained in the present study suggest that ADP plays an important role in mediating platelet aggregation–associated CFVs in experimentally stenosed and endothelium-injured canine coronary arteries. Therefore, it should be included with thromboxane A\textsubscript{2} and serotonin as important mediators of CFVs in this experimental model. Reducing ADP concentrations in the coronary circulation and inhibiting ADP’s ability to stimulate platelet aggregation may prevent coronary artery thrombosis in stenosed and endothelium-injured coronary arteries and provide protection against epinephrine-enhanced platelet aggregation and thrombosis in vivo. A combination of inhibition of ADP-induced platelet aggregation with antagonism of thromboxane A\textsubscript{2} receptors provides more protection than inhibiting ADP’s ability to stimulate platelet aggregation alone in this experimental model. Clopidogrel is effective in preventing CFVs and provides protection against epinephrine-enhancing CFVs. This protective effect appears to result from its ability to antag-
onize the ability of ADP, serotonin, and thromboxane A_2 to stimulate platelet aggregation.

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References

**KEY WORDS**  • apyrase  • clopidogrel  • platelet aggregation  • serotonin  • thromboxane A₂

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