Vascular Prostaglandin and Thromboxane Production in a Canine Model of Myocardial Ischemia

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SUMMARY. Whereas numerous studies have investigated the role of prostacyclin and thromboxane A2 in the maintenance of coronary blood flow, most of these have focused on normal vessels. In the present investigation, we examined the prostaglandin- and thromboxane-synthesizing capacity of isolated coronary artery segments obtained from the site of a critical coronary artery stenosis. Cyclic flow variations were produced by placing a hard cylindrical constrictor on the proximal left anterior descending coronary artery in open-chest, anesthetized dogs. Cyclic flow variations are characterized by progressive declines in coronary blood flow, interrupted by sudden spontaneous restorations of flow. After cyclic flow variations had been induced, the hearts were removed, and the left anterior descending and circumflex coronary arteries were dissected. The vessels were cut into segments and incubated in the presence of increasing concentrations of arachidonic acid (10\(^{-6}\) to 10\(^{-8}\) M). The synthesis of prostaglandin E\(_2\), thromboxane B\(_2\), and 6-keto prostaglandin Fl\(_a\) by the coronary segments was measured by radioimmunoassay. When incubated in the presence of 10\(^{-5}\) M arachidonic acid, coronary artery segments obtained from the left anterior descending coronary artery undergoing cyclic flow variations produced substantially more thromboxane B\(_2\) (142 ± 27 vs. 29 ± 3 pg/mg, \(P < 0.01\)) and less 6-keto prostaglandin Fl\(_a\) (125 ± 12 vs. 350 ± 30 pg/mg, \(P < 0.01\)) than control circumflex coronary artery segments. Circumflex coronary vessels in which the endothelium was removed ex vivo produced 6-keto prostaglandin Fl\(_a\) levels comparable to those found in the left anterior descending coronary artery (147 ± 17 pg/mg), but did not synthesize thromboxane B\(_2\) (23 ± 2.6 pg/mg). These findings were corroborated by incubating either coronary segments, platelets, or neutrophils with \[^{14}C\]arachidonic acid and identifying the radioactive metabolites of \[^{14}C\]arachidonic acid by high performance liquid chromatography. Light microscopic evaluation of the vessel segments revealed platelet aggregates and infiltration of inflammatory cells into the vessel wall in the left anterior descending but not the circumflex coronary artery segments. These data demonstrate that cells capable of thromboxane synthesis accumulate at the site of a coronary artery constriction. In addition, prostacyclin synthesis is reduced due to damage to the endothelium. These factors combine to produce an imbalance in thromboxane A\(_2\) and prostacyclin which favors vasoconstriction and platelet aggregation. These factors may contribute to the production and maintenance of myocardial ischemia in this model. (Circ Res 57: 223–231, 1985)

IN recent years, increasing importance has been ascribed to platelets and the release of platelet-derived vasoactive substances in mediating alterations in coronary vasomotor tone. With atherosclerosis, platelets are exposed to subendothelial surfaces (Ross and Harker, 1976), resulting in platelet activation and aggregation. Thromboxane A\(_2\) (TxA\(_2\)) is synthesized and released from platelets during aggregation (Hamberg et al., 1974, 1975). This naturally occurring substance is a potent coronary vasoconstrictor and further promotes aggregation of circulating platelets. Several studies support a role for TxA\(_2\) in either initiating or propagating increases in coronary tone. For example, several investigators have reported increases in transcardiac TxA\(_2\) during active myocardial ischemia (Lewy et al., 1980; Hirsh et al., 1981). In an experimental model of concentric coronary artery stenosis in the dog (Folts et al., 1976), spontaneous declines in coronary blood flow occur which are interrupted by sudden restorations in flow. These have been referred to as cyclic flow variations or reductions (CFV) and are associated with transient formation of a platelet and red blood cell thrombus at the site of the partial stenosis (Folts et al., 1976; Bush et al., 1984). Similar to humans with active myocardial ischemia (Hirsh et al., 1981), this model is associated with increased transcardiac TxA\(_2\) (Bush et al., 1984). Furthermore, inhibition of either cyclooxygenase (Folts et al., 1976, 1982; Aiken et al., 1980) or TxA\(_2\) synthetase (Aiken et al., 1981; Bush et al., 1984) or infusion of prostacyclin (PGI\(_2\)) (Aiken et al., 1979; Romson et al., 1981), inhibits cyclic flow variations. Taken together, these data suggest that an elevation in TxA\(_2\) without elevations in PGI\(_2\) may be important in sustaining myocardial ischemia.
Evaluation of the role of TxA₂ during myocardial ischemia has been limited to pharmacological interventions and measurements of TxA₂ and prostaglandins in a vessel distal to the stenosis. In an attempt to characterize alterations occurring at a site of a coronary artery stenosis, we have utilized a canine model of coronary stenosis and have measured prostaglandin and thromboxane production in vitro from vessel segments obtained from a stenosed coronary artery undergoing CFV. These vessels were found to synthesize more thromboxane and less PGI₂ than normal coronary vessels. Thus, the local synthesis of TxA₂ at a site of vascular damage, along with the release of other platelet-derived vasoconstrictive substances, may contribute to platelet aggregation and the reduction in coronary blood flow.

Methods

Male mongrel dogs (20–30 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv) and ventilated with room air. Aortic and venous catheters were inserted via the common carotid artery and external jugular vein, respectively. A thoracotomy was produced in the 5th left intercostal space and the heart was suspended in a pericardial cradle. Segments of the left anterior descending (LAD) and circumflex coronary arteries (CCA) were gently dissected away from the surrounding tissue. After the LAD had been adluminally traumatized, a pulsed Doppler flow probe (manufactured by Dr. C. J. Hartley), was placed around it, and control hemodynamic measurements (heart rate, arterial blood pressure, distal coronary artery pressure, and mean and phasic coronary flow) were recorded on a Hewlett Packard (model 7758) eight-channel recorder. After control measurements had been obtained for 30 minutes, a plastic cylindrical constrictor, similar to that described by Fols et al. (1976), was placed on the LAD, 5–15 mm distal to the Doppler flow probe (Bush et al., 1984). The size of the constrictor was carefully selected to prevent the reactive hyperemic response to a 10-second total occlusion. After placement of the coronary constrictor, a pattern of progressive, cyclic declines in coronary blood flow occurred, interrupted by sudden spontaneous restoration of blood flow. In some instances, agitation of the blood vessel was required to restore blood flow. After observing CFV for thirty minutes, we removed the heart and placed it into 0.9% sodium chloride at room temperature. The LAD immediately adjacent to the constrictor and CCA were further dissected free and placed into calcium-free medium 199 containing 3 mM EDTA and UK 38-485 (10⁻³ M). Incubations were subsequently carried out as previously described, but in the presence of 10⁻³ M UK 38-485, and the media were assayed for prostaglandins and thromboxane. Thromboxane B₂ and 6-keto PGF₁α, the stable metabolites of TxA₂ and PGI₂, respectively, and PGE₂ were measured by the method of Dray et al. (1975), as modified by Campbell et al. (1980). Briefly, 0.1 ml of the diluted sample was combined with 0.1 ml of [³H]TxB₂, [³H]-6-keto PGI₁α, or [³H]PGE₂ and 0.1 ml of specific antiserum. After incubation overnight at 4°C, the antibody-bound and -free prostaglandins were separated with dextran-coated charcoal. The bound radioactivity was counted with a Beckman liquid scintillation spectrometer.

To identify the cyclooxygenase metabolites of arachidonic acid by coronary tissue, we incubated segments of coronary arteries in the presence of [¹⁴C]arachidonic acid (10⁻² M) (390 Ci/mol, New England Nuclear) for 10 minutes, with or without the addition of the thromboxane synthetase inhibitor, imidazole (10⁻³ M). The media from five separate incubations were pooled, and 5 µg of unlabeled prostaglandin standards were added. The media were acidified to pH 3.0 with glacial acetic acid and applied to octadecylsilica extraction columns (J.T. Baker) (Powell, 1980). Columns were sequentially washed with 15% ethanol, water, and petroleum ether. Prostaglandins were eluted from the column with ethyl acetate, dried under nitrogen, and chromatographed on a Unimetrics C-18 column, using a Beckman high pressure chromatograph, model 334 (VanKollins, et al., 1980). Solvent A consisted of 0.025 M phosphoric acid in water; solvent B was acetonitrile. The program consisted of a 40-minute isocratic elution with 31% solvent B in A, followed by a 20-minute linear gradient to 100% solvent B and, finally, a 20-minute elution with 100% solvent B. The flow rate was 1 ml/min. Absorbance was monitored at 192 nm. The eluate from the column was collected in 0.5-ml aliquots, scintillation fluid was added, and radioactivity was measured in a Beckman liquid scintillation spectrometer. Identity of the radioactive peaks was established by co-migration with known standards following co-injection.

Similarly, the metabolism of arachidonic acid by either platelets or neutrophils was studied. Two dogs were anesthetized and instrumented with carotid arterial and jugular venous catheters. After a 30-minute stabilization period, stored frozen at −40°C until analyzed for prostaglandins and TxB₂. Preliminary studies revealed that prostaglandin production was linear between 5 and 20 minutes of incubation; therefore, 10-minute incubations were performed in subsequent experiments. The coronary tissue was blotted dry, weighed, and fixed in 10% formalin in phosphate buffer. The segments were subsequently dehydrated, embedded in glycol methacrylate, sectioned, and stained with the “double Lee’s” procedure and examined and photographed with a Zeiss Photomicroscope III (Siler et al., 1984). In a separate set of dogs, after CFV were established, the thromboxane synthetase inhibitor UK 38-485 (generously provided by Dr. P. Urquilla, Pfizer Pharmaceuticals) (Fischer et al., 1983) was administered intravenously at a dose of 5 mg/kg. Hemodynamics were monitored for an additional 30-minute period, during which time CFV were abolished, as has been previously reported (Bush et al., 1984; Apprill et al., 1984). The hearts were removed, and the LAD and CCA were dissected and placed into calcium-free medium 199 containing 3 mM EDTA and UK 38-485 (10⁻³ M). Incubations were subsequently carried out as previously described, but in the presence of 10⁻³ M UK 38-485, and the media were assayed for prostaglandins and thromboxane.
period, arterial blood was collected into either one-tenth volume of isotonic sodium citrate for platelet studies or sodium heparin for neutrophil studies. UK 38-485 was administered at a dose of 5 mg/kg, intravenously, and after 30 minutes, blood samples were again collected. Platelet-rich plasma was obtained by centrifuging citrated blood at 150 g for 10 minutes. The resultant suspension was centrifuged at 1500 g for 10 minutes, and the platelet pellet was resuspended in N-2-hydroxyethylpiperazine-N'2 ethanesulfonic (HEPES) buffer containing 10 mM HEPES, 150 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, and 1 g/liter glucose, pH 7.4. Buffer was added to adjust the final platelet count to approximately 300,000/mm³. Neutrophils were obtained from heparinized arterial blood and were isolated by Ficoll-Hypaque density centrifugation followed by dextran sedimentation (Root et al., 1972). Cells were suspended in HEPES buffer at a final concentration of 40,000/mm³. UK 38-485 (10⁻⁷ M) was added to cells prepared from the blood collected after UK 38-485 treatment, and its vehicle was added to the control cells. Platelets or neutrophils were incubated in the presence of [³⁵C]arachidonic acid (10⁻⁷ M) for 10 minutes with agitation. Incubations were terminated by rapid freezing in a dry ice-acetone bath. Samples were subsequently acidified, extracted, and chromatographed for separation of [³⁵C]-labeled prostaglandins, as described above.

All results are expressed as the mean ± SEM. A one-way analysis of variance was used to determine significance between groups, and an unpaired Student's t-test was used to determine differences from control values. In all cases, P < 0.05 was considered significant.

Results

Cyclic flow variations were produced in 12 dogs. Figure 1 illustrates a representative tracing from a single dog in which a coronary stenosis caused a decrease in distal coronary pressure and resting coronary blood flow. Subsequently, CFV developed and continued unabated for several hours. Except for reducing distal coronary pressure, these CFV did not produce any major hemodynamic changes. After administration of UK 38-485, CFV were abolished in all of the dogs.

In six dogs undergoing CFV, the coronary arteries were removed and evaluated for prostaglandin and thromboxane production in vitro (Fig 2). Under basal conditions, coronary artery segments from the stenosed LAD produced significantly more Txβ than either the control CCA segments or CCA without endothelium (38.3 ± 6.3, 12.8 ± 1.5 and 11.4 ± 1.0, respectively, P < 0.01). When incubated in the presence of increasing concentrations of arachidonic acid, the synthesis of Txβ was stimulated. The stimulation was significantly greater in the stenosed LAD than in the other vessels. Under basal conditions, all three groups of vessels produced similar amounts of 6-keto PGF₁α and PGE₂. Control vessel segments obtained from the CCA demonstrated a dose-dependent increase in 6-keto PGF₁α production.

![Figure 1. Representative tracing of hemodynamic data obtained from a single dog. During the control period, complete occlusion of the left anterior descending coronary artery (LAD), followed by release of the occlusion, produced reactive hyperemia. Between the first and second panels, a constrictor was placed on the LAD. Cyclic flow variations occurred, characterized by spontaneous and gradual declines in coronary flow which were interrupted by sudden restorations in flow. Cyclic flow variations are accompanied by a decrease in distal coronary artery pressure (Distal Cor. Press.) without evidence of other significant hemodynamic changes. Administration of the thromboxane synthetase inhibitor, UK-38-485, at the time indicated abolished CFV.](http://circres.ahajournals.org/Downloadfile/.../fig1.jpg)
in response to exogenous arachidonic acid. Similarly, segments of LAD obtained from the site of the constrictor or segments of circumflex coronary artery in which the endothelium was mechanically removed also synthesized 6-keto PGF₁α when stimulated by arachidonic acid; however, the synthesis was markedly diminished, compared with the control vessel. At the highest concentration of arachidonic acid (100 μM), the synthesis of 6-keto PGF₁α was significantly increased in the LAD segments. Prostaglandin E₂ was produced by each group of vessels when stimulated by arachidonic acid.

In a second set of six dogs, the administration of UK 38-485 abolished the cyclic flow variations. The coronary arteries were removed and incubated in vitro in the presence of UK 38-485 (10⁻³ M) and arachidonic acid (10⁻⁵ M) (Fig. 3). Segments obtained from the LAD produced substantial amounts of TxB₂ in response to arachidonic acid, as previously shown. The thromboxane synthetase inhibitor resulted in a significant decrease in both basal and arachidonic acid-stimulated TxB₂ production (95% and 78% reduction, P < 0.01, respectively). In contrast, the stimulation of 6-keto PGF₁α production by arachidonic acid tended to increase, but failed to reach statistical significance. Segments obtained from the control CCA had a similar response to the thromboxane synthetase inhibitor (Table 1). Basal and arachidonic acid-stimulated TxB₂ production was inhibited by the drug; however, the synthesis of 6-keto PGF₁α was not altered.

To ascertain that the immunoreactive TxB₂ was indeed TxB₂ and to evaluate arachidonic acid metabolism, we incubated coronary arteries with [¹⁴C]-arachidonic acid, extracted the media, and resolved the metabolites by high performance liquid chromatography (Fig. 4). In agreement with the immunoreactive prostaglandin levels, coronary artery segments obtained from the site of a constriction (LAD) produced ¹⁴C-labeled compounds which co-chromatographed upon co-injection with known standards of TxB₂, 6-keto PGF₁α, and PGE₂. Similarly,
FIGURE 4. Separation of the metabolites of \(^{14}\text{C}\)arachidonic acid by high performance liquid chromatography. \(^{14}\text{C}\)Arachidonic acid \((10^{-7} \text{M})\) was incubated with segments of circumflex coronary artery (CCA), left anterior descending coronary artery (LAD), or CCA without endothelium for 10 minutes. The media from several incubations were pooled, extracted, and subjected to high pressure liquid chromatographic purification as described in Methods. The arrows represent the elution times of authentic prostaglandin standards that were co-injected and detected by UV absorbance at 192 nm.

Control circumflex segments primarily produced a radiolabeled compound which co-chromatographed with 6-keto PGF\(_{1\alpha}\). However, \(^{14}\text{C}\)Tx\(_{2}\) was not detected. Lesser quantities of \(^{14}\text{C}\)-6-keto PGF\(_{1\alpha}\) and no \(^{14}\text{C}\)PGE\(_{2\alpha}\) were produced by circumflex segments devoid of the endothelium.

Segments of the LAD were incubated with \(^{14}\text{C}\)-arachidonic acid in the presence and absence of the thromboxane synthetase inhibitor, imidazole (Fig. 5). The untreated, control LAD segments produced \(^{14}\text{C}\)Tx\(_{2}\), \(^{14}\text{C}\)Tx\(_{3}\), and \(^{14}\text{C}\)PGE\(_{2}\) from \(^{14}\text{C}\)arachidonic acid. Imidazole \((10^{-3} \text{M})\) resulted in a substantial reduction in the size of the peak corresponding to \(^{14}\text{C}\)Tx\(_{2}\). Interestingly, the radioactive peak corresponding to 6-keto PGF\(_{1\alpha}\) was also decreased, but the \(^{14}\text{C}\)PGF\(_{2\alpha}\) peak was markedly increased.

When evaluated by light microscopy, segments of the LAD obtained from the site of the constrictor revealed extensive endothelial denudation and damage to the internal elastic lamella (Fig. 6). We observed that focal, small aggregates of platelets adhered to the intimal surface, and that leukocytes, including neutrophils, adhered to the intima and infiltrated the medial layer immediately adjacent to the luminal surface. Further evaluation of the periconstrictor segments revealed small vessels localized within the adventitial layer. However, neither platelets nor inflammatory cells were observed in those vessels. Control vessels obtained from the CCA had only occasional areas of focal loss of endothelium. However, there was no indication of adherent platelet aggregates or of infiltrating leukocytes. In the CCA segments in which the endothelium was mechanically removed ex vivo, microscopic examination confirmed the absence of endothelium. However, there were no platelets, and no inflammatory cells were observed in these segments, as the damage occurred after the vessel had been removed from the heart.

\(^{14}\text{C}\)-Arachidonic acid metabolism was studied in isolated platelets and neutrophils obtained before and after the administration of UK 38-485 (Fig. 7). Platelets produced \(^{14}\text{C}\)Tx\(_{2}\) as the major metabolite of \(^{14}\text{C}\)-arachidonic acid. Two other major nonpolar peaks which comigrated with 12-hydroxyeicosatetraenoic acid (HHT) (fractions 116-118) and 12-hydroxyeicosatetraenoic acid (12-HETE) (fractions 122-124) were also observed. Treatment of the platelets with UK 38-485 inhibited the synthesis of \(^{14}\text{C}\)Tx\(_{2}\) and \(^{14}\text{C}\)HHT. The synthesis of \(^{14}\text{C}\)PGE\(_{2}\), \(^{14}\text{C}\)PGD\(_{2}\), and \(^{14}\text{C}\)-12-HETE was stimulated. In neutrophils, \(^{14}\text{C}\)-arachidonic acid was metabolized to products that comigrated with Tx\(_{2}\) and PGD\(_{2}\) as
Discussion

The roles of PGI₂ and TxA₂ in regulating vascular tone and platelet aggregation has been the subject of numerous investigations. Prostacyclin is the major prostaglandin synthesized by coronary blood vessels (Gryglewski et al., 1976). Its synthesis is greatest in the intimal layer of the blood vessel (Moncada et al., 1977) and, specifically, in the endothelial cell (Weksler et al., 1977; MacIntyre et al., 1978; Revtyak et al., 1984). In the setting of endothelial cell damage, platelets adhere to the subendothelial surfaces (Baumgartner, 1973; Ross and Hacker, 1976). Administration of PGI₂ will prevent this platelet adherence. In the coronary circulation, Folts et al., (1976) discovered that damaging the endothelium and placing a constrictor at the damaged site will result in the accumulation of platelets and white cells and the formation of a white thrombus. The formation and dislodgement of the thrombus produces cyclic variations in coronary blood flow. These flow variations are associated with elevation in the levels of TxB₂ and 6-keto PGF₁α in the blood collected distal to the coronary artery stenosis (Bush et al., 1984). The systemic administration of PGI₂ or a thromboxane synthetase inhibitor will inhibit the cyclic flow variations (Aiken et al., 1981; Bush et al., 1984). Furthermore, Aiken and his colleagues (1980) reported that the local application of PGI₂ or a thromboxane synthetase inhibitor at the site of constriction and damage would prevent cyclic flow variations. These findings emphasize the importance of hormonal events occurring at the constrictor site. Since the ability of damaged coronary arteries to synthesize prostaglandins had not been examined previously, we undertook the present study.

We found that normal canine coronary arteries predominantly synthesize PGI₂ and PGE₂ in response to arachidonic acid. If the endothelium is removed after the vessel has been removed from
the heart, i.e., ex vivo, the ability to synthesize PGI$_2$ is greatly reduced. However, if the endothelium is removed in vivo, a constrictor is placed on the artery, and cyclic flow variation is allowed to occur, the vessel acquires the ability to synthesize thromboxane, and the synthesis of PGI$_2$ is much less than that which occurs in normal vessels. Although the damage to the endothelium of the vessel may explain the lack of PGI$_2$ synthesis, it does not explain the increase in thromboxane synthesis in stenosed vessel segments. Histological evaluation of the coronary arteries revealed at least two potential sources of thromboxane. Stenosed arteries had small focal aggregates of platelets adhering to the intimal surface, and leukocytes adhering to the intima and infiltrating into the media. These cells were not present in control vessel segments. Thus, with perfusion of these stenosed vessels in vivo, platelets and inflammatory cells attach to the damaged site and may contribute to the vasoactive substances released by the damaged, stenotic vessel.

Evaluation of arachidonic acid metabolism in isolated canine platelets and neutrophils demonstrates that both of these populations of cells are capable of synthesizing TxA$_2$. Furthermore, the synthesis of TxA$_2$ in these cells was inhibited by the thromboxane synthetase inhibitor, UK 38-485. The present studies are in agreement with previous ones which demonstrated that platelets produce TxA$_2$ as their major product from the cyclooxygenase pathway (Hamberg et al., 1974, 1975) and that polymorphonuclear leukocytes, as well as macrophages, synthesize both TxA$_2$ and PGE$_2$ (Higgs et al., 1976; Humes et al., 1977; Brune et al., 1978; Murota et al., 1978; Davidson et al., 1980; Walsh et al., 1981). The relative contribution of TxA$_2$ produced by leukocytes and platelets to thrombus formation cannot be stated with certainty. Driscoll et al. (1984) studied the ability of anti-platelet and anti-neutrophil serum to modify acute coronary thrombosis in dogs. Although anti-platelet serum prevented acute coronary thrombosis, anti-neutrophil serum was inactive. These data suggest that platelets rather than neutrophils are responsible for the thrombus formation that occurs following endothelial damage.

Whereas the relative rates of TxA$_2$ synthesis by neutrophils, macrophages, and platelets have not been described, it is clear that TxA$_2$ production by leukocytes can contribute in a major way to the TxA$_2$ release from a tissue. For example, the normal kidney does not appear to synthesize TxA$_2$ from arachidonic acid in appreciable amounts (Morrison et al., 1977). However, with in vivo ureteral obstruction (Morrison et al., 1978) or constriction of the renal vein (Zipser et al., 1980), there is exaggerated prostaglandin synthesis and a progressive increase in synthesis of TxA$_2$ by the kidney. These changes seem to be due to the infiltration of macrophages and lymphocytes and proliferation of fibroblast-like cells (Okegama et al., 1983). An analogous situation may occur in the stenosed or damaged coronary artery.

As in previous studies, treatment of dogs with a stenosed coronary artery with a thromboxane synthetase inhibitor (UK 38-485) prevented cyclic flow variations (Bush et al., 1984; Apprill et al., 1984). In addition, it prevented the synthesis of TxA$_2$ by the stenosed vessel segments without altering the synthesis of PGI$_2$. Similarly, incubating the stenotic vessel in the presence of the thromboxane synthetase inhibitor, imidazole, in vitro, inhibited the conversion of [³⁰C]arachidonic acid to [³⁰C]TxB$_2$ and reduced the conversion to [³⁰C]-6-keto PGF$_{1α}$. The formation of [³⁰C]PGF$_{2α}$ was increased. Previous studies with platelets and normal blood vessels indicate that stimulated platelets release PGH$_2$ in the presence of a thromboxane synthetase inhibitor, and that the vessel can use this source of PGH$_2$ to synthesize PGI$_2$ (Bunting et al., 1976; Moncada et al., 1977; Baenziger et al., 1979; Needleman et al., 1979). Similar results have been obtained with platelets and cultured endothelial cells (Baenziger et al., 1979; Marcus et al., 1980). This transfer of PGH$_2$ has been termed "endoperoxide steal." It seems to be quantitatively significant only in the presence of thromboxane synthetase inhibition (Needleman et al., 1979; Defreyne et al., 1982). In the present study with stenosed coronary artery segments, endoperoxide steal does not appear to occur between the vessel and the adhering platelets, as PGI$_2$ synthesis is not increased in the presence of thromboxane synthetase inhibition. Instead, the synthesis of PGF$_{2α}$ increases, which may represent the non-enzymatic breakdown of PGH$_2$. This difference between normal and stenosed vessels may be due to the absence of the endothelium. The endothelium can utilize PGH$_2$ released from the platelet as a substrate for...
PGI₂ synthesis, whereas other vascular cells may not. We have shown previously that there is an increase in 6-keto PGF₁α in levels of blood obtained distal to a stenosis in dogs receiving a thromboxane synthetase inhibitor (Bush et al., 1984). The data from our present and previous study might be explained by the conversion of platelet-derived PGI₂ to 6-keto PGF₁α by endothelial cells beyond the site of the stenosis while the absence of endothelium in the isolated segments reduces the formation of 6-

keto PGF₁α at the specific site of the stenosis. Thus, endoperoxide steal might be an important mechanism for thromboxane synthetase inhibitors to reduce TXA₂ formation, increase PGI₂ formation, inhibit platelet aggregation, and inhibit thrombus formation in normal vessels. These drugs may exert their anti-platelet and anti-thrombotic effects in stenosed or damaged blood vessels only by reducing TXA₂ formation.

These studies indicate that at a site of endothelial damage and arterial stenosis there is an accumulation of platelets and leukocytes at the subendothelial surface and in the media of the coronary vessel. These cells have the ability to synthesize TXA₂, which may promote the adherence and aggregation of platelets at the stenosis and reduce coronary blood flow. Such a mechanism may contribute to the development of myocardial ischemia and damage.

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