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

James M. Schmitz, Phillip G. Apprill, L. Maximilian Buja, James T. Willerson, and William B. Campbell

From the University of Texas Health Science Center at Dallas, Departments of Internal Medicine (Cardiology Division), Pathology, and Pharmacology, Dallas, Texas

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 constriction 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^-5 to 10^-3 M). The synthesis of prostaglandin E2, thromboxane B2, and 6-keto prostaglandin Flα 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 B2 (142 ± 27 vs. 29 ± 3 pg/mg, P < 0.01) and less 6-keto prostaglandin Flα (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 Flα levels comparable to those found in the left anterior descending coronary artery (147 ± 17 pg/mg), but did not synthesize thromboxane B2 (23 ± 2.6 pg/mg). These findings were corroborated by incubating either coronary segments, platelets, or neutrophils with [14C]arachidonic acid and identifying the radioactive metabolites of [14C]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 A2 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 A2 (TXA2) 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 TXA2 in either initiating or propagating increases in coronary tone. For example, several investigators have reported increases in transcardiac TXA2 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 TXA2 (Bush et al., 1984). Furthermore, inhibition of either cyclooxygenase (Folts et al., 1976, 1982; Aiken et al., 1980) or TXA2 synthetase (Aiken et al., 1981; Bush et al., 1984), or infusion of prostacyclin (PGI2) (Aiken et al., 1979; Romson et al., 1981), inhibits cyclic flow variations. Taken together, these data suggest that an elevation in TXA2 without elevations in PGI2 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 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 performed 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 ethylenediamine tetraacetic acid (EDTA) pH 7.4 (GIBCO) at room temperature. The arteries were cleaned of loose surrounding tissue and carefully cut into 2-mm rings which were subsequently split longitudinally. In a separate set of experiments, CCA were opened longitudinally, and the endothelium was removed by gentle abrasion with a cotton-tipped applicator.

After preparation, pieces of coronary artery were placed into the incubation tube containing 1 ml of medium 199 containing 1.8 mM calcium. The vessels were incubated with various concentrations of arachidonic acid (Sigma Chemical Company) or its vehicle. Incubations were carried out in a shaking water bath at 37°C for 15 minutes and were terminated by removal of the coronary tissue. The media was quickly frozen in a dry ice-acetone bath and 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.

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 PGF₁α, or [³H]PGF₁α 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 (10⁻⁴ 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 Uniminetics C₁₈ 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...
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 [³C]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 [³C]-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 TxB₂ 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 TxB₂ 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
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 PGF\textsubscript{1\alpha} when stimulated by arachidonic acid; however, the synthesis was markedly diminished, compared with the control vessel. At the highest concentration of arachidonic acid (100 \( \mu \)M), the synthesis of 6-keto PGF\textsubscript{1\alpha} was significantly increased in the LAD segments. Prostaglandin E\textsubscript{2} 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\textsuperscript{-3} M) and arachidonic acid (10\textsuperscript{-5} M) (Fig. 3). Segments obtained from the LAD produced substantial amounts of TxB\textsubscript{2} in response to arachidonic acid, as previously shown. The thromboxane synthetase inhibitor resulted in a significant decrease in both basal and arachidonic acid-stimulated TxB\textsubscript{2} production (95% and 78% reduction, \( P < 0.01 \), respectively). In contrast, the stimulation of 6-keto PGF\textsubscript{1\alpha} 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 TxB\textsubscript{2} production was inhibited by the drug; however, the synthesis of 6-keto PGF\textsubscript{1\alpha} was not altered.

To ascertain that the immunoreactive TxB\textsubscript{2} was indeed TxB\textsubscript{2} and to evaluate arachidonic acid metabolism, we incubated coronary arteries with \([^{14}\text{C}]\)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 \( ^{14}\text{C} \)-labeled compounds which co-chromatographed upon co-injection with known standards of TxB\textsubscript{2}, 6-keto PGF\textsubscript{1\alpha}, and PGE\textsubscript{2}. Similarly,

![FIGURE 3. Upper panel: effect of thromboxane synthetase inhibition with UK-38-485 on basal and arachidonic acid-(10\textsuperscript{-5} M) stimulated TxB\textsubscript{2} production by isolated segments of left anterior descending coronary arteries (LAD). Dogs were given 5 mg/kg of UK 38-485, intravenously, 30 minutes before removal of the hearts. Vessel segments were incubated in 10\textsuperscript{-3} M UK 38-485. Lower panel: effect of thromboxane synthetase inhibition on basal or arachidonic acid-(10\textsuperscript{-5} M) stimulated 6-keto PGF\textsubscript{1\alpha} production in the same vessels.](image)

**Figure 3.** Upper panel: effect of thromboxane synthetase inhibition with UK-38-485 on basal and arachidonic acid-(10\textsuperscript{-5} M) stimulated TxB\textsubscript{2} production by isolated segments of left anterior descending coronary arteries (LAD). Dogs were given 5 mg/kg of UK 38-485, intravenously, 30 minutes before removal of the hearts. Vessel segments were incubated in 10\textsuperscript{-3} M UK 38-485. Lower panel: effect of thromboxane synthetase inhibition on basal or arachidonic acid-(10\textsuperscript{-5} M) stimulated 6-keto PGF\textsubscript{1\alpha} production in the same vessels.
FIGURE 4. Separation of the metabolites of \(^1^4\text{C}\)arachidonic acid by high performance liquid chromatography. \(^1^4\text{C}\)arachidonic acid \(10^{-7}\) 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, \(^1^4\text{C}\)TxB\(_2\) was not detected. Lesser quantities of \(^1^4\text{C}\)-6-keto PGF\(_{1\alpha}\) and no \(^1^4\text{C}\)TxB\(_2\) were produced by circumflex segments devoid of the endothelium.

Segments of the LAD were incubated with \(^1^4\text{C}\)-arachidonic acid in the presence and absence of the thromboxane synthetase inhibitor, imidazole (Fig. 5). The untreated, control LAD segments produced \(^1^4\text{C}\)TxB\(_2\), \(^1^4\text{C}\)TxA\(_2\), and \(^1^4\text{C}\)PGE\(_2\) from \(^1^4\text{C}\)arachidonic acid. Imidazole \(10^{-3}\) M resulted in a substantial reduction in the size of the peak corresponding to \(^1^4\text{C}\)TxB\(_2\). Interestingly, the radioactive peak corresponding to 6-keto PGF\(_{1\alpha}\) was also decreased, but the \(^1^4\text{C}\)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 peri-constrictor 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.

\(^1^4\text{C}\)Arachidonic acid metabolism was studied in isolated platelets and neutrophils obtained before and after the administration of UK 38-485 (Fig. 7). Platelets produced \(^1^4\text{C}\)TxB\(_2\) as the major metabolite of \(^1^4\text{C}\)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 \(^1^4\text{C}\)TxB\(_2\) and \(^1^4\text{C}\)HHT. The synthesis of \(^1^4\text{C}\)PGE\(_2\), \(^1^4\text{C}\)PGD\(_2\), and \(^1^4\text{C}\)-12-HETE was stimulated. In neutrophils, \(^1^4\text{C}\)arachidonic acid was metabolized to products that comigrated with TxB\(_2\) and PGD\(_2\), as
well as a variety of unidentified nonpolar metabolites. UK 38-485 inhibited the synthesis of \( [14C]TxB_2 \) and increased the synthesis of \( [14C]PGE_2 \) and \( [14C] - PGF_{2\alpha} \).

**Discussion**

The roles of PGI\(_2\) and TxA\(_2\) 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 PGI\(_2\) 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 TxB\(_2\) and 6-keto PGF\(_{1\alpha}\) in the blood collected distal to the coronary artery stenosis (Bush et al., 1984). The systemic administration of PGI\(_2\) 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 PGI\(_2\) 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 constractor 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 PGI\(_2\) and PGE\(_2\) in response to arachidonic acid. If the endothelium is removed after the vessel has been removed from
Davidson et al., 1980; Walsh et al., 1981). The relative contribution of TxA2 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 TxA2 synthesis by neutrophils, macrophages, and platelets have not been described, it is clear that TxA2 production by leukocytes can contribute in a major way to the TxA2 release from a tissue. For example, the normal kidney does not appear to synthesize TxA2 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 TxA2 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 TxA2 by the stenosed vessel segments without altering the synthesis of PGI2. Similarly, incubating the stenotic vessel in the presence of the thromboxane synthetase inhibitor, imidazole, in vitro, inhibited the conversion of [14C]arachidonic acid to [14C]TxB2 and reduced the conversion to [14C]-6-keto PGI2. The formation of [14C]PGF2α was increased. Previous studies with platelets and normal blood vessels indicate that stimulated platelets release PGH2 in the presence of a thromboxane synthetase inhibitor, and that the vessel can use this source of PGH2 to synthesize PGI2 (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 PGH2 has been termed "endoperoxide steal." It seems to be quantitatively significant only in the presence of thromboxane synthetase inhibition (Needleman et al., 1979; Defreyn 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 PGI2 synthesis is not increased in the presence of thromboxane synthetase inhibition. Instead, the synthesis of PGF2α increases, which may represent the non-enzymatic breakdown of PGH2. This difference between normal and stenosed vessels may be due to the absence of the endothelium. The endothelium can utilize PGH2 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 levels in 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 PG₂ 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.

We express our appreciation to Mary Beth Santawski, Judy Ober, and Janice McNatt for their technical assistance, and to Stephanie Woolen for her secretarial assistance.

Supported by National Institutes of Health Ischemic SCGRants HL-17669 and HL-25471, and by the Moss Heart Fund.

Dr. Campbell is the recipient of Research Career Development Award K04-HL-00801 from the National Institutes of Health.

Address for reprints: Dr. James T. Willerson, The University of Texas Health Science Center at Dallas, Department of Internal Medicine (Cardiology Division), 5323 Harry Hines Boulevard, Dallas, Texas 75235.

Received October 1, 1984; accepted for publication April 29, 1985.

References


Gryglewski RJ, Bunting S, Moncada S, Flower RJ, Vane JR (1976) Arterial walls are protected against platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. Prostaglandins 12: 685-697


Marcus AJ, Weksler BB, Jaffe EA, Broekman MJ (1980) Synthesis of prostacyclin from platelet-derived endoperoxides by cultured...
Moncada S, Herman AG, Higgs EA, Vane JR (1977) Differential
formation of prostacyclin (PGX or PGI₂) by layers of the arterial
wall. An explanation for the anti-thrombotic properties of
Moncada S, Vane JR, Higgs EA (1977) Human arterial and venous
tissues generate prostacyclin (prostaglandin X), a potent inhibitor
of platelet aggregation. Lancet 1:18–20
Morrison AR, Nishikawa K, Needleman P (1977) Unmasking of
thromboxane A₂ synthesis by ureter obstruction in the rabbit
Morrison AR, Nishikawa K, Needleman P (1978) Thromboxane
A₂ biosynthesis in the ureter obstructed isolated perfused kid-
ney of the rabbit. J Pharmacol Exp Ther 205:1–8
Murota S, Kawamura M, Motta I (1978) Transformation of
arachidonic acid into thromboxane B₂ by the homogenates of
activated macrophages. Biochim Biophys Acta 528:507–511
Needleman P, Wyche A, Raz A (1979) Platelet and blood vessel
arachidonate metabolism and interactions. J Clin Invest 63:
345–349
Okegawa T, Jones PE, de Schryver K, Kawasaki A, Needleman
P (1983) Metabolic and cellular alterations underlying the
exaggerated renal prostaglandin and thromboxane synthesis in
ureter obstruction in rabbits: Inflammatory response involving
Powell W (1980) Rapid extraction of oxygenated metabolites of
arachidonic acid from biological samples using octadecylsilic.
Prostaglandins 20:947–955
synthesis by coronary vascular endothelium (abstr). Fed Proc
43:757
Romson JL, Haack DW, Abrams GD, Lucchesi BR (1981) Pre-
vention of occlusive coronary artery thrombosis by prostacyclin
infusion in the dog (abstr). Circulation 64:906A
Root RK, Rosenthal AS, Bolstrom PJ (1972) Abnormal bactericidal
metabolic and lysosomal function of Chediak-Higashi syn-
Science 193:1094–1100
Siler AC, Thomas D, Reynolds NC, Buja LM (1984) Glycol meth-
acy late for histology: Problems and solutions. Lab Med 15:
338–341
VanRollins M, Ho SHR, Greenwald JE, Alexander M, Darman
high performance liquid chromatography of metabolites of
arachidonic acid from incubation with human and rabbit plate-
lets. Prostaglandins 20:571–580
and metabolism of arachidonic acid in human neutrophils. J
Biol Chem 256:7228–7233
Weksler BB, Marcus AJ, Jaffe EA (1977) Synthesis of prostaglan-
din I₂ (prostacyclin) by cultured human and bovine endothelial
cells. Proc Natl Acad Sci USA 74:3992–3996
Zipser R, Myers S, Needleman P (1980) Exaggerated prostaglan-
din and thromboxane synthesis in the rabbit with renal vein

INDEX TERMS: Myocardial ischemia • Prostaglandins • Throm-
boxane • Coronary spasm • UK 38-485
Vascular prostaglandin and thromboxane production in a canine model of myocardial ischemia.
J M Schmitz, P G Apprill, L M Buja, J T Willerson and W B Campbell

Circ Res. 1985;57:223-231
doi: 10.1161/01.RES.57.2.223

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/57/2/223

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/