Cardiology.

CYCLIC FLOW VARIATIONS WITH CORONARY ARTERIAL STENOSIS: DOES THROMBOXANE A_2 ACCUMULATION PLAY A ROLE?

by

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Grand Rounds

Pathophysiological Alterations Associated with Unstable Angina Pectoris (Rest Angina)

In 1979, we postulated that alterations in prostaglandins play an important role in mediating the syndrome of unstable angina pectoris in patients (1). Previous studies had demonstrated that unstable angina pectoris (increasingly frequent angina occurring at minimal activity and at rest) is caused by primary decreases in myocardial oxygen delivery rather than major increases in myocardial oxygen demand (2,3) (Table 1).

TABLE 1

Potential Causes of Unstable Angina Pectoris

1. Progressive decreases in coronary arterial lumen with increasing atherosclerosis
2. Hemorrhage into an atherosclerotic plaque with partial obstruction of the vessel lumen by thrombus
3. Coronary arterial spasm
4. Local increases in thromboxane A_2 associated with platelet aggregation at sites of severe coronary arterial stenosis

Prostaglandins are naturally occurring compounds that are potent modulators of vascular smooth-muscle tone and platelet aggregability (4) (Figures 1 and 2). Prostaglandin I_2 or prostacyclin (PGI_2) is a powerful coronary vasodilator and inhibitor of platelet aggregation, and it is the predominant prostaglandin synthesized by the heart (5,6). Prostaglandin E_2 (PGE_2) is also synthesized in the heart and it dilates coronary vascular smooth muscle but promotes platelet aggregation (7). In contrast, thromboxane A_2 (TxA_2) is a potent vasoconstrictor released by platelets and by vascular endothelium that may cause further aggregation of circulating platelets (8). Both PGI_2 and TxA_2 are unstable and spontaneously convert to the inactive metabolites 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TxB_2), respectively (8,9). Figure 1 demonstrates the specific pathways involved in the formation of these prostaglandins. Figure 2 emphasizes the physiologic roles for prostacyclin (PGI_2) and thromboxane A_2 in altering platelet aggregation and modulating vascular smooth muscle tone.

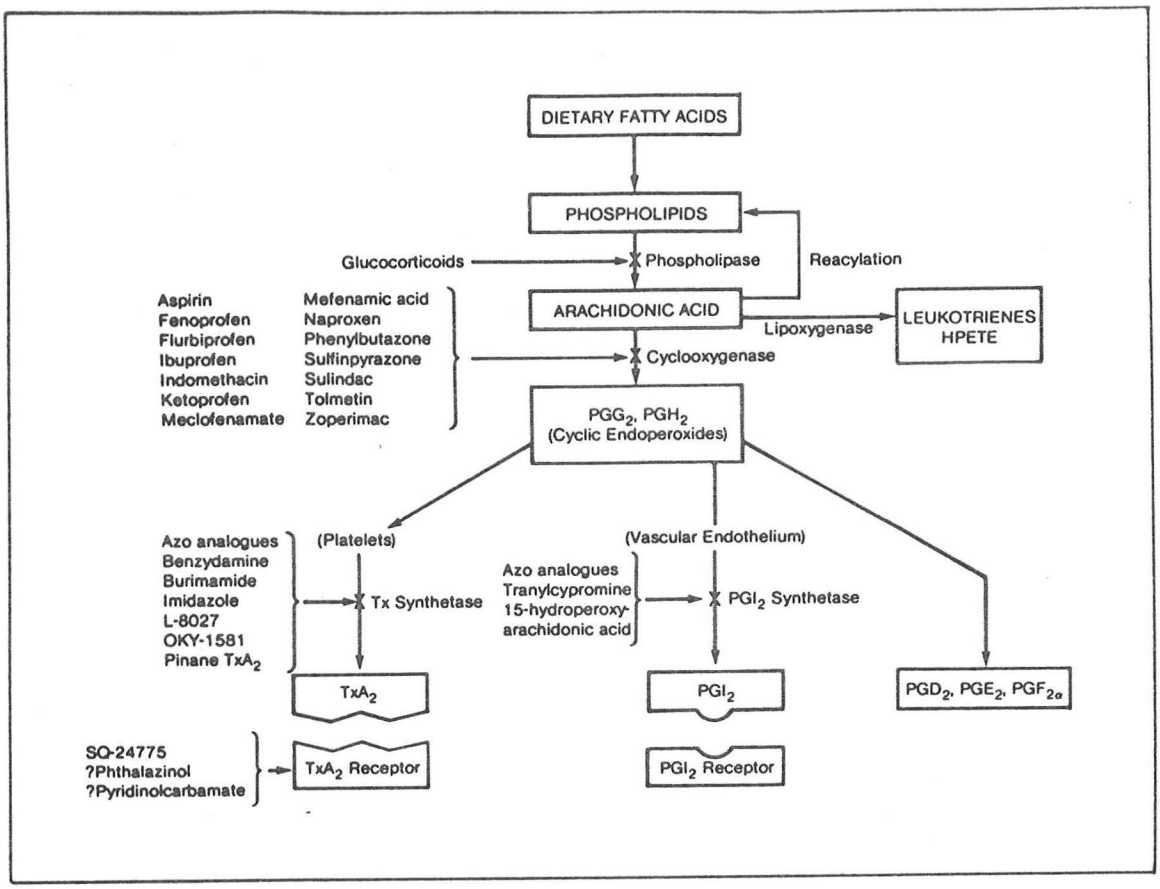

FIGURE 1

The pathway of arachidonic acid metabolism and the pharmacologic agents that block it. Arachidonic acid is enzymatically released from cell membrane phospholipids by a series of phospholipases, after which it enters one of three pathways: (1) reacylation into the phospholipid storage pool, (2) metabolism by the lipoxygenase enzymes to the leukotrienes and hydroperoxy-eicosatetraenoic acid (HPETE), and (3) conversion by cyclooxygenase to the prostaglandins (PGD_2, PGE_2, $PGF_{2\alpha}$) as well as to prostacyclin (PGI_2) and thromboxane A_2 (TxA_2). Glucocorticoids interfere with the phospholipases. The nonsteroidal antiinflammatory drugs inhibit cyclooxygenase activity, thereby preventing the synthesis of all prostaglandins and thromboxanes. Prostacyclin synthetase is found principally in vascular endothelial cells; there are several inhibitors of this enzyme that prevent the formation of PGI_2. Thromboxane synthetase is concentrated within platelets; inhibitors of this enzyme prevent TxA_2 formation. Finally, there are several compounds that inhibit TxA_2 activity by blocking the TxA_2 receptor. (This figure was reproduced from Reference 29).

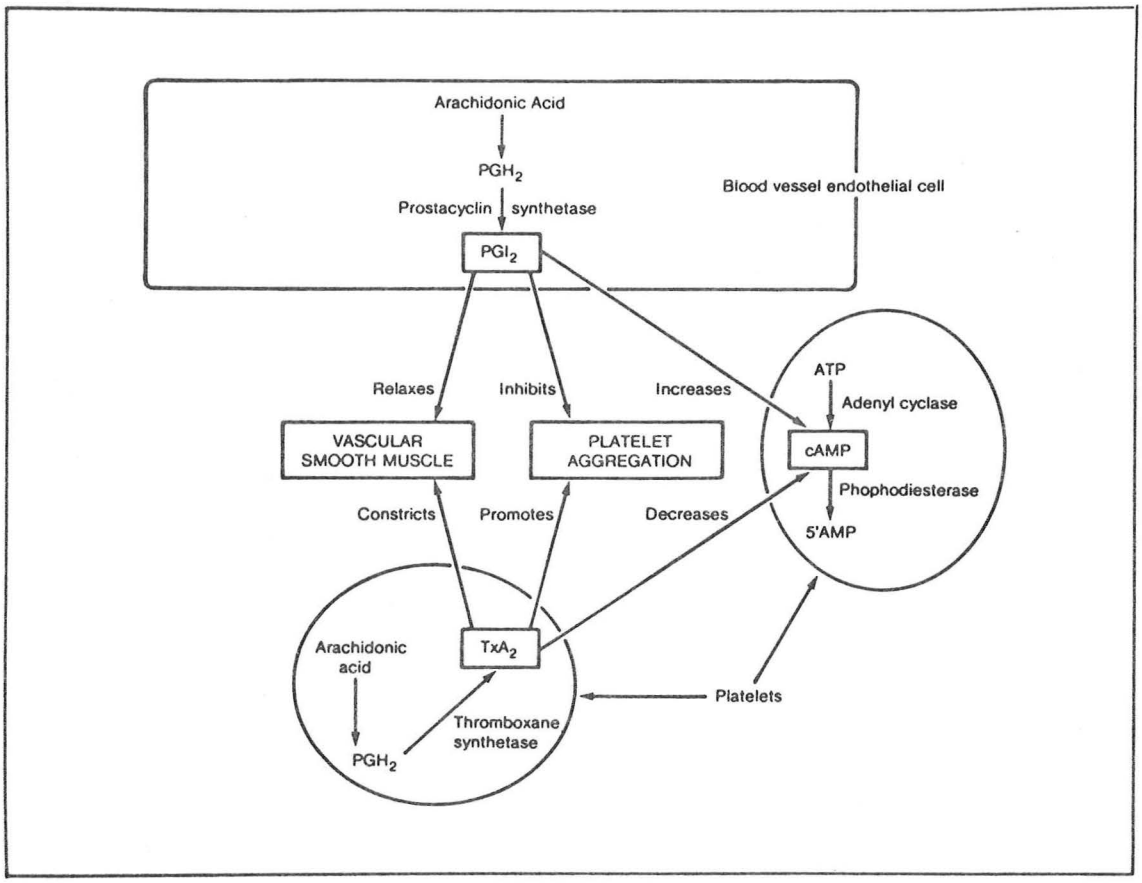

FIGURE 2

A schematic representation of the TxA_2:PGI_2 balance within the circulation. An endothelial cell of the blood vessel wall is on top; the blood vessel lumen below contains two platelets. In the endothelial cell, arachidonic acid is converted to PGH_2 (a cyclic endoperoxide) and then to PGI_2 (prostacyclin), which increases intracellular cAMP levels, relaxes vascular smooth muscle and inhibits platelet aggregation. In contrast, in the platelet, arachidonic acid is converted to PGH_2 and then to TxA_2, which decreases intracellular cAMP levels, constricts vascular smooth muscle and promotes platelet aggregation. (This figure was reproduced from Reference 29).

Release of Prostaglandins and Thromboxane into the Coronary Circulation in Patients with Ischemic Heart Disease

A. Clinical Protocol. To test the hypothesis that unstable angina pectoris is associated with alterations in prostaglandin metabolism, 60 patients undergoing cardiac catheterization from September, 1979 to September, 1980 were evaluated (1). Drs. L. David Hillis and Brian Firth obtained blood samples from the coronary sinus (CS) and ascending aorta (AO) at the time of routine cardiac catheterization in 31 women and 29 men (29-76 years old) without any attempt to control or alter the patients' medications prior to cardiac catheterization. None of the patients received premedication at cardiac catheterization. After informed consent was obtained, a No. 7 or 8 French woven-Dacron (Goodale-Lubin) catheter was positioned in the coronary sinus through a brachial vein under fluoroscopic control and its location was confirmed by both fluoroscopy and blood sampling for oxygen saturation. A No. 7 or 8 French polyurethane pigtail catheter was introduced percutaneously into the femoral artery and advanced to the ascending aorta adjacent to the coronary ostia. All subsequent blood samples were obtained before systemic heparinization or injection of radiographic contrast material.

Patient Groups

On the basis of the history, noninvasive evaluation, and results of cardiac catheterization (and without knowledge of the prostaglandin, thromboxane, or lactate results) each patient was assigned to one of five groups (Figures 3 and 4). Patients with ischemic heart disease were defined as those with fixed atherosclerotic coronary artery disease in which at least one coronary artery had $\geq$50% narrowing of the luminal diameter, angiographically documented coronary arterial spasm, or electrocardiographic evidence of previous myocardial infarction with a corresponding abnormality in segmental wall motion as determined with left ventriculography.

Group A (six patients) had nonischemic heart disease, which included a variety of congenital and acquired noncoronary cardiac lesions. Specifically, these patients had patent ductus arteriosus, atrial-septal defect, mitral stenosis, mitral regurgitation, idiopathic (nonischemic) cardiomyopathy, or cor pulmonale secondary to primary pulmonary hypertension. Group B (14 patients) comprised those with chest pain without objective evidence of cardiac disease on resting electrocardiogram, ambulatory two-channel electrocardiographic monitoring, exercise-tolerance testing with simultaneous radionuclide equilibrium gated blood pool scintigraphy, and cardiac catheterization, including selective coronary arteriography with ergonovine provocation. In Group C (18 patients), each patient had ischemic heart disease, with the most recent episode of chest pain occurring more than 96 hours before study. In Group D (15 patients), each patient had ischemic heart disease, and the most recent chest pain had occurred 24 to 96 hours before study. Finally, Group E (7 patients) comprised patients with unstable angina pectoris who had experienced chest pain within 24 hours before study. One patient who had an acute subendocardial myocardial infarction 12 hours after the study [confirmed by serum enzyme determinations, including creatine kinase B (10) and serial ^{99m}Tc stannous pyrophosphate myocardial scintigraphy (11)] was included in Group E. There were two patients with evidence of coronary arterial spasm (one in Group D and one in Group E).

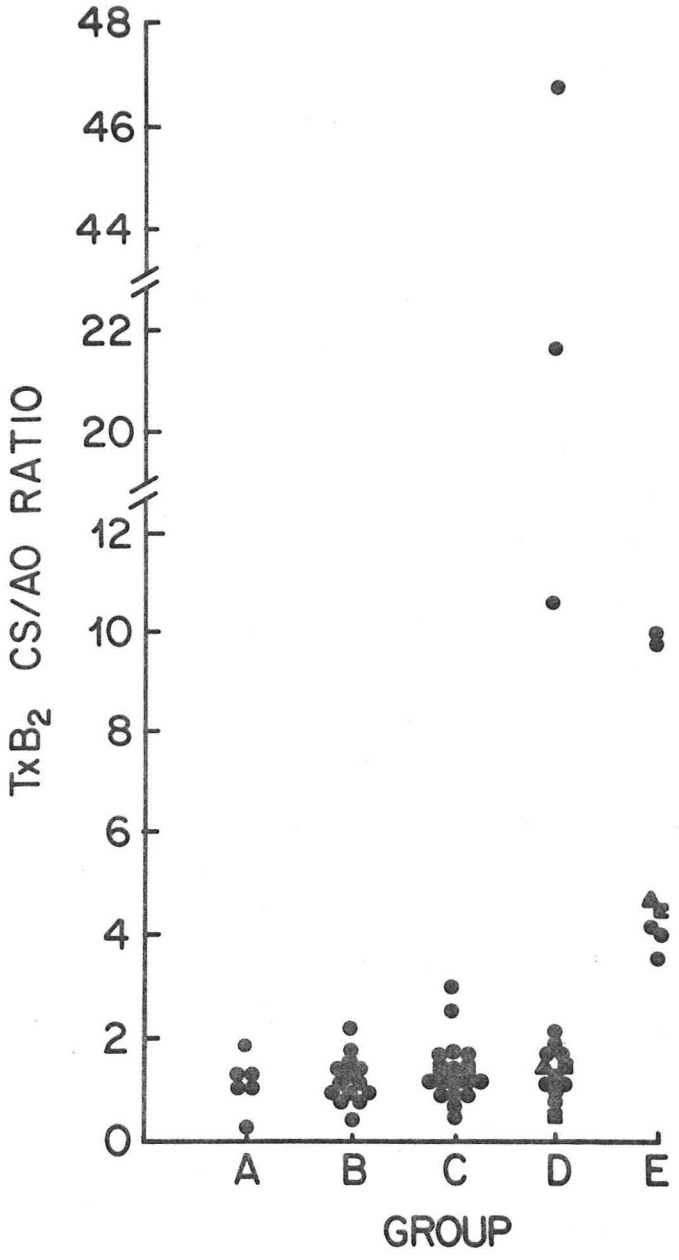

FIGURE 3

Ratios of thromboxane B_2 (TxB_2) in coronary sinus and in ascending aorta (CS/AO) in the five groups of patients. Each point represents the data from one patient. Squares identify patients who received a cyclooxygenase inhibitor within 5 days of study, and triangles patients with coronary arterial spasm. In Groups A (valvular and congenital nonischemic heart disease), B (chest pain syndrome without ischemic heart disease), and C (ischemic heart disease without chest pain for at least 96 hours), all patients had TxB_2 CS/AO ratios of 3.1 or lower. Group D (ischemic heart disease with chest pain 24 to 96 hours before study) had a bimodal distribution: 12 patients had low TxB_2 CS/AO ratios, whereas three had very high ratios. Group E (ischemic heart disease and chest pain within 24 hours before study) had TxB_2 CS/AO ratios (range, 3.5 to 9.9) that were higher than those of Groups A, B, and C ($p<0.05$). (This figure was reproduced from Reference 1).

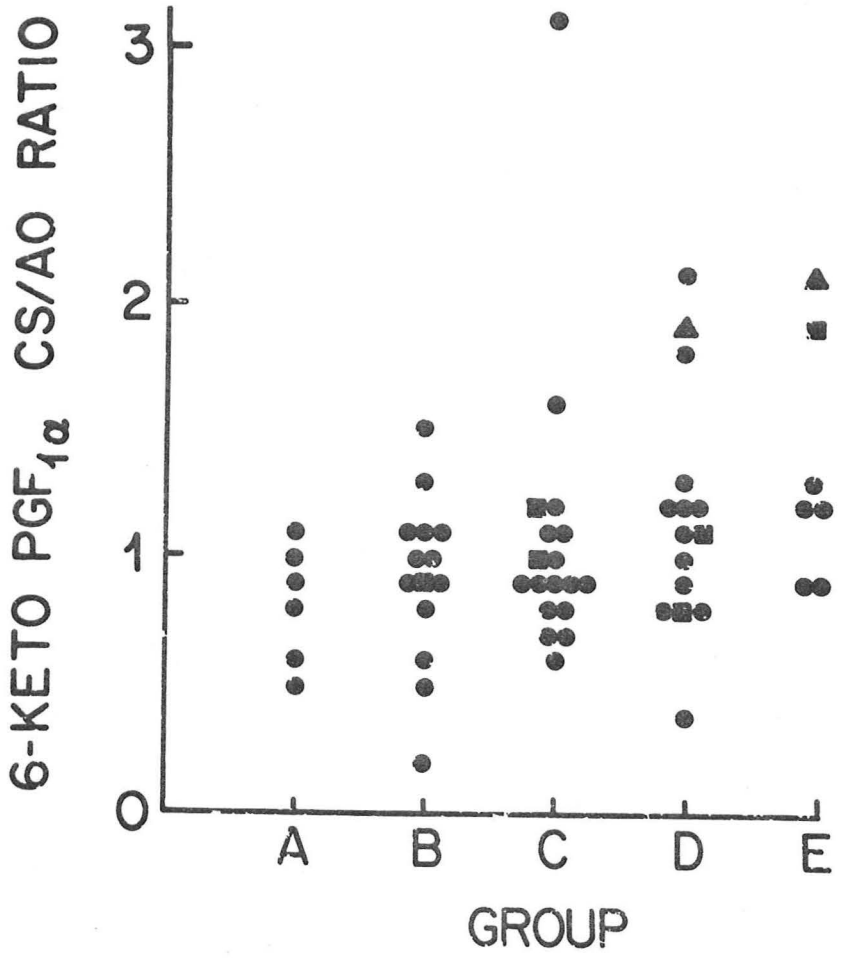

FIGURE 4

Ratios of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) in coronary sinus and in ascending aorta (CS/AO) in the 5 groups of patients. Each point represents the data from one patient. Squares identify patients who received a cyclooxygenase inhibitor within 5 days of study, and triangles patients with coronary arterial spasm. Results in the 5 groups were not statistically different from one another. (This figure was reproduced from Reference 1).

B. Chemical Analyses. The blood samples for prostaglandin and thromboxane analysis were drawn into heparinized plastic syringes and transferred quickly into iced 10-ml tubes containing indomethacin (10μg) and heparin (1000 units). They were immediately centrifuged at 2000xg for 15 minutes at 4°C. The supernatants were separated and stored at -20°C for subsequent analysis. Concentrations of 6-keto-$PGF_{1\alpha}$ and TxB_2 in aortic and coronary-sinus blood were analyzed in all 60 patients, and PGE_2 and lactate concentrations were measured in the first 45 patients. The prostaglandins were measured by Drs. Hirsh and Campbell at this institution.

Prostaglandins were measured with the radioimmunoassay method of Dray et al (12), as modified by Campbell et al (13). Briefly, this method involves adding the appropriate [^{3}H] prostaglandin or thromboxane to 4 ml of plasma and extracting the plasma with 20 ml of petroleum ether. The aqueous phase is then extracted with 20 ml of 50:50 solution of ethyl acetate and cyclohexane after acidification to pH 3 with glacial acetic acid. The organic phase is removed and evaporated to dryness under a stream of nitrogen at 30°C. Then, the extract is separated into its prostaglandin components by means of silicic acid chromatography with solvents of increasing polarity. Free fatty acids, PGA_2, and PGB_2 are first eluted from the silicic acid with a 60:40 solution of toluene and ethyl acetate. Then PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 are eluted in the second fraction with a 60:40:5:1 solution of toluene, ethyl acetate, methanol, and water. The second fraction is evaporated to dryness under nitrogen, reconstituted in phosphate-buffered saline containing gelatin, and assayed for prostaglandins by radioimmunoassay.

The antibodies for the radioimmunoassay were produced in rabbits. The rabbits were immunized against a prostaglandin-thyroglobulin conjugate produced with the mixed-anhydride method of Jaffe and Behrman (14). The levels of sensitivity of the assays are less than 5 pg for PGE_2 and 6-keto-$PGF_{1\alpha}$ and less than 1 pg for TxB_2. The anti-PGE_2 serum cross-reacted 14 per cent with PGE_1 and less than 0.6 per cent with the other known prostaglandins or PGE_2 metabolites. The anti-6-keto-$PGF_{1\alpha}$ serum cross-reacted 2% with PGE_1, 14% with $PGF_{1\alpha}$, and less than 0.6% with the other known prostaglandins and 6-keto-$PGF_{1\alpha}$ metabolites. The anti-TxB_2 serum cross-reacted less than 0.003% with all known prostaglandins. The results obtained with the radioimmunoassay were corrected for recoveries (65.4$\pm$12.0%, mean $\pm$S.D.) and expressed in picograms per milliliter of plasma.

The blood samples for lactate determinations were immediately deproteinated in iced perchloric acid, centrifuged, decanted, and stored in a frozen state. Lactate concentration was assayed enzymatically with the Sigma Chemical kit (St. Louis); spectrophotometric measurements were performed at 340 nm.

C. Statistical Analyses. Because of unequal variances among groups, concentrations in the coronary sinus and ascending aorta and the CS/AO concentration ratios for the prostaglandins and thromboxane were analyzed for intergroup differences with a nonparametric analysis (the Kruskal-Wallis procedure) (15). For comparison of the CS/AO ratios of 6-keto-$PGF_{1\alpha}$ and PGE_2, a regression analysis was performed, and Pearson's correlation coefficient was determined. An analysis of variance was performed as a test for intergroup differences in the mean percentage of myocardial lactate extraction.

All values were expressed as mean $\pm$S.D. with the median and range reported for additional comparison because of the skewed distributions in some groups.

D. Thromboxane B_2. When the concentrations of TxB_2 in the coronary sinus and ascending aorta were expressed as the CS/AO ratio, significant intergroup differences were observed (Figure 3). Group A (valvular and congenital nonischemic heart disease) had a TxB_2 CS/AO ratio of 1.2±0.6 (median, 1.2; range, 0.2 to 1.9); Group B (chest-pain syndrome without ischemic heart disease) had a ratio of 1.2±0.4 (median, 1.2; range, 0.4 to 2.2); and Group C (ischemic heart disease without chest pain for at least 96 hours) had a ratio of 1.3±0.6 (median, 1.2; range, 0.5 to 3.0). Although the overall mean TxB_2 CS/AO ratio for Group D (ischemic heart disease with chest pain 24 to 96 hours before study) was 6.3±12.5 (median, 1.5; range, 0.5 to 46.6), this group had a distinct bimodal distribution: 12 patients had low ratios (mean, 1.3±0.4; range, 0.5 to 2.1), whereas three patients had markedly elevated values (mean, 26.2±18.5; range, 10.5 to 46.6). The patients in Group D with low and high TxB_2 CS/AO ratios could not be distinguished from each other on the basis of any clinical criteria. Group E (ischemic heart disease and chest pain within 24 hours before study) had a TxB_2 CS/AO ratio of 5.8±2.8 (median, 4.5; range, 3.5 to 9.9), which was significantly higher than those of Groups A, B, and C ($p<0.05$).

The aortic concentrations of TxB_2 allowed no distinction among any of the groups. As shown in Table 2, there was no association between the aortic TxB_2 concentration and the presence or degree of clinical activity of ischemic heart disease. Similarly, neither the coronary-sinus TxB_2 concentrations alone nor the absolute changes in TxB_2 concentration across the coronary bed corresponded to the clinical groups of the patients (Table 2).

TABLE 2

Prostaglandin and Thromboxane Profile of the Five Patient Groups, Including Levels in Coronary Sinus (CS) and Ascending Aorta (AO) and CS/AO Ratios.

Group *	Thromboxane B_2			6-Keto-Prostaglandin $F_{1\alpha}$			Prostaglandin E_2			Ratio of Thromboxane B_2 CS/AO to 6-Keto-Prostaglandin $F_{1\alpha}$ CS/AO
	CS	AO	CS/AO †	CS	AO	CS/AO †	CS	AO	CS/AO †	
	pg/ml	*pg/ml*		*pg/ml*	*pg/ml*		*pg/ml*	*pg/ml*		
A										
Mean	133±104	117±69	1.2±0.6 ‡	167±74	240±177	0.8±0.2	146±84	187±138	0.9±0.1	1.5±0.8
Median	99	102	1.2 ‡	157	166	0.9	121	124	1.0	1.8
Range	30–314	47–246	0.2–1.9	64–257	60–515	0.5–1.1	50–280	52–399	0.7–1.0	0.2–2.2
B										
Mean	98±57	97±72	1.2±0.4 ‡	115±53	140±71	0.9±0.3	113±50	118±42	1.0±0.2	1.5±0.9
Median	79	72	1.2 ‡	118	117	1.0	110	120	1.0	1.3
Range	41–245	27–274	0.4–2.2	19–205	51–277	0.2–1.5	48–220	55–195	0.7–1.3	0.3–4.0
C										
Mean	104±105	74±65	1.3±0.6 ‡	143±99	135±58	1.1±0.6	131±41	139±55	1.0±0.4	1.5±0.8
Median	73	52	1.2 ‡	127	131	0.9	136	134	0.8	1.2
Range	13–375	10–267	0.5–3.0	47–492	57–253	0.6–3.1	52–187	60–222	0.7–2.0	0.5–3.6
D										
Mean	228±290	108±224	6.3±12.5	144±78	139±122	1.2±0.5	169±131	146±129	1.3±0.5	4.4±7.0
Median	123	42	1.5	125	119	1.1	123	91	1.2	1.4
Range	8–980	13–908	0.5–46.6	57–333	49–565	0.4–2.1	53–446	41–421	0.6–2.4	0.6–22.2
E										
Mean	405±381	67±60	5.8±2.8 ‡	168±86	129±55	1.4±0.5	165±102	114±47	1.4±0.6	4.7±2.6
Median	329	54	4.5 ‡	174	145	1.2	133	105	1.4	4.4
Range	54–1044	11–180	3.5–9.9	78–324	53–205	0.9–2.1	58–304	59–193	0.6–2.2	2.2–8.3

*Patient groups A-E are described in detail in the text. For thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ values, 60 patients were studied (Group A, 6; Group B, 14; Group C 18; Group D, 15; and Group E, 7). For prostaglandin E_2 values, 45 were studied (Group A, 6; Group B, 10; Group C, 11; Group D, 12; and Group E, 6). Mean data are ±S.D.

†These calculations represent the mean, median, and range of the individual patient CS/AO ratios in each group.

‡Results in Group E were different from those of Groups A, B, and C ($P<0.05$).

(This Table was reproduced from Reference 1).

E. **6-keto-PGF$_{1\alpha}$ and PGE$_2$.** The CS/AO ratios of prostaglandin 6-keto-PGF$_{1\alpha}$ (an inactive metabolite of PGI$_2$) and PGE$_2$ showed no significant intergroup differences (Figure 4 and Table 2). However, a good correlation was obtained between the 6-keto-PGF$_{1\alpha}$ and PGE$_2$ CS/AO ratios for the 45 patients in whom both were measured ($r=0.88$, $p<0.0001$) (Figure 5). As observed with the absolute TxB$_2$ values, there were no significant differences among the groups in the absolute 6-keto-PGF$_{1\alpha}$ and PGE$_2$ concentrations from the coronary-sinus and aortic blood (Table 2) or in the absolute difference of each substrate across the coronary bed.

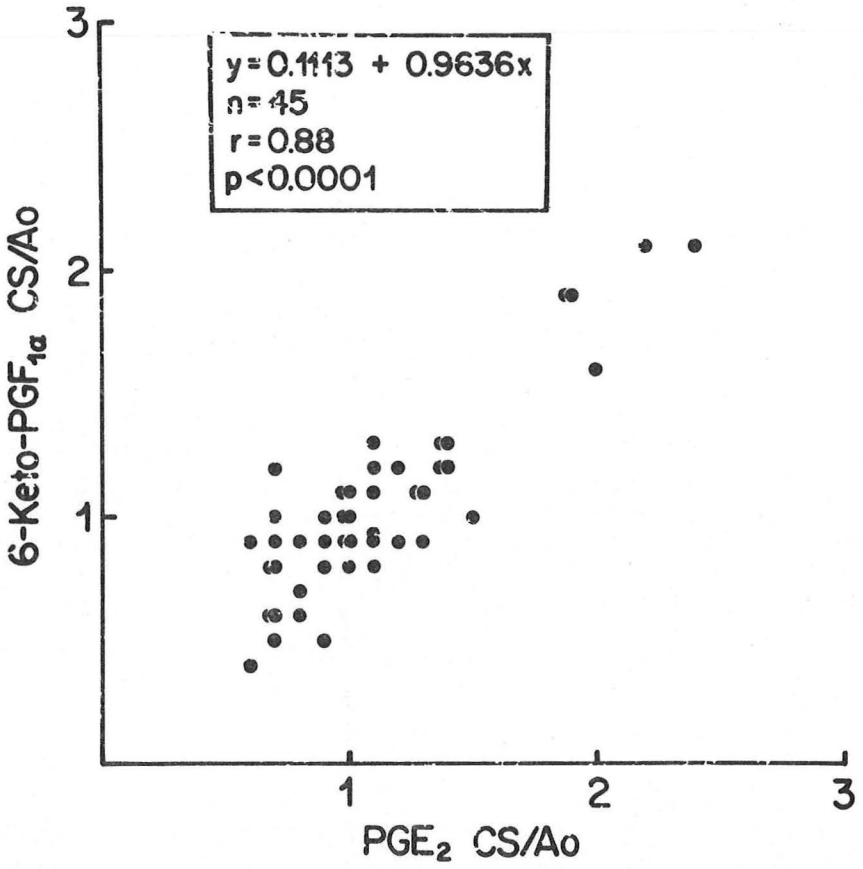

FIGURE 5

Comparison of the coronary sinus/ascending aorta (CS/AO) ratios of 6-keto-prostaglandin F$_{1\alpha}$ (6-keto-PGF$_{1\alpha}$) and prostaglandin E$_2$ (PGE$_2$) in 45 patients. Each point represents the data from 1 patient. As noted, there is a good correlation betwen the 6-keto-PGF$_{1\alpha}$ and PGE$_2$ CS/AO ratios ($r=0.88$, $p<0.0001$). (This figure was reproduced from Reference 1).

F. **TxB$_2$/6-keto-PGF$_{1\alpha}$ Ratio.** The CS/AO ratio of TxB$_2$ divided by the CS/AO ratio of 6-keto-PGF$_{1\alpha}$ did not allow better discrimination among the five groups of patients than did the TxB$_2$ CS/AO ratio alone (Table 2).

G. Effects of Medications. Of the 60 patients, 47 (78%) were receiving long-acting nitrates, 21 (35%) were receiving propranolol (dose range, 30 to 240 mg per 24 hours; mean dose, 120 mg per 24 hours), and two (3%) were receiving metoprolol, 100 mg per 24 hours, at the time of catheterization. In addition, six patients had received cyclooxygenase inhibitors within 5 days of study (aspirin in 2, aspirin plus ibuprofen in 1, sulfinpyrazone in 1, indomethacin in 1, and sulindac in 1). These patients were approximately equally distributed among the groups: 1 in Group B, 2 in Group C, 2 in Group D, and 1 in Group E (Figure 3). Exclusion of these 6 patients did not alter the conclusions that were based on statistical analysis at the $p<0.05$ confidence level.

H. Influence of Number of Abnormal Coronary Arteries. Figure 6 demonstrates that the number of angiographically abnormal coronary arteries did not appear to be an important factor in explaining the increased transcardiac thromboxane B_2 concentration in patients with unstable angina pectoris. Previous studies at this institution and elsewhere have demonstrated that there is no consistent relationship between the number of coronary arteries with significant stenoses and the development of unstable angina pectoris (16).

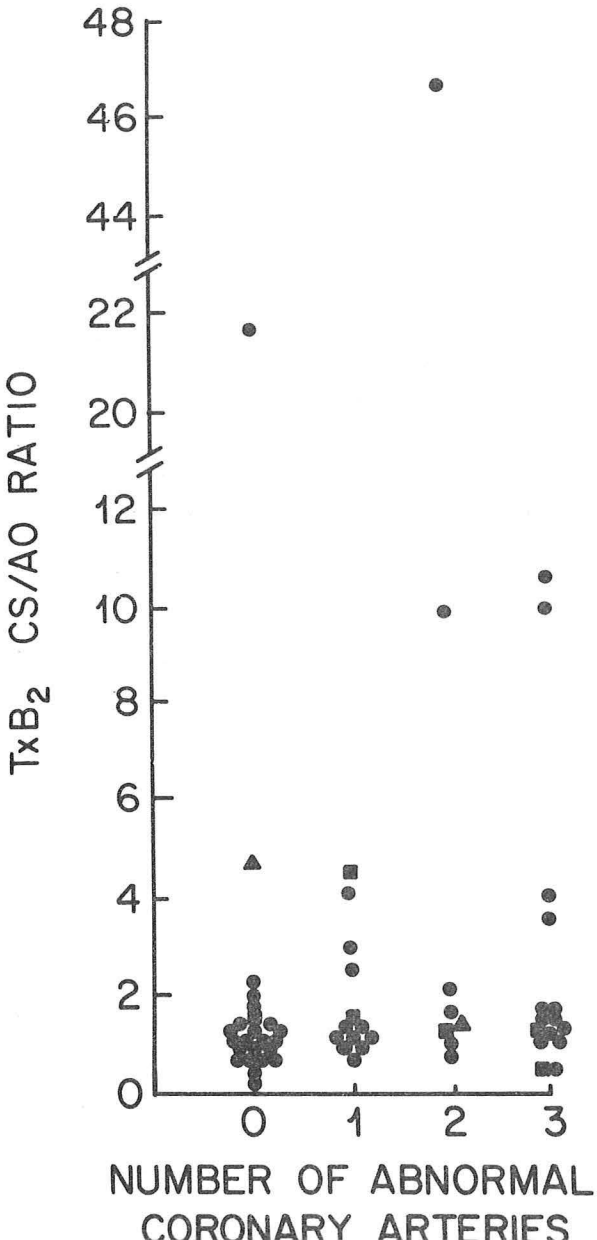

FIGURE 6
Ratios of thromboxane B_2 (TxB_2) in coronary sinus and in ascending aorta CS/AO), plotted for the presence and degree of coronary artery disease. Each point represents the data from 1 patient. Squares identify patients who received a cyclo-oxygenase inhibitor within 5 days of study, and triangles patients with coronary arterial spasm. The TxB_2 CS/AO ratios were similar among patients with and without underlying coronary artery disease. (This figure was reproduced from Reference 1).

Summary

The data obtained in this study demonstrated a temporal but not necessarily causal relationship between increases in transcardiac thromboxane B_2 concentration and the presence of active unstable angina pectoris in patients. In order to determine possible causal relationships, it would be necessary to a) determine whether there are increases in transcardiac thromboxane concentration with important coronary arterial narrowing in an experimental model and b) whether such increases in transcardiac thromboxane concentration are associated with decreases in coronary blood flow and/or enhanced platelet aggregation.

Cyclic Flow Variations in a Canine Model with a Partial Coronary Arterial Constriction

Recently, we have utilized an experimental animal model initially developed by Folts, et al (17,18). Figure 7 demonstrates the surgical instrumentation of the heart utilized in this experimental model (19).

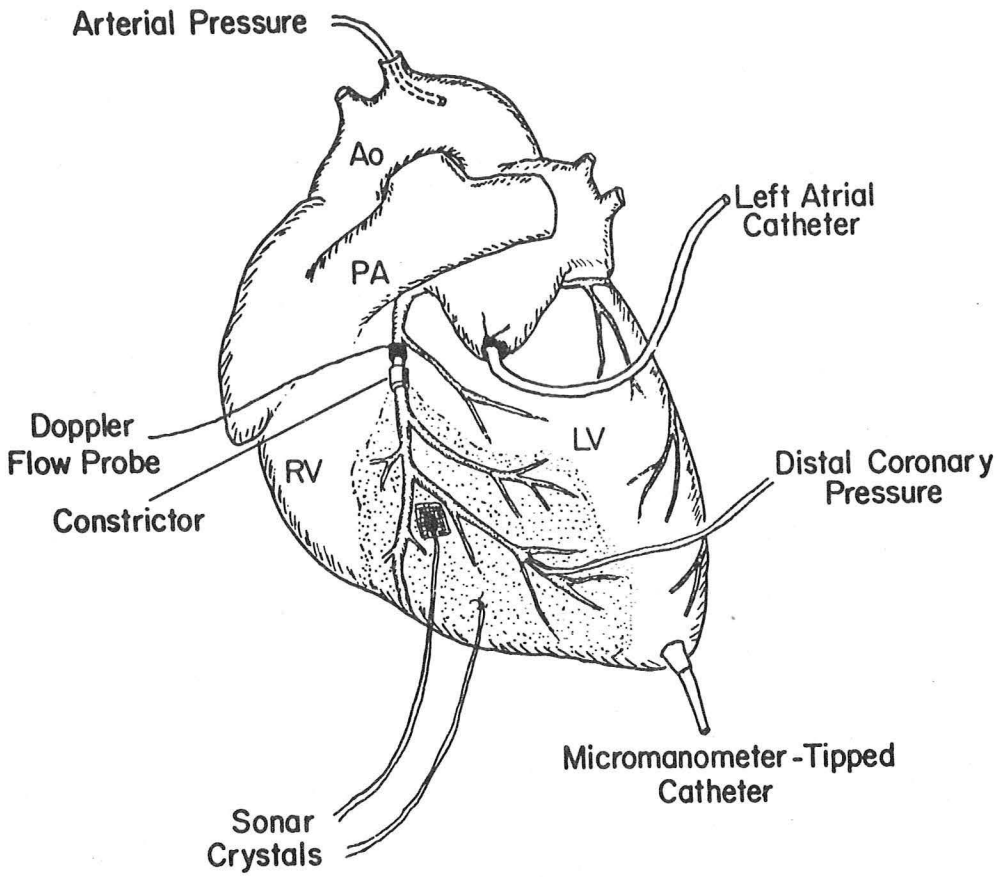

FIGURE 7

Schematic diagram of cardiac preparation for studying cyclic coronary flow variations. Stippled area represents area made ischemic by coronary constrictor and intravascular platelet aggregation. Ao=aorta; PA=pulmonary artery; RV=right ventricle; LV=left ventricle.

In this experimental model, a partial coronary arterial stenosis is created around the proximal left anterior descending or circumflex coronary artery. The coronary arterial stenosis is severe enough to prevent hyperemic flow after a transient coronary arterial occlusion, and it reduces resting coronary blood flow by approximately 50%. Angiographic studies of such narrowed coronary arteries indicate approximately 75% luminal diameter narrowing. A flow probe is positioned proximal to the coronary arterial stenosis and a catheter is placed into the distal portion of the same coronary artery to allow a) measurements of distal coronary arterial pressure and b) blood samples to be obtained for prostaglandin measurements. Left ventricular pressure is measured by a high fidelity pressure transducer (Konigsberg) inserted into the left ventricular apex, and another catheter is positioned in the left atrium to allow measurement of left atrial pressures and to serve as a site for the injection of radioactive microspheres thus allowing regional myocardial blood flow to be measured. In some experiments, a pair of 5mHz ultrasonic crystals are placed in the area of the left ventricle to be made ischemic to measure transmural systolic wall thickening (20).

Dogs with proximal partial coronary arterial stenosis similar to that described above develop cyclic coronary blood flow alterations spontaneously (Figure 8). Folts, et al (17) and our laboratory (19) have demonstrated that the cyclic flow variations are caused by platelet, red cell, white cell thrombi that develop at the site of proximal coronary arterial narrowing and then spontaneously break loose allowing restoration of coronary blood flow (Figures 8 and 9).

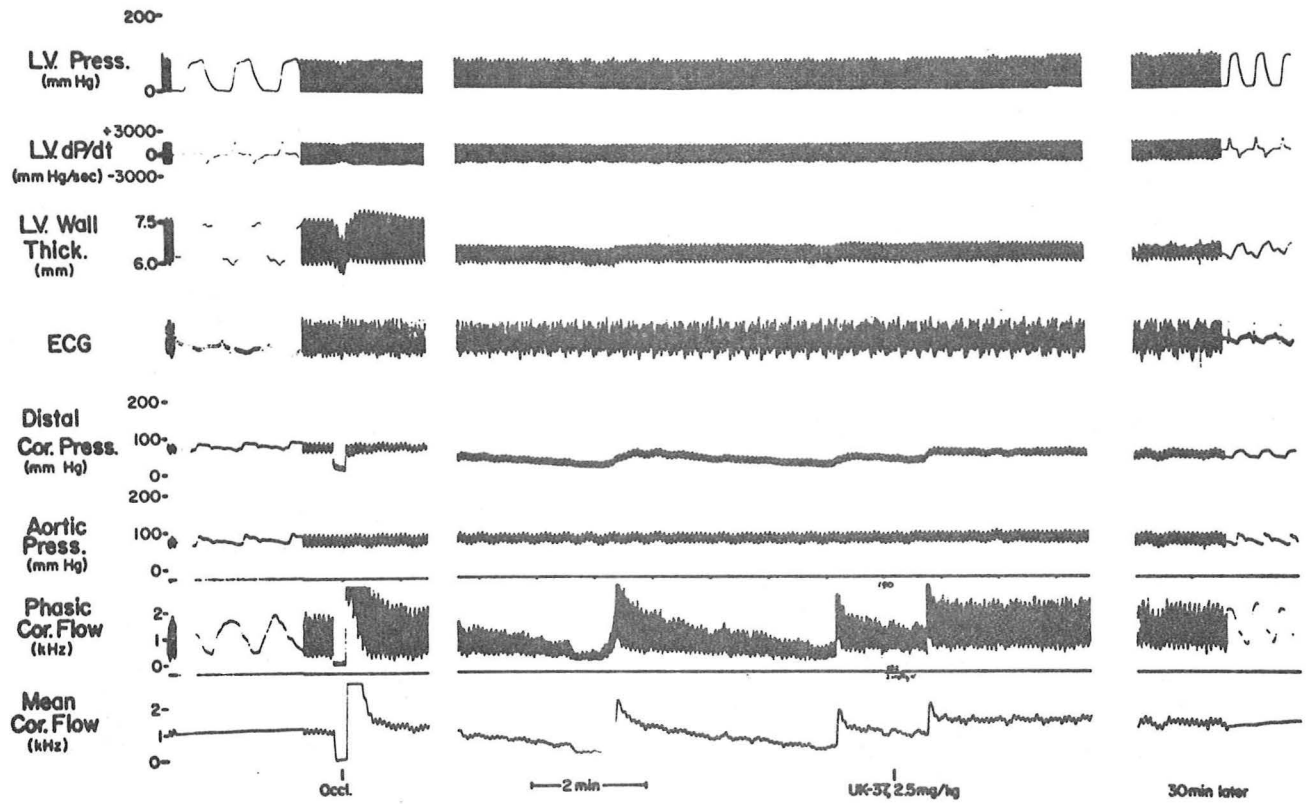

FIGURE 8

Legend for Figure 8

Representative recording from a dog with a severe coronary stenosis (panels 2 and 3). A 10 second total occlusion of the coronary artery produces a decline in LV dp/dt, LV wall thickening (and end-diastolic wall thickness), and distal coronary (cor) pressure (press). Release of the occlusion restores LV wall thickening and distal coronary pressure to normal and produces a reactive hyperemia (note overshoot in coronary blood flow). Placement of a severe stenosis (panel 2) causes a decrease in LV systolic wall thickening and distal coronary pressure and produces cyclic flow variations (CFRs), characterized by progressive declines, interrupted by sudden, spontaneous restorations of flow. After 60 minutes of CFRs, 2.5 mg/kg (iv) of the selective TxA_2 synthetase inhibitor dazoxiben, was given and the CFRs were abolished. This effect lasted for over 30 min (3rd panel).

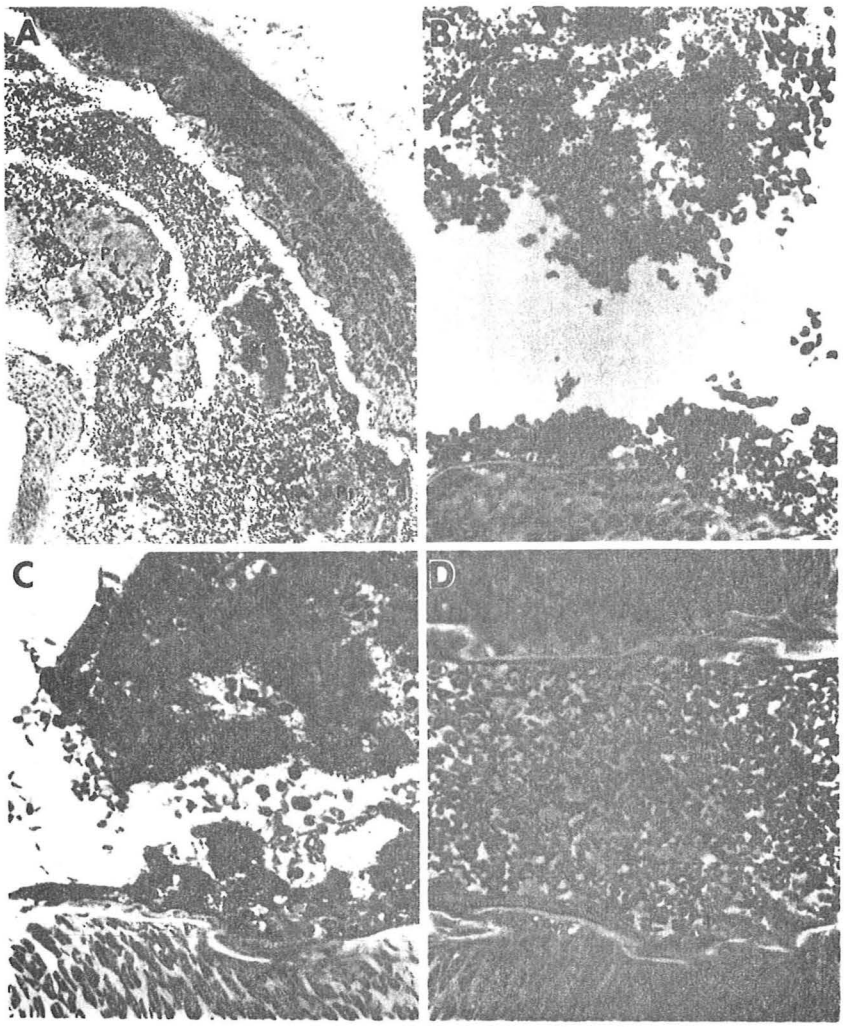

FIGURE 9

Morphology of coronary arteries. A. Peri-constrictor segment of LAD obtained at a point of marked cyclic blood flow reduction contains several masses of aggregated platlets (Pt) as well as erythrocytes (X60). B. Higher magnification view shows the small granular platelets, some of which are attached to the arterial wall (X370). C. In another arterial segment, leukocyte-fibrin aggregate is attached to the arterial wall (X370). D. Control coronary artery contains loosely arranged erythrocytes and a few leukocytes, but no platelet aggregates (X370). Toluidine blue-stained sections of methacrylate-embedded coronary arteries.

Effects of the Selective Thromboxane Synthetase Inhibitor, Dazoxiben, (UK-37,248)* on Cyclic Blood Flow Variations in Stenosed Canine Coronary Arteries

Cyclic flow variations developed in 28 animals instrumented as described previously (Figures 7 and 8). Placement of the constrictor around the proximal coronary artery reduced mean and phasic coronary blood flow 42±6 and 53±5%, respectively in saline-treated (control) dogs and 35±4% and 48±5% prior to dazoxiben administration. Compared to control (preconstriction), the hyperemic response following a 10 second total coronary occlusion was blunted or abolished (236±11 vs 82±7% of control, basal coronary blood flow, $p<0.001$). Initially, sudden spontaneous restorations of coronary blood flow alternated with progressive decreases in coronary blood flow and distal coronary artery pressure (Figure 8). The frequency of coronary flow variations continued unabated for several hours in the untreated animals.

We utilized radiolabeled microspheres to measure changes in regional myocardial blood flow in the control and dazoxiben-treated dogs. Table 3 describes the regional blood flow values for both groups of animals. Both subendocardial and subepicardial flow fell significantly during nadirs of coronary blood flow during the cyclic flow variations (Table 3). Blood flow in the subepicardium increased to control values during restorations of flow. Total coronary occlusion at the end of the experiment produced significantly greater declines in both subepicardial and subendocardial blood flow. Blood flow in nonischemic LV myocardium did not change significantly throughout these time periods.

TABLE 3

Regional Myocardial Blood Flow (RMBF; ml/min/g) in Ischemic and Nonischemic LV Myocardium Before and During Cyclic Flow Reductions and After Total Coronary Occlusion

LV Region	Level	Control (Pre-constriction)	CFR	Flow Restorations	Total LAD Occl
Ischemic	Epi	1.00 ± 0.08^a	0.37 ± 0.06^b	1.03 ± 0.16^a	0.15 ± 0.05^c
	Endo	0.90 ± 0.09^a	0.26 ± 0.07^b	0.72 ± 0.12^a	0.06 ± 0.02^c
Nonischemic	Epi	0.89 ± 0.08	0.84 ± 0.07	0.86 ± 0.08	0.91 ± 0.09
	Endo	0.99 ± 0.10	0.89 ± 0.07	0.90 ± 0.09	0.96 ± 0.10

CFR = cyclic flow reductions; Endo = endocardium; epi = epicardium; LV = left ventricular; Occl = occlusion.

All time points except total LAD occlusion were before saline or dazoxiben administration. RMBF was measured during total coronary occlusion at the end of the study. Within the same region and transmural layer of the LV, values with the same letter superscript are not significantly different from each other (ANOVA, Duncan's Multiple Range Test; $p < 0.05$; n=25 dogs).

The thromboxane synthetase inhibitor, dazoxiben (UK-37) (2.5 mg/kg), either completely abolished or severely attenuated the frequency and severity of cyclic flow variations (Table 4) (Figures 8 and 10). The nadir of coronary blood flow also increased significantly after dazoxiben administration (8.6±2.2 to 48.8±5.4% of control coronary blood flow). The frequency of cyclic flow variations remained unchanged after saline

*Kindly provided by Dr. Pedro Urquilla at Pfizer Pharmaceuticals in Groton, Connecticut.

administration and the nadir of coronary blood flow declined somewhat (18.7±5.7 to 13.4±4.1% of control coronary blood flow values). There was no difference in the original severity of coronary arterial constriction, nor in the frequency nor magnitude of cyclic flow variations between saline and dazoxiben-treated dogs during the first hour of observation and prior to the administration of either saline or dazoxiben.

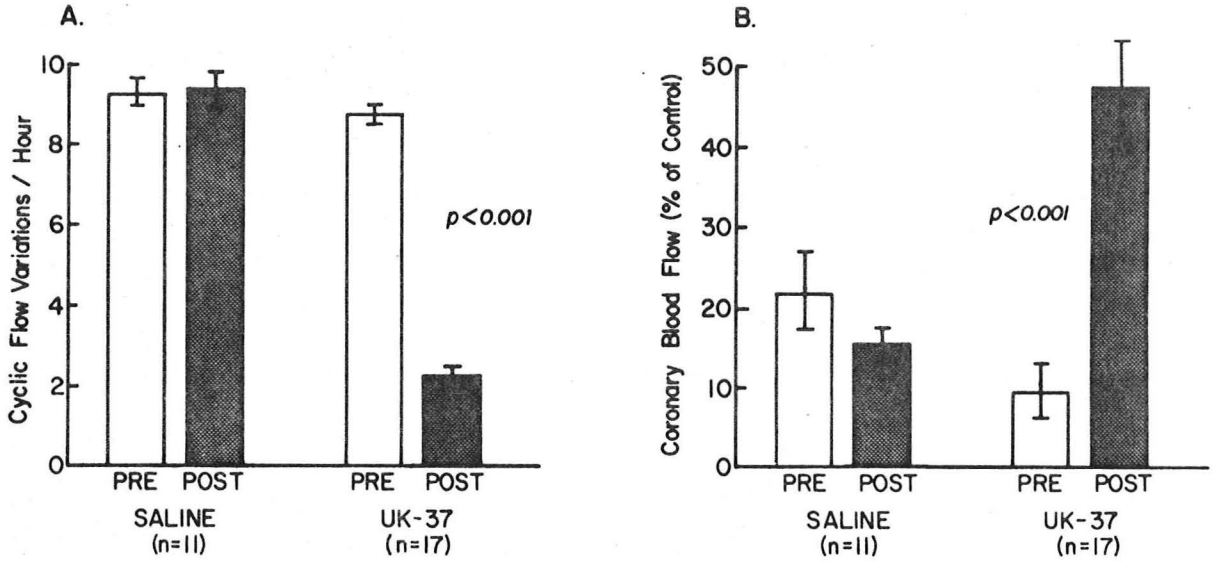

FIGURE 10

The number of cyclic flow variations per hour prior to and following saline administration in 11 dogs and prior to and following dazoxiben administration (UK-37) in 17 dogs is shown in the far left panel. Mean values and standard errors of the mean are demonstrated. Dazoxiben administration significantly reduced the number of cyclic flow variations as shown in the figure. In panel B, the nadir of coronary blood flow during cyclic flow variations prior to and following saline administration is shown in the left panel and prior to and following dazoxiben administration in the right panel. Note that dazoxiben increased the nadir of coronary blood flow during cyclic blood flow variations significantly.

TABLE 4

Frequency and Severity of Cyclic Flow Reductions (CFRs) Before and After Saline or Dazoxiben Administration

	Frequency (CFRs/hr)		Severity (nadir of CBF % control)	
	Pre-Rx	Post-Rx	Pre-Rx	Post-Rx
Saline (n=13)	8.8 ± 0.8	9.0 ± 1.0	18.7 ± 5.7	13.4 ± 4.1
	NS		NS	
Dazoxiben (n=28)	10.1 ± 0.8	3.2 ± 1.0	8.6 ± 2.2	48.8 ± 5.4
	$p < .001$		$p < .001$	

The average of 3 lowest nadirs were used to obtain a mean value for this parameter. When total abolition of CFRs occurred after dazoxiben administration, the 3 lowest values for CBF were used to compute a post-treatment value in this group of dogs.

Restoration of Coronary Blood Flow Variation with a Stable Endoperoxide Analog with Thromboxane-Like Effects

Figure 11 demonstrates a representative tracing in which cyclic flow variations, abolished completely by dazoxiben, were restored by the intra-atrial administration of U46619, a stable endoperoxide analog, which exerts thromboxane-like effects on platelet aggregation and coronary vascular resistance (21). U46619 was given to dogs whose cyclic flow variations were abolished completely by dazoxiben, and in 5 of 7 animals, the stable endoperoxide analog restored the cyclic flow variations.

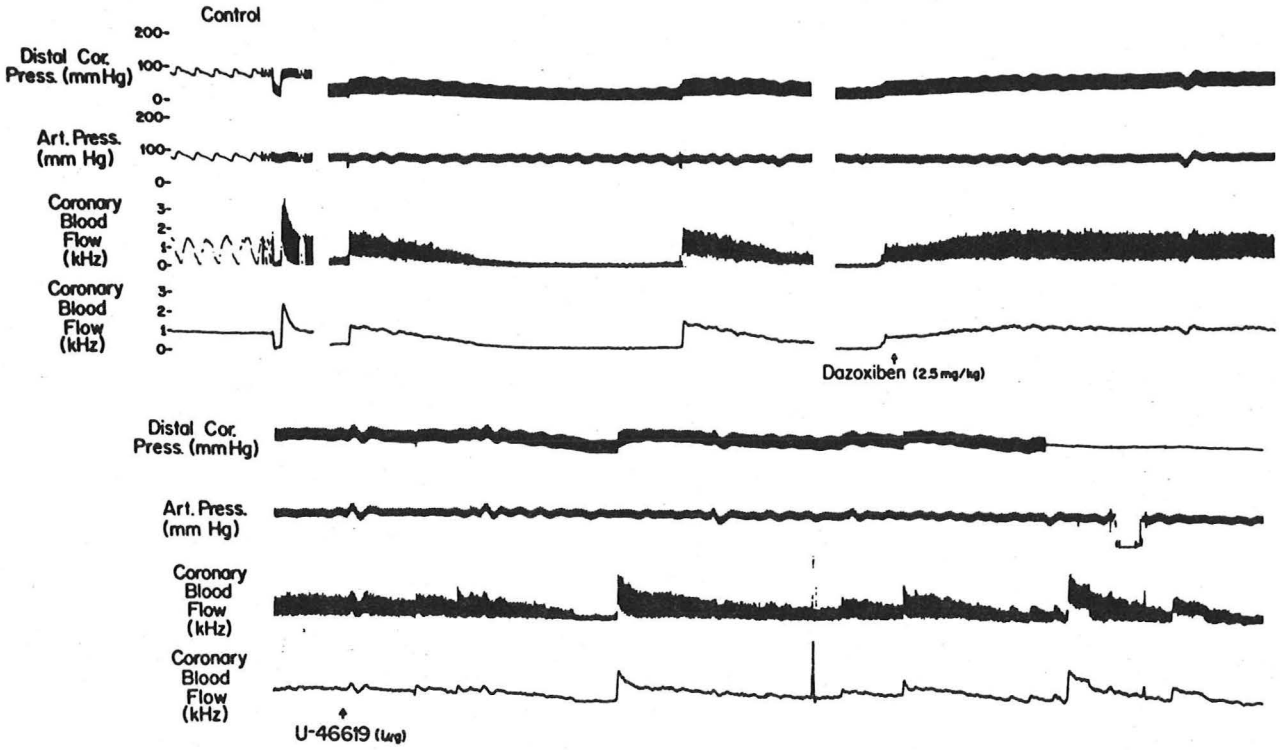

FIGURE 11

An original strip recording from an experiment in which CFRs, produced by constriction of the LAD coronary artery (second panel) and abolished by dazoxiben (third panel), were restored 1 hour later by the intra-atrial administration of U46619 (stable endoperoxide analog, 1 µg) as shown in the bottom 3 panels that demonstrate distal coronary pressure, systemic arterial pressure, and coronary blood flow alterations (top to bottom, respectively). The repeated administration of U46619 produced CFRs which equaled those observed before dazoxiben administration in their severity.

Hemodynamic Changes During Cyclic Flow Variations

Table 5 demonstrates the hemodynamic changes that occurred during the cyclic flow variations with dazoxiben or saline treatment. There were no significant differences between dazoxiben-treated and saline-treated groups in the control values of any hemodynamic variable. However, with time there were significant decreases in aortic systolic and diastolic pressures and in LV dP/dt in the dazoxiben-treated animals, although the magnitude of change was small.

TABLE 5

Hemodynamic Changes in Dogs with Coronary Arterial Stenosis

	TRT	Control	Immed	30 min	60 min	65 min	90 min	120 min
Heart Rate (beats/min)	Saline	147 ± 5	144 ± 8	150 ± 6	142 ± 8	136 ± 11	153 ± 8	142 ± 10
	Dazoxiben	146 ± 6	140 ± 5	147 ± 6	146 ± 6	143 ± 9	145 ± 5	149 ± 6
AOS (mmHg)	Saline	117 ± 4	111 ± 4	114 ± 4	121 ± 5	111 ± 4	114 ± 3	118 ± 5
	Dazoxiben	114 ± 2	108 ± 2[a]	109 ± 2[a]	106 ± 3[a,b]	109 ± 4[a]	104 ± 3[a]	102 ± 4[a,b]
AOD (mmHg)	Saline	93 ± 4	89 ± 4	91 ± 5	99 ± 5	90 ± 5	92 ± 4	93 ± 6
	Dazoxiben	91 ± 3	85 ± 3[a]	87 ± 3[a]	85 ± 3[a,b]	86 ± 4[a]	82 ± 4[a]	81 ± 4[a]
LV dP/dt_{max} (mmHg/sec)	Saline	1949 ± 96	1780 ± 101	1646 ± 56	1798 ± 63[a]	1609 ± 76	1784 ± 62	1890 ± 84
	Dazoxiben	1873 ± 59	1789 ± 81	1720 ± 58[a]	1606 ± 62[a]	1706 ± 93[a]	1697 ± 72[a]	1663 ± 78[a]

Dazoxiben (2.5 mg/kg, in 20 ml saline [n=28 dogs]) or saline (n=13 dogs) was given immediately after measurements were obtained at 60 minutes.

AOS = aortic systolic pressure; AOD = aortic diastolic pressure; LV dP/dt_{max} = maximal rate of rise of left ventricular pressure; Immed = immediately after producing coronary constriction; TRT = treatment.

[a]Significantly different from control ($p < 0.05$, ANOVA, and Duncan's multiple range test).

[b]Significantly different from the variable immediately above it.

Hemodynamic Effects of Dazoxiben

Dazoxiben did not produce hemodynamic changes in control animals without coronary constrictions. Figure 12 shows that dazoxiben, at doses of 1.0, 2.5, and 5.0 mg/kg did not affect heart rate, arterial (systolic or diastolic) pressure, coronary blood flow, or LV dP/dt, nor did it affect the transmural distribution of blood flow in 5 open-chest, anesthetized dogs.

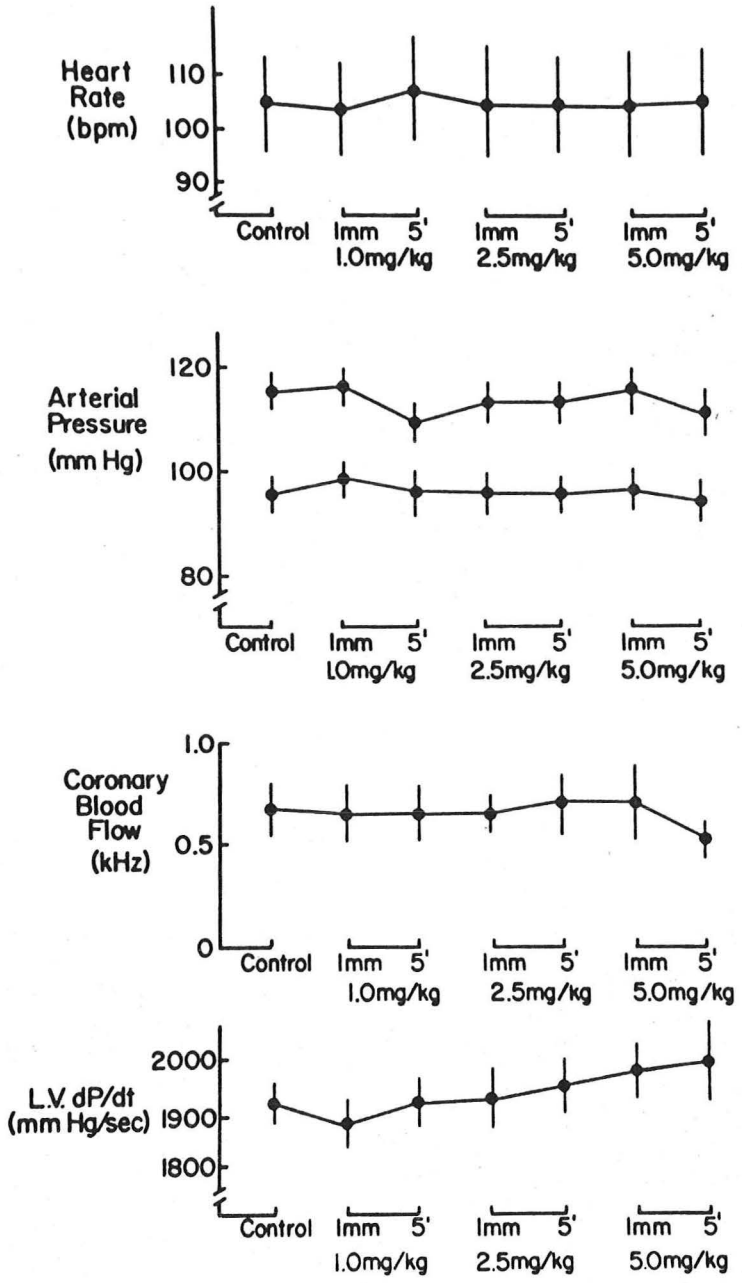

FIGURE 12

Dazoxiben was administered to 5 open-chest, pentobarbital anesthetized dogs without coronary constrictors in cumulative doses of 1.0, 2.5, and 5.0 mg/kg. There was no significant change in any hemodynamic variable.

Effects of Dazoxiben on Plasma Aortic and Distal Coronary Arterial TxB_2 and 6-keto $PGF_{1\alpha}$ Levels During Intravascular Platelet Aggregation

Figures 13 and 14 show the plasma TxB_2 levels proximal (aortic) and distal to the coronary stenosis, before, during cyclic flow variations, and after the administration of dazoxiben. Levels of TxB_2 in plasma sampled from the distal coronary catheter increased significantly from a control value of 71±18 pg/ml to 352±126 during cyclic flow variations. Aortic TxB_2 levels also increased significantly during cyclic flow variations from 73±12 to 145±32 pg/ml. As dazoxiben abolished cyclic flow variations, there was also a marked decrease in aortic and distal coronary arterial TxB_2 levels to control values (57±13, respectively). The levels of 6-keto $PGF_{1\alpha}$ in the distal coronary arterial blood also increased 259% from control levels of 133±22 to 344±41 pg/ml during cyclic flow variations (Figure 14B). In addition to decreasing TxB_2 levels, the administration of dazoxiben did not change the elevated prostacyclin values (343±82) (Figure 14B). The ratio of 6-keto $PGF_{1\alpha}$ (prostacyclin) to TxB_2 concentrations at the 3 time points studied is shown in Figure 14D. 6-keto $PGF_{1\alpha}$ and TxB_2 ratios declined from a control value of 3.0±0.67 to 2.9±0.9 with cyclic flow variations; this ratio increased significantly to 7.1±1.6 after dazoxiben administration. The increased ratio of 6-keto $PGF_{1\alpha}$ to TxB_2 after dazoxiben was due to the reduction in TxB_2 concentration.

Plasma Arterial Levels of TXB_2 (pg/ml) Proximal and Distal to the Site of Coronary Constriction and Platelet Aggregation ***In Vivo***

	N	Control	During Cyclic Coronary Flow Variation	Post Dazoxiben
Aortic	5	65 ± 17	187 ± 46	116 ± 70
Distal Coronary	5	47 ± 9	308 ± 110*	44 ± 12

Values shown are mean ± SE.

*Significantly different from levels at control and after dazoxiben administration (ANOVA [p=0.033]), Neuman-Keuls).

FIGURE 13

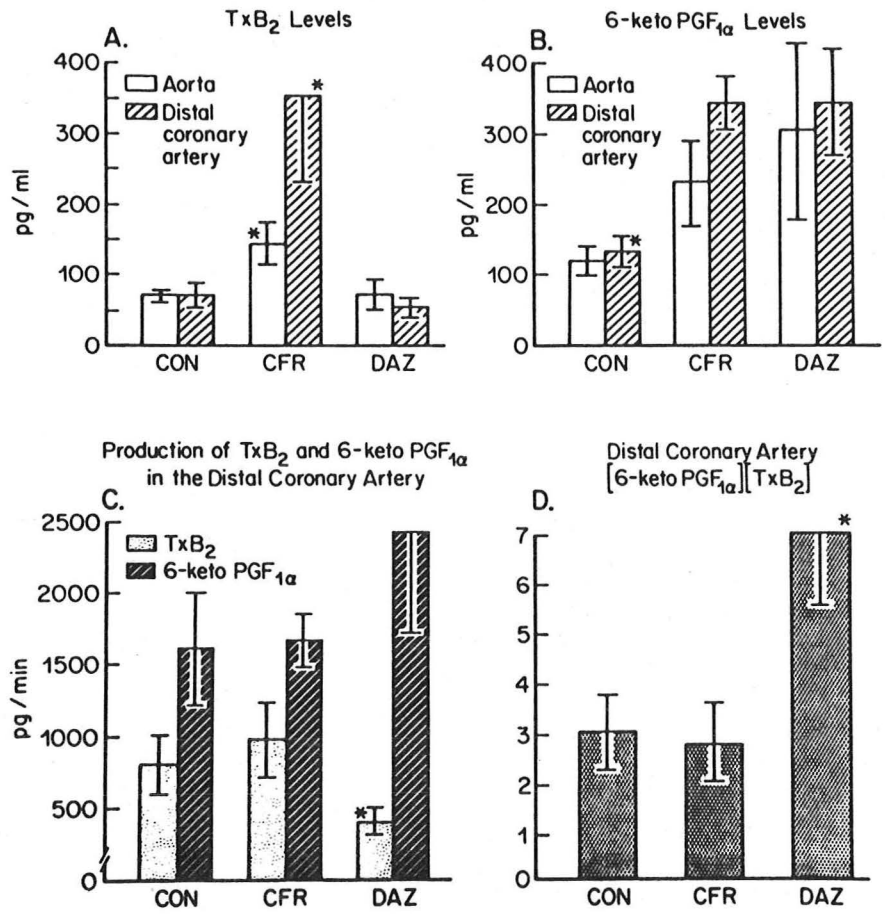

FIGURE 14

Changes in aortic and distal coronary arterial TxB_2 (A) and 6-keto $PGF_{1\alpha}$ (B) levels, before and during CFRs, and after dazoxiben administration. Production of TxB_2 and 6-keto $PGF_{1\alpha}$ (C) was calculated as the product of plasma concentration X blood flow rate from the distal coronary catheter. The ratio of 6-keto $PGF_{1\alpha}/TxB_2$ (D) in blood collected from the distal coronary artery catheter was obtained from values of concentrations (n=15). The n for all figures except C (where n=10) is 15 dogs. * = significantly different ($p<0.05$) vs other 2 time points (ANOVA and Duncan's multiple range test).

Effect of Dazoxiben on Aggregability and Prostaglandin and Thromboxane Production by Platelets In Vitro

Arachidonic acid-induced platelet aggregation as well as TxA_2 and PGE_2 production by platelet rich plasma prepared from blood samples taken before and after dazoxiben administration were measured in 3 dogs. Exogenously-added arachidonic acid stimulated PGE_2 and TxB_2 production by platelet rich plasma prepared from control (pre-dazoxiben) blood samples in all 3 dogs (Figure 15). TxB_2 (but not PGE_2) production was suppressed or eliminated in platelet rich plasma isolated from blood samples taken 5 and 30 minutes after dazoxiben administration in all 3 dogs. Arachidonic acid-induced platelet

aggregation also was suppressed by dazoxiben. Systemically-administered dazoxiben suppressed thrombin-induced platelet aggregation in vitro in 3 of 3 dogs.

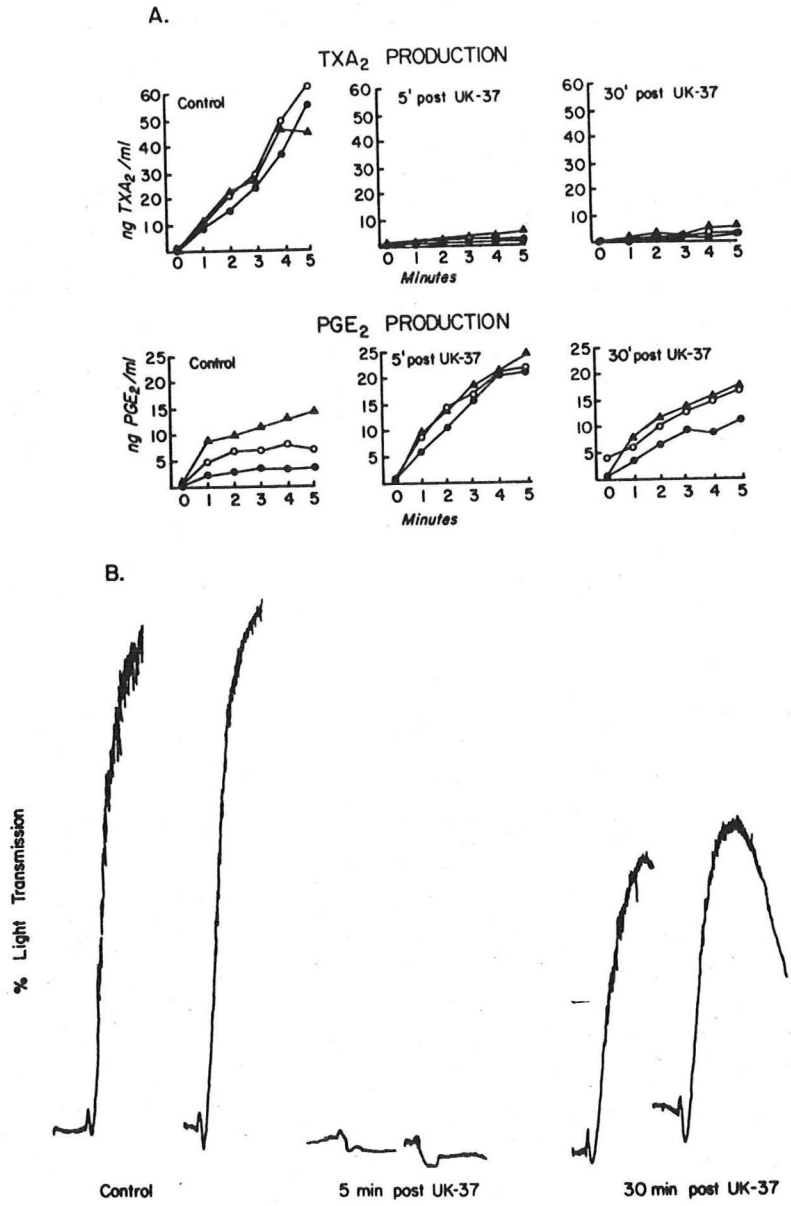

FIGURE 15

Panel A. Arachidonate-induced TxA_2 and PGE production by platelets isolated from blood obtained from 5 dogs before and 5 and 30 minutes after systemically administered dazoxiben, 2.5 mg/kg. Three concentrations of arachidonate were used: 3×10^{-4}M, —●—; 7.5×10^{-4}M, —O—; and 7.5×10^{-3}M,—▲—. Dazoxiben completely suppressed TxA_2 production, while PGE_2 production was unaffected. Panel B. The in vivo administration of dazoxiben also reduced platelet aggregation in vitro as shown. This effect appeared to be diminishing 30 minutes after dazoxiben administration.

Effects of Dazoxiben on Prostacyclin and PGE_2 Production by Canine Coronary Arteries In Vitro

Table 6 shows the effects of dazoxiben on endogenous PGE_2 and PGI_2 (measured as 6-keto $PGF_{1\alpha}$) production in vitro in canine coronary arterial rings. Dazoxiben did not alter the production of PGE_2 or 6-keto $PGF_{1\alpha}$ significantly.

TABLE 6

Effects of Dazoxiben on PGE_2 and 6 keto $PGF_{1\alpha}$ Production by Canine Coronary Arterial Segments In Vitro

	N	Control		Dazoxiben
PGE_2 (ng/mg)	8	0.20 ± 0.02		0.24 ± 0.03
			p = 0.336	
6 keto $PGF_{1\alpha}$ (ng/mg)	8	3.37 ± 0.25		4.52 ± 0.51
			p = 0.060	

Dazoxiben was added in vitro to yield a final concentration of 10^{-6}M.

N = number of coronary arterial segments.

Morphologic Observations

Peri-constrictor segments of the LAD obtained at the nadir of blood flow during cyclic flow variations contained microthrombi composed of masses of aggregated platelets, leukocyte-fibrin clumps, densely adherent erythrocytes, and variable consolidation of these components (Figure 9). Arterial walls exhibited multiple foci of endothelial denudation and damage to the internal elastic lamella. Platelets, fibrin, and leukocytes frequently were adherent to the arterial wall in these areas. Control vessels contained loosely arranged erythrocytes and a few other blood elements, but no aggregates of platelets were identified.

Summary of the Observations from the Experiments in Dogs with Proximal Coronary Arterial Narrowing and the Administration of a Specific Thromboxane Synthetase Inhibitor (Dazoxiben)

The data obtained in the studies described above demonstrate that the thromboxane synthetase inhibitor, dazoxiben, abolishes or markedly attenuates cyclic blood flow variations produced, presumably by alternating formation and dislodgement of platelet-red blood cell thrombi. These cyclic flow variations were accompanied by physiologically important changes in endocardial and epicardial blood flow and by contractile changes in myocardium rendered ischemic during the decreases in coronary blood flow. Therefore, the ability to prevent cyclic flow variations in this experimental model would be expected to translate into an important preservation of blood flow and contractile function.

Morphologic examination of coronary arteries at the site of constriction revealed platelet thrombi with blood erythrocyte and leukocytic involvement. These findings confirm the morphologic and radiographic observations of Folts et al (17,22) and support the view that cyclic flow variations in this model are due to platelet aggregation. Dazoxiben's elimination of stenosis-induced cyclic flow variations together with inhibition of TxB_2 synthesis in vivo and in vitro, and antiaggregatory effects in vitro argue strongly in favor of platelet aggregation as an important cause of the cyclic flow variations observed in this model.

The beneficial effects of dazoxiben do not appear to be due to hemodynamic effects, since the drug did not produce any systemic hemodynamic effects nor did it increase coronary blood flow in open-chest, anesthetized dogs not subjected to coronary arterial constriction. Folts et al have also shown that coronary vasodilators do not reverse cyclic flow variations in similar, experimentally-stenosed coronary artery preparations (17,22).

The cyclic flow variations were associated with 5 and 2 1/2 fold increases in the plasma concentrations of TxB_2 and 6-keto $PGF_{1\alpha}$, respectively in blood collected in the catheter draining the distal coronary arterial bed. Figure 14C shows the change in the "production" (concentration X distal coronary flow rate) of TxB_2 and 6-keto $PGF_{1\alpha}$ before and during cyclic flow variations and after dazoxiben administration. There was no significant change in the production of either TxB_2 or 6-keto $PGF_{1\alpha}$ during the cyclic flow variations. Therefore, the increase in concentration of TxB_2 and 6-keto $PGF_{1\alpha}$ appears to be more importantly related to the reduction in coronary blood flow than a major increase in thromboxane synthesis. Needleman et al have presented evidence from in vitro studies that endoperoxides formed by imidazole (a less potent TxA_2 synthetase inhibitor) treated platelets can be converted to prostacyclin by the endothelium of exogenously added bovine coronary arterial segments or microsomes (23). Aiken and associates (24) have provided indirect evidence that conversion of platelet-derived cyclic endoperoxides to prostacyclin does occur and plays a significant role in the antithrombotic effects of OKY1581, a selective TxA_2 synthetase inhibitor (24). Aiken et al have also shown that the administration of prostacyclin prevents cyclic flow variations in a similar experimental animal model (25). However, the fact that distal coronary arterial levels of 6-keto $PGF_{1\alpha}$ increased with cyclic flow variations in our study and remained elevated after dazoxiben administration, while TxA_2 levels also increased during cyclic flow variations and returned to normal after dazoxiben, argues for an important role of TxA_2 in producing platelet aggregation in this model. The ability of an exogenously-administered endoperoxide analog to restore cyclic flow variations after their abolition by

dazoxiben also suggests that TxA_2, per se, contributes importantly to the intravascular platelet aggregation in severely stenosed and (at least partially) de-endothelialized coronary arteries. Collectively, these findings indicate that cyclic flow reductions are associated with increases in TxA_2 concentrations and that their abolition by a selective TxA_2 synthetase inhibitor is associated with both a decrease in platelet TxA_2 synthesis and a beneficial intracoronary balance in the ratio of PGI_2 and TxA_2 concentrations. Moreover, in recent in vitro studies performed by Dr. James Schmitz, Dr. Philip Apprill, and Dr. William Campbell at this institution, it has been possible to demonstrate that prostacyclin production is markedly reduced at the site of the arterial constrictor and partial endothelial damage, but remains normal more distally in the same coronary artery, whereas, PGE_2 production remains normal or above normal at the site of the coronary arterial constrictor (unpublished observations). Therefore, the increases in prostacyclin found in the distal coronary artery in our model most likely originates distal to the site of coronary arterial narrowing, and presumably the increases in thromboxane are important in causing platelet aggregation and reductions in coronary blood flow at the site of the coronary arterial constrictor.

Other Alternatives for Altering Platelet Aggregation and Preventing Cyclic Flow Reductions with Experimental Coronary Arterial Stenosis

Table 7 demonstrates that several substances are capable of causing platelet aggregation (26). In particular, serotonin released from aggregating platelets might cause further platelet aggregation and alter coronary vascular tone in a manner similar to thromboxane. Indeed, we have found that thromboxane synthetase inhibitors abolish cyclic flow variations in approximately 90% of dogs with proximal coronary arterial stenoses. In the remaining 10% of dogs, ketanserin, a serotonin antagonist, abolishes the cyclic flow variations (27). Therefore, it appears that in selected canine models with proximal coronary arterial narrowing, serotonin antagonism is necessary to eliminate platelet aggregation at the site of a narrowed coronary artery and the resultant cyclic flow variations.

TABLE 7

Inducers of human platelet responses

Low molecular weight substances	Proteolytic enzymes	Particulate matter	Agglutinating agents
ADP	Thrombin	Collagen fibrils	Ristocetin + human factor VIII
Epinephrine	Trypsin	Polystyrene latex	Bovine factor VIII
			Zymosan
Thromboxane A_2		Glass	Antiplatelet antibody
			Aggregated immune complexes
Prostaglandins G_2 and H_2[a]	Snake venom proteases	Virus	Polylysine
Vasopressin			
Serotonin			

Relevance to Patients and Future Directions

In summary, our recent studies have demonstrated that thromboxane does accumulate across the coronary bed in patients with active unstable angina pectoris. Thromboxane accumulation also occurs in canine models with a severe, proximal coronary arterial stenosis, and endothelial denudation and a specific inhibitor of thromboxane synthesis prevents the platelet aggregation at the site of coronary arterial narrowing and the attendant cyclic flow reductions. However, it remains to be shown whether cyclic flow reductions occur in the patient with a tight proximal coronary arterial lesion or with multivessel coronary arterial stenoses. Moreover, it remains to be determined whether a specific inhibitor of thromboxane synthesis, i.e. dazoxiben would prevent such cyclic flow variations and platelet aggregation in patients just as occurs in the canine model. However, it should be noted that a recent multicenter Veterans Administration Hospital study has demonstrated that the equivalent of one aspirin per day reduces the frequency of myocardial infarction ($p=0.005$) and of death ($p=0.054$) in the 12 week period following its administration in patients with unstable angina pectoris (28) (Figure 16). These data suggest that platelet aggregation and thromboxane increases may be important in causing acute myocardial infarction and even death in such patients, but until similar studies are performed with a more specific thromboxane synthetase inhibitor this possibility remains unproved. Nevertheless, our recent findings and those of others are consistent with the possibility that platelet aggregation and thromboxane A_2 accumulation may play a causal role in initiating and/or sustaining unstable angina pectoris in patients (Figure 17) (29,30).

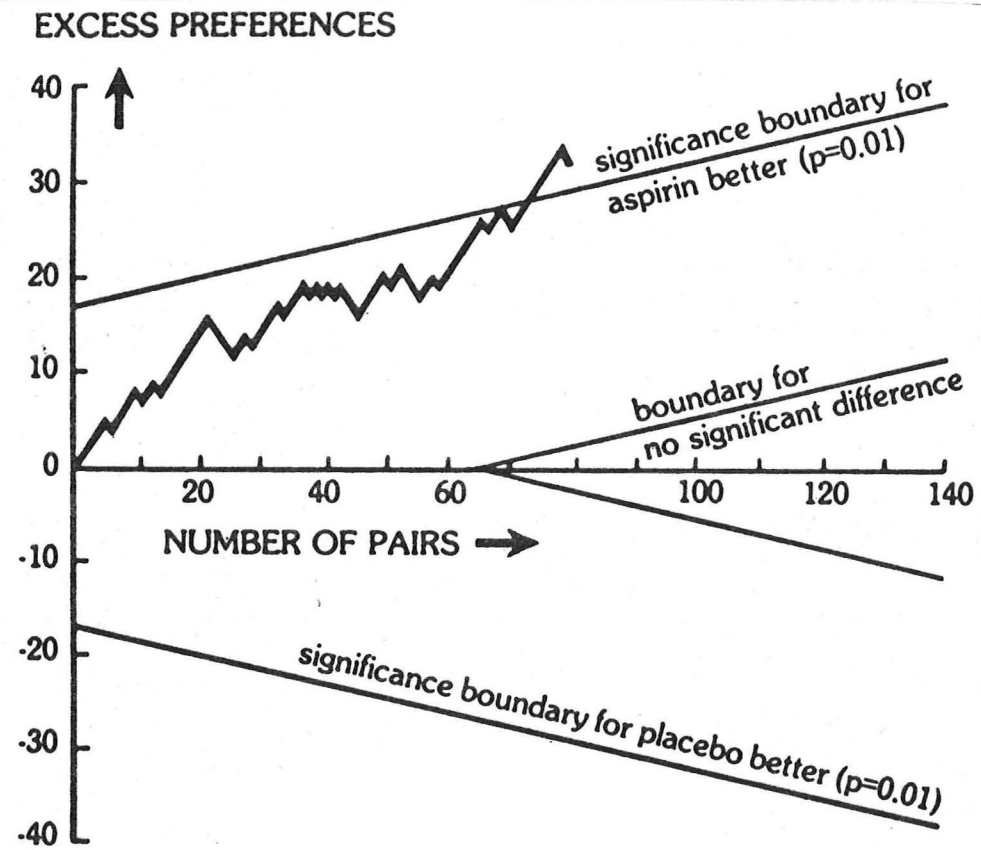

FIGURE 16

Legend for Figure 16

Sequential analysis for the combined end points death or acute myocardial infarction in patients with unstable angina. This is a sequential plan with a two-sided overall significance level of 0.01. Each patient receiving aspirin is paired with a patient receiving placebo on the basis of entry date. The abscissa represents the number of pairs in which a preference occurred (within a pair, the patient receiving one treatment died or had an acute myocardial infarction, whereas the other did not). The ordinate represents the director of the preferene (plot moves up one unit if preference is in favor of aspirin, down one unit if preference is in favor of placebo). If the sample path had crossed either boundary for no significant difference, one would have had 95% confidence that the reduction in the event rate is not as large as 50%. The significance boundary was crossed on the 75th pair when there were +29 excess preferences (52 preferences in favor of aspirin and 23 in favor of placebo). This ensured that the difference between the treatment groups for the end point death or acute myocardial infarction was significant at the 0.01 level even after the authors allowed for multiple tests during the trial. (This figure was reproduced from Reference 28).

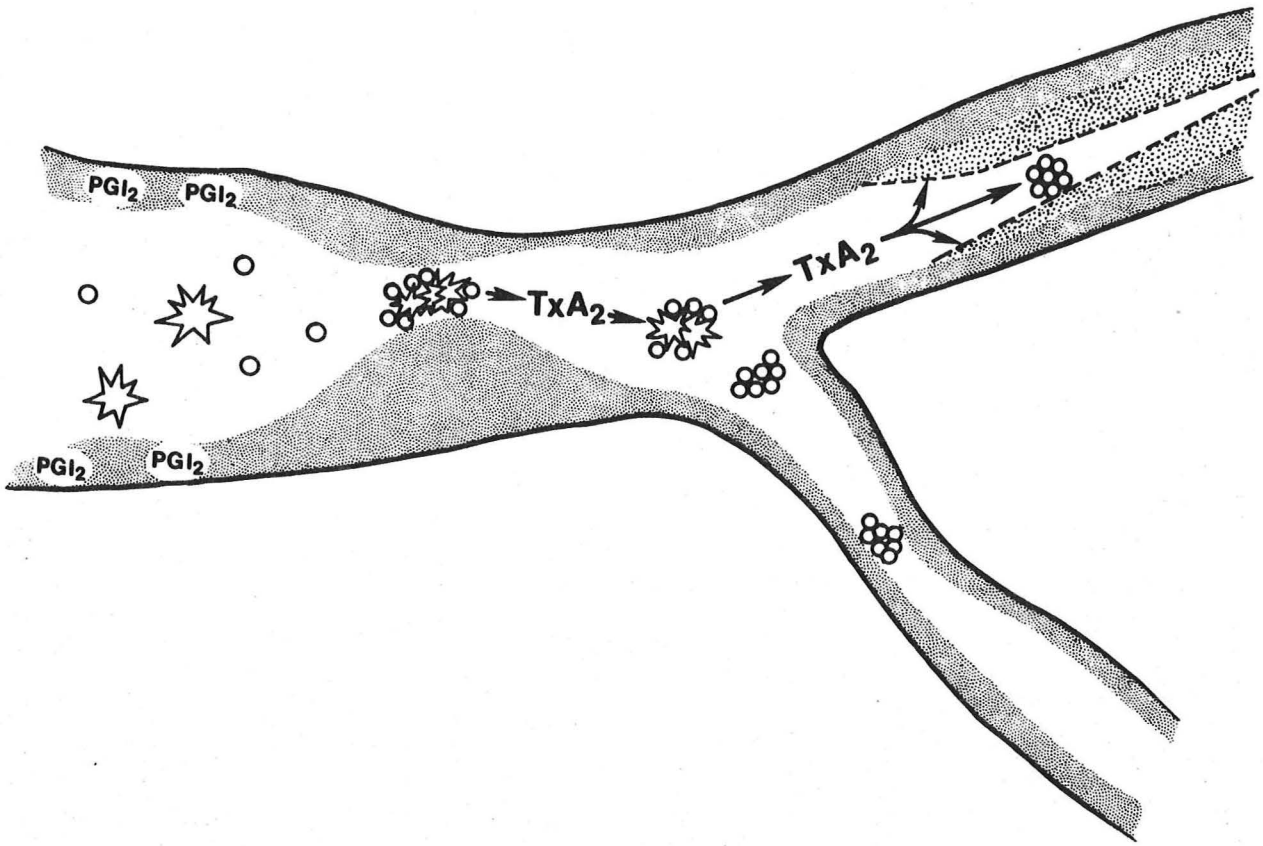

FIGURE 17

Legend for Figure 17

A schematic representation of the proposed interaction among prostacyclin, thromboxane, platelets, and vascular smooth muscle in the pathophysiology of unstable angina pectoris and acute myocardial infarction. Circulating platelets (O) become activated (*) and adhere to exposed collagen at the site of an atherosclerotic lesion. Adhering platelets undergo a release reaction: thromboxane A_2 (TxA_2) is produced and released, and platelet aggregation is initiated. Platelet aggregates may "plug" a distal coronary artery at the site of a stenosis, or TxA_2 may cause vasoconstriction, allowing platelet "plugs" to obstruct even a normal coronary artery. Thus, increases in TxA_2 may initiate or sustain the syndrome of unstable angina pectoris. Prostacyclin may prevent platelet adherence to normal vascular endothelium, and reduced amounts of PGI_2 in atherosclerotic coronary arteries may be important in the initiation of this pathologic process. (This figure was reproduced from Reference 29).

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