Elevation of Thromboxane B₂ Levels in Patients with Classic and Variant Angina Pectoris

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SUMMARY  Thromboxane A₂ (TXA₂), a vasoconstrictive prostanoid, causes intense spasm of isolated coronary vessels and increases platelet aggregability. To define the role of TXA₂ in the pathogenesis of angina pectoris, plasma levels of thromboxane B₂ (TXB₂), a biologically inactive product of TXA₂, were determined in the coronary sinus (CS), aorta (AO), and peripheral vein in 30 patients with angina pectoris. Determinations were made by radioimmunoassay using anti-TXB₂ antisera and [¹⁴C]TXB₂. Acidic lipids were extracted from plasma after treatment of samples with ethylenediamine tetraacetic acid (EDTA) and indomethacin. The 18 patients with effort angina and angiographically documented coronary stenosis (≥ 75%) showed a marked increase in peripheral TXB₂ (mean ± SD 505 ± 178 pg/ml plasma) compared with 24 normal subjects (254 ± 89 pg/ml plasma; p < 0.01). When AO and CS TXB₂ levels were determined in 10 cases with simultaneous measurements of CS blood flow during atrial pacing, calculated TXB₂ release in coronary circulation at rest (−2.3 ± 14.8 ng/min) markedly rose during pacing-induced myocardial ischemia (34.7 ± 50.6 ng/min; p < 0.01), while in four control subjects with normal coronary arteries the values at rest (−1.0 ± 5.0 ng/min) did not change significantly at peak pacing (−1.5 ± 10.9 ng/min). All 12 patients with variant angina had a marked increase in peripheral TXB₂ (802 ± 249 pg/ml plasma; p < 0.01); two of five cases who were subjected to coronary sampling showed increased TXB₂ levels both in CS and AO during a spontaneous attack or attacks induced by ergonovine or by atrial pacing, which were accompanied by coronary vasospasm and fluctuation of CS blood flow. These results indicate that increased TXA₂ production in the coronary circulation may be at least partly responsible for coronary vasospasm and angina.

MYOCARDIAL OXYGEN DEMANDS are met by variations of coronary blood flow, which may be mediated by endogenous chemical substances, such as adenosine, prostaglandins and catecholamines. Among these, prostaglandin-like substances (prostanoids) have recently been considered in the ischemic myocardium to serve as potent determinants of coronary vascular tone and regional blood flow.¹⁻³ Such vasoactive prostanoids were identified as thromboxane A₂ and prostaglandin I₂, which possess opposite effects on the control of vascular tone. Thus, thromboxane A₂, which is produced by an enzyme of platelet microsomes,⁴ causes vasoconstriction⁵ and increases platelet aggregability;⁶ prostaglandin I₂, which is produced by an enzyme of vascular microsomes,⁷ causes vasodilation⁸ and decreases platelet aggregability.¹⁰,¹¹ Thromboxane A₂ and prostaglandin I₂ are extremely labile substances, with half-lives of 30 seconds and 10 minutes, respectively. Thus, they are readily converted to thromboxane B₂ and 6-keto-prostaglandin (PG) F₁₀₂ respectively, biologically inactive and chemically stable catabolites, which are

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assayable through radioimmunoassay\textsuperscript{12, 13} or gas chromatography–mass spectrometry.\textsuperscript{14}

Coronary vasospasm, or abnormally increased coronary vascular tone, has recently been postulated to initiate the pathologic events associated with angina pectoris\textsuperscript{15, 16} and acute myocardial infarction;\textsuperscript{17, 18} however, acute coronary thrombosis is also considered a pathogenic trigger\textsuperscript{19, 20} in these diseases. In variant angina, the occurrence of coronary vasospasm is widely recognized.\textsuperscript{21–24}

Experimental evidence suggests that the contraction of coronary vessels and aggregability of blood platelets are interrelated and can be mediated by vasoactive prostanooids such as thromboxane A\textsubscript{2} and prostaglandin I\textsubscript{2} in the coronary circulation under normal or ischemic conditions.\textsuperscript{25–27} Therefore, it is clinically important to determine whether these prostanooids are associated with pathologic events, such as coronary vasospasm and thromboembolism, in patients with coronary artery disease. In cases with effort angina, platelet counts in the coronary sinus are significantly lower than those in the root of the aorta,\textsuperscript{28} possibly resulting from altered biosynthesis of vasoactive prostanooids within atherosclerotic vasculature that could increase platelet destruction. In our preliminary work determining plasma levels of radioimmunoassayable thromboxane B\textsubscript{2}, a circulating cataloible of thromboxane A\textsubscript{2}, we found that most patients with effort angina have increased thromboxane B\textsubscript{2} levels in peripheral venous blood and coronary sinus blood, both at rest and during atrial pacing stress.\textsuperscript{29, 30} Lewy et al.\textsuperscript{31} reported that patients with unstable angina have increased thromboxane B\textsubscript{2} in the coronary sinus during pacing-induced ischemia. Even greater increases in peripheral levels of thromboxane B\textsubscript{2} have been found in patients with variant angina.\textsuperscript{29, 32}

The present study was undertaken to further delineate the role of altered thromboxane metabolism in patients with classic effort angina and in patients with variant angina. Thromboxane B\textsubscript{2} levels were measured in the peripheral and coronary circulation under resting conditions and during angina that occurred spontaneously or was induced by atrial pacing or ergonovine.

**Materials and Methods**

**Patient Population**

Thirty patients with angina pectoris (23 males and seven females; mean age 56 years, range 41–68 years) were studied. Eighteen patients had effort angina and 12 had variant angina. The patients with effort angina had typical angina on exertion documented by supine bicycle ergometer with evidence of ischemic ST-segment depression, and had angiographically documented stenosis (more than 75%) in one, two or all of the major coronary arteries. Those with variant angina had spontaneous angina with ST-segment elevation (more than 0.2 mV), and seven of these patients had no significant stenosis in any of the major coronary arteries. Twenty healthy male volunteers (mean age 30 years, range 25–40 years) and four patients with normal coronary arteries without variant angina served as control subjects. Blood was taken from the antecubital vein of all subjects. Ten patients with effort angina and four patients with variant angina were subjected to an atrial pacing stress test, followed by coronary angiography. Four patients with variant angina were also subjected to an ergonovine test. During the pacing stress and ergonovine tests, blood was sampled from the coronary sinus and the aortic root. None of these subjects received antianginal drugs, such as propranolol, calcium antagonists (diltiazem or nifedipine) and nitrates, for 72 hours before the study, nor had they taken aspirin for at least 2 weeks.

**Procedures for the Stress Tests and Blood Sampling**

A #7F coronary sinus flow catheter (Webster Co.) was positioned in the coronary sinus and its position was confirmed by injection of Urografin 76. This catheter was used for coronary sinus sampling and measurement of coronary sinus blood flow through the thermodilution method using a Webster model CBA-210 flowmeter.\textsuperscript{33} A #8F pigtail catheter was also positioned in the sinus of Valsalva for aortic sampling and pressure recording. For ECG recordings, leads I–III and V\textsubscript{4–6} were used. Ten milliliters of blood were withdrawn at rest simultaneously from the coronary sinus and the sinus of Valsalva into heparinized plastic syringe. Coronary sinus blood flow was measured immediately after blood sampling.

Atrial pacing was initiated at a rate of 90 beats/min and increased in increments of 20 beats/min every 3 minutes. Pacing was stopped at the time of induction of angina or after maximal pacing at a rate of 150 beats/min. Simultaneous coronary arteriovenous sampling and measurements of coronary sinus blood flow were carried out immediately after completion of each pacing sequence and several minutes after completion of the entire test.

Ergonovine maleate was administered intravenously in the superior vena cava through a Swan-Ganz catheter as 0.1-mg bolus injections every 3–4 minutes until induction of typical angina or a maximal total dose of 0.4 mg. When angina was induced, a coronary angiogram was taken immediately after blood sampling and measurement of coronary sinus blood flow. Immediately after withdrawal into the syringe, 5-ml aliquots were transferred into polypropylene tubes containing 1-mM ethylenediamine tetraacetic acid (EDTA) and 0.1 mM indomethacin (in final concentration) for assays of thromboxane B\textsubscript{2}. Other aliquots were transferred to determine lactate, oxygen and carbon dioxide. Samples were stored on ice until completion of the stress tests. After the test, plasma was separated by centrifugation at 1500 g for 20 minutes.

**Radioimmunoassay for Thromboxane B\textsubscript{2}**

Anti-thromboxane B\textsubscript{2} antisera were supplied by the Upjohn Company, Kalamazoo, Michigan. The [5,6,8,9,11,12,14,15-\textsuperscript{3}H]arachidonic acid (98.5 Ci/mmol)
was obtained from New England Nuclear Corporation. Authentic thromboxane B₂ was obtained from the Ono Pharmaceutical Company. Biosynthesis of [³H]thromboxane B₂ was carried out according to the procedures of Fitzpatrick et al.,¹² with slight modifications. For [³H]thromboxane B₂ synthesis, [³H]arachidonic acid (120 µCi) was incubated with 25 mg of sheep seminal vesicles (Ran Biochemicals, Israel) and 18 mg of horse platelet microsomes, which were prepared from platelet-rich plasma by a previously described method,¹⁴ for 20 minutes at 37°C and at pH 7.5. The reaction mixture was acidified to pH 3.5 with 2 N hydrochloric acid and extracted twice with 2 volumes of ethyl acetate. The [³H]thromboxane B₂ was purified by thin layer chromatography on silica gel plates, which were developed by the solvent system, containing benzene: dioxane: acetate (60:30:3). Overall yield was 5.9% and purity was greater than 95%. For standardization of radioimmunoassay for thromboxane B₂ solutions of authentic thromboxane B₂ in 99% methanol were evaporated to dryness under nitrogen and were reconstituted in 0.1 M phosphate-buffered saline (0.9%), pH 7.4, with gelatin (0.1%) to concentrations of 5–1000 pg/ml. These samples were incubated with antisera and tritiated compounds as described below.

To determine thromboxane B₂ in plasma, each 1-ml sample of plasma was acidified by 0.1 ml of 1 N hydrochloric acid and extracted by 4 ml of ethyl acetate (vortex 20 sec × 2, centrifugation 3000 rpm, 15 minutes) and dehydrated by anhydrous Na₂SO₄. One-milliliter plasma extracts were evaporated to dryness under nitrogen at 40–55°C and resuspended in 0.1 M phosphate-buffered saline (0.9%), pH 7.4, with gelatin (0.1%), diluted appropriately (usually 1:4), and subjected to incubation with antisera and tritiated compounds. Then, 0.05 ml of [³H]thromboxane B₂ (15,000 cpm) was added to antiserum (0.05 ml), which was appropriately diluted (usually 1:400 to 1:1000). The final assay volume was 0.15 ml. The mixtures were incubated for 60 minutes at room temperature and left standing at 4°C for 16–24 hours. Antibody-bound thromboxane B₂ was separated from unbound compound with 0.1 ml of dextran-coated charcoal (mixture of 0.375 mg of dextran and 3.75 mg of charcoal) by centrifugation at 12,000 g for 30 seconds and supernatant containing antibody-bound thromboxane B₂ was counted. All assays had 95% recovery efficiency. The sensitivity of the assay was 10 pg per plasma sample (0.25 ml), and a slight cross-reactivity was seen only with prostaglandin D₂ with which relative crossreaction was less than 1%. The ability of the assay to detect known amounts of thromboxane B₂ is shown in figure 1. Plasma thromboxane B₂ determinations were expressed in pg/ml of plasma. In our laboratory, this method has a coefficient of variation of 10% for duplicate determination. Net thromboxane B₂ flux from coronary circulation was calculated by arteriovenous difference in thromboxane B₂ levels multiplied by coronary sinus blood flow.

**Plasma Lactate Determinations**

Samples for lactate were taken, immediately deproteinized in an equal volume of 0.6 M perchloric acid, and subsequently analyzed by the method of Rosenberg.¹⁸ Percent lactate extraction was calculated as the ratio of arteriovenous difference to the arterial level (A−V)/A × 100.

**Coronary Sinus Blood Flow Measurements**

Coronary sinus blood flow was measured by the continuous thermodilution technique.¹⁹ Five percent dextrose in water at room temperature was injected into the coronary sinus using a Harvard pump at a rate of 38 ml/min. Coronary sinus blood flow (CSBF) was calculated using the formula²⁰: (T₀ − Tᵢ) / (T₀ − Tₘ) − 1)(1.08), where T₀, Tᵢ and Tₘ are the temperature of blood, indicator and a mixture of blood and indicator, respectively, and 1.08 is a constant derived from the density and specific heat of the mixture of dextrose solution and blood.

Statistical analysis was performed using the t test with paired and unpaired comparisons where appropriate.

**Results**

**Peripheral Plasma Levels of Thromboxane B₂ in Normal Subjects and Patients with Angina Pectoris**

Blood was taken at rest from the antecubital vein of the 20 normal subjects and four patients with atypical
During a nonanginal period (usually 10 a.m. to noon), all cases of variant angina had a marked increase in peripheral vein thromboxane $B_2$ levels (802 ± 249 pg/ml; p < 0.01). Serial samples at 4-hour intervals from a patient with variant angina revealed a marked increase in the thromboxane $B_2$ level in peripheral vein at midnight (820 pg/ml) and 4 a.m. (616 pg/ml) compared with 8 a.m. (560 pg/ml), noon (476 pg/ml), 4 p.m. (528 pg/ml) and 8 p.m. (484 pg/ml). The patient had no attacks of angina during this 24-hour period.

Before the atrial pacing stress and ergonovine test, coronary sinus levels of thromboxane $B_2$ showed the same levels as the corresponding peripheral samples for normal subjects, patients with effort angina and patients with variant angina (fig. 2).

**Thromboxane $B_2$ Production in the Coronary Circulation During Atrial Pacing**

Ten patients with effort angina were subjected to atrial pacing. In all cases, percent lactate extraction at rest (33 ± 14%) markedly reduced at peak pacing (4 ± 20%), which was accompanied, in eight cases, by typical angina. Another four cases with a chest pain syndrome who had normal coronary arteriograms and did not develop angina or produce lactate during pacing served as control subjects. Representative findings in a patient with effort angina are shown in figure 3. The thromboxane $B_2$ levels in the coronary sinus and in the aorta at rest were significantly higher than in normal subjects. Coronary sinus blood flow increased gradually from the control value of 53 ml/min to 96

**FIGURE 2.** Comparison of plasma thromboxane $B_2$ levels in a peripheral vein and the coronary sinus (Cs) in normal subjects and patients with angina pectoris. Twenty normal subjects (open circles) and four patients with normal coronary arteries (open triangles) with a "chest pain syndrome" served as controls. All of the patients with effort angina (closed circles) had angiographically documented stenosis (≥ 75%) in one, two or all of the major coronary arteries. All of the patients with variant angina showed typical ECG changes (ST-segment elevation of more than 0.2 mV) during spontaneous angina; closed squares and closed triangles represent the cases with and without significant stenosis (≥ 75%) in major coronary arteries. In five cases with stenosis (closed squares), spontaneous or ergonovine-induced vasospasm was demonstrated during a typical anginal attack. Horizontal bars represent mean values. Numbers at the bottom indicate the mean ± SD (pg TXB2/ml plasma).

chest pain and normal coronary arteries. Mean thromboxane $B_2$ levels were 254 ± 89 pg/ml (mean ± SD) (fig. 2). When serial samples at 8-hour intervals within 2 days were obtained from a normal subject, thromboxane $B_2$ levels of the intradividual samples were 191 ± 31 pg/ml, and thus, peripheral thromboxane $B_2$ levels did not fluctuate significantly during a day or between days. The thromboxane $B_2$ levels were not affected by the age of the subject.

Even when blood was taken at rest, many patients with effort angina had significantly greater than normal thromboxane $B_2$ levels in the peripheral circulation (antecubital vein) (fig. 2). The mean thromboxane $B_2$ level for the 18 patients with effort angina was 505 ± 178 pg/ml ($p < 0.01$). Seventy percent of these patients had had angina in the 48 hours before sampling. None of the patients had had an attack within 1 hour of sampling.

The 12 cases with variant angina had reversible ST-segment elevation of more than 0.2 mV associated with angina during a spontaneous attack. In the five cases with ≥ 75% stenosis of a major artery, spontaneous or ergonovine-induced vasospasm was demonstrated during a typical anginal attack. Even

**FIGURE 3.** Effects of atrial pacing on plasma thromboxane $B_2$ levels in the aorta (Ao) and coronary sinus (Cs) in a patient with 90% stenosis of the proximal portion of left anterior descending coronary artery and the right coronary artery. The abscissa represents time after onset of pacing. Pacing rates are indicated at the bottom of the figure. CsBF = coronary sinus blood flow.
ml/min at peak pacing and then decreased to the basal level after pacing. Thromboxane B₂ production in coronary circulation was estimated by multiplying the arterial–coronary sinus difference in thromboxane B₂ levels by coronary sinus blood flow. Production was 37.3 ng/min at peak pacing in the case shown in figure 3, in contrast to the four controls, who did not show thromboxane B₂ production during pacing. For the group of patients with effort angina, arterial thromboxane B₂ levels were either constant or slightly decreased during atrial pacing, whereas thromboxane B₂ levels in coronary sinus showed moderate to marked increases with increased coronary sinus blood flow. In 10 patients with effort angina, thromboxane B₂ production in coronary circulation at rest (−2.3 ± 14.8 ng/min) rose markedly during pacing-induced ischemia (34.7 ± 50.6 ng/min; p < 0.01), while in four controls the value at rest (−1.0 ± 4.0 ng/min) did not change significantly at peak pacing (−1.5 ± 10.9 ng/min) (fig. 4). Six of the 10 patients with effort angina had markedly increased thromboxane B₂ production in the coronary circulation during peak pacing.

Thromboxane B₂ Alterations During Ischemia in Patients with Variant Angina

Atrial pacing was performed in four patients with variant angina who had exertional angina as well as rest angina accompanied by typical ST-segment elevation. In none of these cases were significant stenotic lesions demonstrable during coronary angiography performed after blood sampling. The changes in thromboxane B₂ in the aorta and coronary sinus in patients with variant angina were less consistent than those observed in patients with classic effort angina and obstructive coronary atherosclerosis. Even before spasm, unusual discrepancies were present between aortic and coronary sinus thromboxane B₂ levels (table 1). Figure 5 presents data for two patients who developed severe angina at pacing rates of 110 and 150 beats/min, respectively. In both cases, thromboxane B₂ levels in the aorta and coronary sinus at rest were markedly higher than normal, and aortic levels greatly exceeded coronary sinus levels. Marked increases in aortic thromboxane B₂ levels were detected before (case 1) or almost simultaneously with (case 2) induction of angina. In case 1, aortic thromboxane B₂ markedly increased and then gradually decreased, while thromboxane B₂ in the coronary sinus gradually increased. Termination of angina coincided with reduction of aortic thromboxane B₂ to its original level, which was similar to that of the final level of thromboxane B₂ in the coronary sinus. Coronary sinus blood flow markedly decreased and lactate production began at the onset of anginal pain. In case 2, aortic and coronary sinus thromboxane B₂ levels did not change up to the pacing rate of 150 beats/min, when an abrupt increase in aortic levels occurred concomitant with the onset of angina. Despite this increase, coronary sinus thromboxane B₂ levels remained unchanged, but the increased thromboxane B₂ could have been produced in the portion of the coronary circulation that does not drain into the coronary sinus. Termination of pacing resulted in a precipitous decrease in aortic thromboxane B₂ levels. A slight and transient increase in coronary sinus thromboxane B₂ occurred immediately after the peak of aortic thromboxane B₂ levels.

Coronary angiograms were performed immediately after blood sampling and measurements of coronary sinus blood flow. In case 1 (fig. 6), spontaneous vasoconstriction (fig. 6B) accompanied a marked increase in thromboxane B₂ level in the coronary sinus. Sublingual nitroglycerin, 0.3 mg, resulted in reduction of the thromboxane B₂ level to the control state, with simultaneous relief of the vasospastic obstruction. Intravenous ergonovine maleate induced a second episode of vasoconstriction (fig. 6C), during which the thromboxane B₂ level in the coronary sinus again increased. In the other patient (case 2) with variant angina, in whom atrial pacing induced vasoconstriction in the left anterior descending coronary artery, the thromboxane B₂ level increased significantly in the coronary sinus. No significant coronary sinus thromboxane B₂ increase was seen in a patient in whom ergonovine provoked spasm in the right coronary artery (case 3) or in two other patients to whom ergonovine was administered (cases 4 and 5).

**Discussion**

Thromboxane A₂ is a short-lived, biologically active prostanoid, considered to be degraded in a 1:1 ratio to thromboxane B₂, which is biologically inactive and
TABLE 1. Plasma Thromboxane B₂ in Aorta and Coronary Sinus During a Vasospastic Attack in Patients with Variant Angina

<table>
<thead>
<tr>
<th>Case</th>
<th>Induction of angina</th>
<th>Spastic vessels</th>
<th>TXB₂ levels (pg/ml)</th>
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<tr>
<td></td>
<td></td>
<td>Ao</td>
<td>Cs</td>
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<td></td>
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<td>Before spasm</td>
<td>During spasm</td>
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<tr>
<td>1</td>
<td>Pacing</td>
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<td></td>
<td>Spontaneous</td>
<td>LAD</td>
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<tr>
<td></td>
<td>Ergonovine (0.4 mg)</td>
<td>LAD</td>
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<tr>
<td>2</td>
<td>Pacing</td>
<td>LAD</td>
<td>1320</td>
</tr>
<tr>
<td>3</td>
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<td>RCA</td>
<td>405</td>
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<tr>
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<td>LAD</td>
<td>740</td>
</tr>
<tr>
<td>5</td>
<td>Ergonovine (0.1 mg)</td>
<td>LAD</td>
<td>672</td>
</tr>
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Abbreviations: Ao = aorta; Cs = coronary sinus; LAD = left anterior descending coronary artery; RCA = right coronary artery; TXB₂ = thromboxane B₂.

Chemically stable. Intravenously injected [³H]thromboxane B₂ has been found to remain in the intravascular space for a considerable period; after 13 hours, 74% was secreted into the urine. Thromboxane B₂ is not readily degraded in the pulmonary circulation, unlike other prostanoic substances. The relatively rapid decline in thromboxane B₂ levels in aortic samples obtained during pacing-induced angina in the patients with variant angina could be explained by a rapid distribution phase, which may be followed by a more stable phase.

Blood platelets are labile corpuscles that can readily aggregate and produce an artifactual increase in thromboxane levels during and after blood sampling. Therefore, in the present study, samples were mixed with EDTA and indomethacin in polypropylene tubes immediately after withdrawal. This technique halted platelet aggregation and inhibited the enzyme cyclo-

**FIGURE 5.** Elevation of thromboxane B₂ levels in patients with variant angina induced by atrial pacing. In case 1, angina was induced at a pacing rate of 110 beats/min, which was incompletely relieved by nitroglycerin (NTG) administered at 13.5 minutes. In case 2, angina was induced at the pacing rate of 150 beats/min, which was immediately relieved by cessation of pacing. The pacing rates are indicated at the bottom of the figure. Ao = aorta; Cs = coronary sinus; CsBF = coronary sinus blood flow.
ELEVATED THROMBOXANE B₂ IN ANGINA/Tada et al.

FIGURE 6. Representative cine frames from the left coronary angiograms in the 30° right anterior oblique projection, taken in a patient with variant angina during nonanginal period (control) (A) and during spontaneous (B) and ergonovine-induced (C) angina. (A) Almost normal coronary arteries, except that the diameter in left main coronary artery and proximal portion of the left anterior descending coronary artery (LAD) are slightly narrowed. Spontaneous angina accompanied severe vasospastic obstruction (≥90%) in the LAD and slight obstruction in left circumflex coronary artery (LCX). Sublingual nitroglycerin (0.3 mg) relieved spasm. Subsequent i.v. injection of ergonovine maleate (0.4 mg) induced the second anginal attack, which accompanied complete obstruction in the LAD (C).

oxygenase. We and others have demonstrated that plasma thromboxane B₂ levels and platelet aggregability are not affected by these sampling procedures. Silver et al. showed that ex vivo prostaglandin formation by human platelets could not be demonstrated when platelets were removed by centrifuging blood in the presence of EDTA. Therefore, values probably represent true levels of plasma thromboxane B₂ rather than artifacts that might be introduced during and after blood sampling.

Plasma levels of thromboxane B₂ were determined in the peripheral and coronary circulation in patients with angina pectoris to define the possible involvement of this prostanoid in the pathologic events associated with angina. In patients with severe coronary artery stenosis and effort angina, thromboxane B₂ in a peripheral vein and in the coronary sinus were significantly increased at rest. During myocardial ischemia produced by atrial pacing, a significant increase in thromboxane B₂ production coincided with the onset of angina in more than 50% of cases. The observed thromboxane production during pacing-induced myocardial ischemia is essentially in accord with the report by Lewy et al., who showed by using slightly different assay procedures that atrial pacing stress resulted in significant release of thromboxane B₂ in almost 60% of cases with severe coronary stenosis (unstable angina). However, their thromboxane assay was not sensitive enough to detect thromboxane B₂ levels below 200 pg/ml (0.5 pmol/ml), probably because they used antisera with sensitivity relatively lower than ours, and they incubated the antisera with patient plasma without extracting thromboxane B₂. The sensitivity of our assay method exceeds that of Lewy et al., in that as little as 40 pg/ml (0.1 pmol/ml) of thromboxane B₂ could be detected, enabling us to define normal range of peripheral thromboxane B₂ levels (254 ± 89 pg/ml, or 0.69 ± 0.24 pmol/ml) and to detect subtle increments in peripheral and coronary sinus levels of thromboxane B₂ in angina pectoris.

The present report indicates that many, but not all, patients with effort angina have significant increases in peripheral thromboxane B₂ levels during nonanginal periods. Atrial pacing-induced augmentation of thromboxane B₂ in the coronary sinus reported by Lewy et al. (260–4800 pg/ml, or 0.7–13 pmol/ml) was well above their assay threshold, and was on the same order of magnitude as that in the present report (300–1300 pg/ml). Also, in the present study, coronary sinus blood flow was measured during atrial pacing, which substantiated the increased production of thromboxane B₂ in coronary circulation. Thus, using more sensitive assay procedures, we defined normal levels of thromboxane B₂ and could examine more quantitatively the increased production of thromboxane B₂ in coronary circulation during pacing-induced ischemia.

All patients with variant angina showed markedly increased thromboxane B₂ levels in peripheral blood during nonanginal periods. In some cases with variant angina, pacing stress induced a marked increase in thromboxane B₂ in the coronary sinus and in the aortic root. This increase occurred before or with the onset of angina. An increase in thromboxane B₂ in the coronary sinus was also found during spontaneously or ergonovine-induced vasospastic attacks. These results suggest that an increased production of thromboxane A₂ is associated with angina in patients with variant angina. From the present study, we do not know
whether increases in plasma thromboxane B₂ are of primary or secondary significance. Excessive thromboxane release could cause vasospasm and/or increased platelet aggregability. Alternatively, coronary vasospasm could be the primary event: The spasm-induced regional ischemia might lead to an increase in thromboxane formation derived from circulating blood platelets. The observed thromboxane increases in the coronary sinus during coronary spasm is in accord with the report by Robertson et al., who showed that coronary sinus blood in patients with variant angina may contain increased numbers of platelet aggregates during vasospastic attack. During spasm and myocardial ischemia, levels of thromboxane B₂ in both aortic and coronary sinus are augmented in almost all cases. These observations led us to speculate that a significant amount of thromboxane A₂ in patients with variant angina may be derived from a tissue such as pulmonary tissue that is proximal to the heart. These findings explain why Raynaud's phenomenon is associated with variant angina. It would be interesting to examine whether thromboxane B₂ flux from the pulmonary tissue is increased, particularly under conditions where hyperventilation is the predominant cause in inducing coronary vasospasm. The present indication that increased peripheral thromboxane B₂ levels are associated with variant angina may be clinically significant in that vasospastic disorders are related to the altered metabolism of thromboxanes. It is not known if the observed elevation of thromboxane B₂ is primarily derived from the altered platelet function or is caused secondarily by the altered metabolism of prostaglandin I₂ in the vascular wall.

Mehta et al. found that platelet counts in the coronary sinus in patients with severe coronary stenosis are markedly decreased. Thus, the production of thromboxane B₂ in patients with effort angina may be associated with increased destruction of blood platelets as they circulate through impaired coronary vessels. This contention may be supported by the present findings that in patients with effort angina, the pacing stress induced marked increases in net release of thromboxane B₂ from the coronary circulation (figs. 3 and 4). However, the increase could also be produced by increased arachidonic acid release into the coronary circulation during ischemia. Increased arachidonic acid levels may produce an increase in thromboxane A₂ by serving as a substrate for tissue cyclooxygenase, resulting in increased prostaglandin H₂, a precursor of thromboxane A₂.

That some patients with effort angina do not have increased thromboxane B₂ levels in peripheral blood (fig. 2) and thromboxane B₂ release in coronary circulation (fig. 4) indicates that in some cases, increased thromboxane A₂ production is not required for the pathogenesis of angina. In such patients, exertional angina may be produced by relative ischemia caused by stenotic arteries rather than coronary vasospasm. Thromboxane B₂ production may also reflect the state of impairment of the vascular wall; a metabolically active vascular lesion may induce platelet aggregibility, while an inactive lesion may not. Prostaglandin I₂, a potent coronary vasodilator, may also be altered in these patients.

Our data have important clinical implications for the pathophysiologic occurrence associated with impaired interactions between functions of vascular disorders. Perhaps the greatest significance of our results is related to recently evolving clinical concepts, in which coronary vasospasm and/or increased aggregability of platelets (leading to thromboembolism) may be caused by altered thromboxane metabolism. Thus, the finding that plasma thromboxane B₂ levels increase in peripheral and coronary circulation of many patients with effort angina and variant angina might be closely related to the pathologic events, such as coronary vasospasm and thromboembolism. It is of clinical interest to examine whether platelet-suppressant drugs may antagonize such pathologic events found in coronary artery disease. Frishman et al. documented that propranolol abolishes platelet hyper-responsiveness to ADP in patients with angina pectoris, but it is not well known whether propranolol could reduce thromboxane metabolism or modulate another important mechanism that governs functions of platelets or other cardiovascular tissues. It is also undefined whether thromboxane metabolism in platelets could be altered by the platelet suppressants, which are functionally varied, such as β-blocking agents, cyclooxygenase inhibitors and phosphodies- terase inhibitors. Therefore, the therapeutic significance of the present study must be examined in terms of the role of platelet-suppressant therapy.

Further work may demonstrate that determination of plasma thromboxane B₂ levels may be of clinical diagnostic value, permitting assessment of severity of disease by the evaluation of the extent to which vascular wall and platelet functions are impaired. It is also important to define plasma levels of 6-keto-PGF₁α, a catabolite of prostaglandin I₂ that is supposed to counteract thromboxane A₂, in patients with impaired coronary arteries.

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