Circadian Variation of Plasma Fibrinopeptide A Level in Patients With Variant Angina

Hisao Ogawa, MD, Hirofumi Yasue, MD, Shuichi Oshima, MD, Ken Okumura, MD, Koshi Matsuyama, MD, and Kenji Obata, MD

Plasma levels of fibrinopeptide A (FPA), β-thromboglobulin (BTG), and platelet factor 4 (PF4) were examined on venous plasma samples taken every 4 hours for 24 hours in 20 patients with variant angina and 20 patients with stable exertional angina together with 24-hour Holter recordings. The mean plasma FPA levels (ng/ml) at 2:00 PM, 6:00 PM, 10:00 PM, 2:00 AM, 6:00 AM, and 10:00 AM were 4.6±1.0, 3.1±0.5, 6.1±1.6, 9.9±2.4, 8.7±1.4, and 4.2±0.8 in patients with variant angina (p<0.01) and 1.8±0.2, 2.3±0.3, 1.9±0.2, 1.9±0.2, 1.9±0.3, and 2.3±0.2 in those with stable exertional angina. In seven patients with variant angina, we also examined the effects of heparin (3,000 units), given subcutaneously at 6:00 PM, 10:00 PM, and 2:00 AM, on the plasma FPA levels and the anginal attacks. Although heparin suppressed the elevation and circadian variation of plasma FPA levels, it did not suppress the attacks and their circadian variation in these patients. Plasma FPA levels increased significantly from 3.7±0.5 to 12.5±2.7 ng/ml during or immediately after an attack in the seven patients with no heparin. On the other hand, the plasma levels of BTG and PF4 were increased in patients with variant angina as compared with those with stable exertional angina but did not show a significant circadian variation in both groups. We conclude that 1) plasma levels of FPA, BTG, and PF4 were increased in patients with variant angina as compared with those with stable exertional angina; 2) there was a significant circadian variation in the plasma levels of FPA in parallel with that of the frequency of the attacks with the peak level occurring from midnight to early morning in patients with variant angina; and 3) elevated levels of plasma FPA are the result and not the cause of coronary spasm. (Circulation 1989;80:1617–1626)

It is established that variant angina is caused by coronary artery spasm.1–4 Coronary artery spasm has also been implicated in the pathogenesis of unstable angina5,6 or acute myocardial infarction.7–9 There is now increasing evidence that coronary thrombosis plays a pivotal role in the production of unstable angina10–13 or acute myocardial infarction.14–16 Although factors precipitating coronary thrombosis have not yet been fully elucidated, activation of the coagulation system and platelets is common to thrombosis in general.17

It is not known whether coronary spasm of itself can cause sufficiently severe and prolonged ischemia so as to precipitate unstable angina or acute myocardial infarction or whether coronary spasm predisposes to the formation of fibrin or platelet aggregates, which then lead to prolonged total coronary obstruction and myocardial infarction. Coronary spasm may also be the result, and not the cause, of the acute coronary thrombosis or platelet aggregation because vasoconstrictive substances such as thromboxane A2 and serotonin are released at the time of thrombosis.5,18

Recent studies19–22 have indicated that the plasma level of fibrinopeptide A (FPA), a specific product of fibrinogen cleavage by thrombin, is a useful index of fibrin formation in vivo and that plasma FPA levels are increased in patients with unstable angina and acute myocardial infarction. β-Thromboglobulin (BTG) and platelet factor 4 (PF4) are platelet-specific proteins that are stored in platelet α-granule and secreted during the activation of platelets, and their plasma levels have been used as indexes of platelet activation in vivo.23,24

It has been shown that there is a marked circadian variation in the frequency of attacks of variant angina, with a peak frequency in the early morning and a low frequency in the afternoon.25–27 A similar circadian rhythm has also been reported for the
There is an elevation of variant angina (19 patients) and elevated frequency of acute myocardial infarction and sudden cardiac death.

The present study was designed to examine 1) whether there is an elevation or a circadian variation in the plasma levels of FPA, BTG, and PF4 in patients with variant angina and 2) if so, whether the elevated levels or circadian variation are the cause or the result of the attack of coronary spasm.

### Patients and Methods

**Patient Population**

Twenty patients with variant angina (19 men and one woman, mean age, 58 years; range, 45–72 years) were studied. Their age, gender, electrocardiographic changes during attack, and results of coronary arteriography are shown in Table 1. All patients had attacks of chest pain occurring at rest, usually from midnight to early morning, and associated with ST segment elevation on the electrocardiogram (ECG). No patients had old myocardial infarction. Cardiac catheterization including coronary arteriography was done within 1 week after the study. Coronary arteriography demonstrated coronary artery spasm during the attacks of angina in all the 18 patients in whom coronary arteriography was done during the attack. The patients with new Q waves on the ECG or an increase in plasma creatine kinase level were excluded from the study. In principle, all drugs except nitroglycerin were stopped for at least 3 days before the study day. However, three patients (patients 11, 13, and 16) had severe attacks and were also treated with antianginal drugs on the study day. Patient 11 was treated with long-acting nitrate, diltiazem, and long-acting nifedipine every 6 hours and long-acting pindolol once a day; patient 13 with long-acting nitrate and nifedipine every 12 hours; and patient 16 with long-acting nitrate every 6 hours.

### Table 1. Electrocardiographic and Coronary Arteriographic Findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)/gender</th>
<th>ECG changes during attack</th>
<th>Coronary arteriogram (% stenosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Attack</td>
</tr>
<tr>
<td>1</td>
<td>56/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>100% S6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST ↑ in V1-5</td>
<td>90% S3</td>
</tr>
<tr>
<td>2</td>
<td>45/M</td>
<td>ST ↑ in II, III, aVF, V1</td>
<td>100% S2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST ↑ in V1-6</td>
<td>99% S7</td>
</tr>
<tr>
<td>3</td>
<td>70/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>100% S1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99% S12</td>
</tr>
<tr>
<td>4</td>
<td>58/M</td>
<td>ST ↑ in II, III, aVF or</td>
<td>100% S1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST ↑ in V1-6</td>
<td>90% S7</td>
</tr>
<tr>
<td>5</td>
<td>48/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>100% S1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100% S13</td>
</tr>
<tr>
<td>6</td>
<td>65/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>100% S4</td>
</tr>
<tr>
<td>7</td>
<td>72/M</td>
<td>ST ↑ in II, III, aVF, V1</td>
<td>100% S1</td>
</tr>
<tr>
<td>8</td>
<td>72/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>100% S3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST ↑ in V1-4</td>
<td>99% S6</td>
</tr>
<tr>
<td>9</td>
<td>57/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>90% S2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>90% S13</td>
</tr>
<tr>
<td>10</td>
<td>64/M</td>
<td>ST ↑ in V1-4</td>
<td>99% S9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>90% S4</td>
</tr>
<tr>
<td>11</td>
<td>59/M</td>
<td>ST ↑ in V1-5</td>
<td>...</td>
</tr>
<tr>
<td>12</td>
<td>46/M</td>
<td>ST ↑ in V1-5</td>
<td>99% S7</td>
</tr>
<tr>
<td>13</td>
<td>60/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>100% S2</td>
</tr>
<tr>
<td>14</td>
<td>67/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>...</td>
</tr>
<tr>
<td>15</td>
<td>52/M</td>
<td>ST ↑ in V1-4</td>
<td>100% S6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>75% S2</td>
</tr>
<tr>
<td>16</td>
<td>45/M</td>
<td>ST ↑ in V1-4</td>
<td>100% S7</td>
</tr>
<tr>
<td>17</td>
<td>64/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>90% S1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99% S7</td>
</tr>
<tr>
<td>18</td>
<td>64/M</td>
<td>ST ↑ in II, III, aVF</td>
<td>90% S4</td>
</tr>
<tr>
<td>19</td>
<td>55/F</td>
<td>ST ↑ in II, III, aVF or</td>
<td>100% S8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST ↑ in V1-5</td>
<td>90% S2</td>
</tr>
<tr>
<td>20</td>
<td>48/F</td>
<td>ST ↑ in V1-4</td>
<td>100% S7</td>
</tr>
</tbody>
</table>

ECG, electrocardiographic; NTG, nitroglycerin; ST ↑, ST segment elevation; S1–S15, segments of the coronary arteries as defined by the American Heart Association Committee Report.
Twenty patients with stable exertional angina (16 men and four women, mean age, 61 years; range, 45–71 years) were studied as control subjects. All patients with stable exertional angina had no episodes of rest angina and had advanced organic coronary artery stenosis of 90% or greater of the luminal diameter. In principle, these patients did not receive antianginal drugs for 2 weeks before the study. However, five patients were also treated with antianginal drugs on the study day. Three patients were treated with long-acting nitrate, diltiliazem, and propranolol every 6 hours and two patients with long-acting nitrate and diltiazem every 6 hours.

None of the 40 study patients were receiving heparin or coumarin anticoagulant agents and antiplatelet agents; were administered thrombolytic agents such as urokinase or streptokinase or tissue plasminogen activator; had thromboembolism or collagen disease, disseminated intravascular coagulation, advanced liver disease, or renal failure; had a prosthetic heart valve or a pacemaker; or had pathologic states that exhibit increased plasma levels of polymorphonuclear elastase such as acute leukemia, sepsis, or other inflammations.

**Study Protocol**

The study was done within 1 week before the day of cardiac catheterization to eliminate the possible confounding effects of cardiac catheterization and residual hematomas on the plasma levels of FPA, BTG, and PF4. Blood samples were obtained from patients every 4 hours at 2:00 PM, 6:00 PM, 10:00 PM, 2:00 AM, 6:00 AM, and 10:00 AM. All patients underwent 24-hour Holter recordings with Del Mar Avionics, Irvine, California Model 447 two-channel recorder for at least 24 hours on the day of blood sampling. An ischemic attack was defined by more than 2.0 mm ST segment elevation or by more than 1.0 mm horizontal or downsloping ST segment depression 80 msec after the J point, lasting for at least 1 minute.

In seven patients with variant angina shown in Table 2 (patients 1, 2, and 3 in Table 1 and an additional four patients) we also examined the effects of heparin (3,000 units), given subcutaneously at 6:00 PM, 10:00 PM, and 2:00 AM, on the plasma FPA levels and anginal attacks. This method of administration of heparin was designed to suppress the elevation of plasma FPA levels from midnight to early morning. All drugs except nitroglycerin were withdrawn for at least 3 days before the study. We compared plasma FPA levels and frequency of attacks between those administered heparin with those not administered heparin in these seven patients. We performed blood samplings before and after ischemic attacks from 12:00 midnight to 6:00 AM in addition to routine samplings every 4 hours in these seven patients. The study protocol was approved by the Kumamoto University Medical School Ethics Committee, and informed consent was obtained from each patient.

**Blood Sampling Procedures and Assays**

Samples were drawn by venipuncture by two specially trained physicians (H.O. and S.O.) who were evaluated with quality control procedures before and during the study. The first 2–3 ml blood was discarded, and the subsequent sample was collected in a sequential manner directly into syringes containing the appropriate anticoagulant mixtures and processed immediately. The sites of venipuncture of each patient were changed at each time because there was the possibility that slight residual hematomas would confound some of the data. Quality of the sampling procedure was criti-
cally noted prospectively, and inappropriate samples were discarded. The anticoagulant mixtures for FPA contained 1,000 kallidinogenase inaktivator einheiten (KIE)/ml aprotinin, 1,000 units/ml heparin, and 0.11 M/l Na citrate; those for BTG and PF4 contained 15 mM/l theophylline, 3.7 mM/l adenosine, 0.198 mM/l dipyriramole, and 0.11 M/l Na citrate. They were provided by Diagnostica Stago, Inc, Franconville, France. The mixtures of samples for FPA and anticoagulants were promptly centrifuged 10 minutes at 3,000 rpm at 4°C. Those of samples for BTG, PF4, and anticoagulants were centrifuged at least 15 minutes later, but within 1 hour, at 3,000 rpm at 4°C for 30 minutes. Plasma was separated immediately and fast frozen at −20°C for no more than 24 hours and subsequently at −80°C before assay. The assay was performed within 1 month.

FPA, BTG, and PF4 levels were measured by the commercial enzyme-linked immunosorbent assay (ELISA) kits produced by Diagnostica Stago Inc. The procedures outlined by the manufacturer were meticulously followed. The plasma samples were measured in duplicate.

FPA levels were measured on plasma from which fibrinogen and large fibrinogen degradation products were completely removed by adsorption with bentonite, according to the method of Kockum and Frebelius. Recovery rate of FPA was more than 97% at four different concentrations (0, 3.12, 6.25, and 12.50 ng/ml). The detection limit of the ELISA for FPA was 0.25 ng/ml. Variation coefficients of intra-assay and interassay for FPA were 5.7% and 9.2%, respectively. The normal value for FPA in our laboratory was 1.6±0.1 (SEM) ng/ml.

Because the antibody (rabbit antiserum) against FPA that we used in this study has the same specificity to the C-terminal and N-terminal regions of FPA, and there is the possibility that the antibody could detect the 1 to 21 elastase product or the 1 to 23 plasmin product of fibrinogen in the plasma, we also measured the plasma levels of polymorphonuclear leukocyte elastase and those of plasmin in the samples, which showed maximum plasma FPA values in all 20 patients with variant angina.

The plasma level of polymorphonuclear leukocyte elastase was assessed by measurement of human polymorphonuclear elastase complexed with α1-proteinase inhibitor with ELISA (PMN Elastase, E. Merck, Darmstadt, FRG). Recovery rate of human polymorphonuclear leukocyte elastase/α1-proteinase inhibitor complex was more than 75%, and variation coefficients of intra-assay and interassay were 7.7% and 8.2%, respectively. Normal value was 67±2.3 (SEM) µg/l.

The plasma level of plasmin generation was assessed by determination of the plasmin-α2- plasmin inhibitor complex with a one-step sandwich ELISA (TD-80C, Teijin Ltd, Tokyo, Japan) with polyclonal rabbit antibody against human plasmin- and peroxidase-conjugated monoclonal anti-α2-plasmin inhibitor antibody. Intra-assay and interassay coefficients of variation in this assay were 6% and 10%, respectively. Normal range was less than 0.8 mg/l.

Recovery rate of BTG was greater than 94% and that of PF4 was greater than 92%. The detection limits of the ELISA were 0.5 IU/ml for BTG and 0.2 IU/ml for PF4, respectively. Variation coefficients of intra-assay and interassay for BTG were 2.8% and 8.1%, and those for PF4 were 4.5% and 6.7%, respectively. The normal values for BTG and PF4 in our laboratory were 22.8±1.0 (SEM) IU/ml and 3.4±0.2 (SEM) IU/ml, respectively.

ELISA methods for FPA, BTG, and PF4 used in this study were developed by Amiral and his coworkers and were comparable with commercially available radioimmunoassay (RIA) kits in both the clinical and normal samples.

In seven patients with variant angina treated with heparin, the effect of heparin was estimated by effective heparin activity and activated partial thromboplastin time. Effective heparin activity was measured by the Du Pont (Wilmington, Delaware) Aca discrete clinical analyzer with a chromogenic substrate. Activated partial thromboplastin time was expressed in seconds, and normal range in our laboratory was 30.5–41.2 seconds.

Statistical Analysis

Analysis of variance with repeated measurements and other parametric tests were performed after logarithmic transformation of the FPA, BTG, and PF4 data because these data have been noted to be skewed in past studies. Statistical significance of changes in the time course was evaluated with pairwise comparisons using Bonferroni criterion. The comparison for plasma FPA, BTG, and PF4 levels of the patients with variant angina and stable exertional angina at each sampling time was performed by unpaired t test. Furthermore, the comparison for peak plasma FPA levels of the patients with 75% or more organic coronary artery stenosis and those with less than 75% organic stenosis in variant angina was also performed by use of the unpaired t test. Frequency of episodes of attacks during each 4-hour period was tested with Friedman test. In seven patients treated with heparin, change of activated partial thromboplastin time, comparison of plasma FPA levels and frequency of attacks under heparin treatment with those under no heparin treatment in the same seven patients, and change of plasma FPA levels before and after ischemic attacks were estimated by paired t test. p values were considered statistically significant at less than 0.05. Data were expressed as mean±SEM.

Results

Characteristics of the Study Groups

Coronary arteriography was done in all patients with variant angina except one (patient 14) and in all
patients with stable exertional angina. Coronary artery spasm was induced by intracoronary injection of acetylcholine in all of the 18 patients with variant angina in whom coronary arteriography was done. Only one patient (patient 11) had severe organic stenosis, and intracoronary injection of acetylcholine was not done in this patient. Fourteen patients had between one and 16 attacks on the study day. One patient (patient 20) had no attacks over 6 months. Forty (56%) of 71 episodes of attacks occurred between 10:00 PM and 6:00 AM on the examination day and demonstrated significant circadian variation (p<0.05) (Figure 1).

Figure 2. Line graph showing plasma fibrinopeptide A (FPA) levels (ng/ml) in patients with variant angina and stable exertional angina. Data are expressed as mean±SEM. FPA levels in patients with variant angina demonstrated significant circadian variation (p<0.01). *p<0.05, **p<0.01, difference of the levels between patients with variant angina and those with stable exertional angina at each sampling time.

Plasma Fibrinopeptide A Levels

The mean plasma FPA levels (ng/ml) in the patients with variant angina and in those with stable exertional angina at 2:00 PM, 6:00 PM, 10:00 PM, 2:00 AM, 6:00 AM, and 10:00 AM are shown in Figure 2 and Table 3. The plasma FPA levels in patients with variant angina demonstrated significant circadian variation (p<0.01). The mean level at 6:00 AM was significantly higher than those at 10:00 AM, 2:00 PM, and 6:00 PM; the mean level at 2:00 AM was also higher than that at 6:00 PM (p<0.01). Thus, plasma FPA levels from midnight to early morning were significantly higher than those at daytime in patients with variant angina. In contrast, the plasma FPA levels of stable exertional angina were almost constant throughout 24 hours. The plasma FPA levels of one patient with variant angina (patient 20) who had had no attacks during 6 months were almost constant during the day.

Table 3. Plasma Levels of Fibrinopeptide A, β-Thromboglobulin, and Platelet Factor 4

<table>
<thead>
<tr>
<th></th>
<th>2:00 PM</th>
<th>6:00 PM</th>
<th>10:00 PM</th>
<th>2:00 AM</th>
<th>6:00 AM</th>
<th>10:00 AM</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Fibrinopeptide A (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Variant angina</td>
<td>4.6±1.0</td>
<td>3.1±0.5</td>
<td>6.1±1.6</td>
<td>9.9±2.4</td>
<td>8.7±1.4</td>
<td>4.2±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Exertional angina</td>
<td>1.8±0.2</td>
<td>2.3±0.3</td>
<td>1.9±0.2</td>
<td>1.9±0.2</td>
<td>1.9±0.3</td>
<td>2.3±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
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<tr>
<td>β-Thromboglobulin (IU/ml)</td>
<td></td>
<td></td>
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<tr>
<td>Variant angina</td>
<td>39.8±4.8</td>
<td>35.8±3.4</td>
<td>37.7±4.7</td>
<td>41.3±4.2</td>
<td>40.6±5.5</td>
<td>37.2±4.0</td>
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<td>Exertional angina</td>
<td>26.4±5.3</td>
<td>28.1±5.8</td>
<td>25.6±4.8</td>
<td>27.2±5.4</td>
<td>31.4±7.7</td>
<td>23.7±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<td>&lt;0.01</td>
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<tr>
<td>Platelet factor 4 (IU/ml)</td>
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<tr>
<td>Variant angina</td>
<td>3.9±0.6</td>
<td>3.8±0.6</td>
<td>4.6±1.1</td>
<td>5.3±1.0</td>
<td>4.3±0.8</td>
<td>3.8±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Exertional angina</td>
<td>2.6±0.7</td>
<td>2.7±0.7</td>
<td>2.4±0.5</td>
<td>2.8±0.7</td>
<td>3.0±0.8</td>
<td>1.8±0.3</td>
<td>NS</td>
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<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
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</tr>
</tbody>
</table>
The mean values of plasma FPA levels in the patients with variant angina were significantly higher than those of stable exertional angina at 2:00 PM, 10:00 PM, 2:00 AM, and 6:00 AM (p<0.01). There were no significant differences in the values at 6:00 PM and 10:00 AM between the two groups (Figure 2 and Table 3).

Of the 19 patients with variant angina examined by coronary angiography, seven patients had significant organic coronary artery stenosis of more than 75% of luminal diameter and 12 had no significant organic stenosis. The mean value of peak plasma FPA levels in seven patients with significant organic stenosis was 15.3±3.5 ng/ml and that in 12 without significant stenosis was 12.7±3.4 ng/ml. Thus, there was no significant difference of FPA levels between the two groups.

Effects of Heparin on Plasma Fibrinopeptide A Levels and Attacks

The effect of heparin in seven patients with variant angina treated with heparin was estimated by effective heparin activity and activated partial thromboplastin time. Effective heparin activity (units/ml) at 2:00 PM, 6:00 PM, and 10:00 AM was not detected but was 0.05 at 10:00 PM, 0.14 at 2:00 AM, and 0.18 at 6:00 AM. Activated partial thromboplastin time (seconds) at 10:00 PM, 2:00 AM, and 6:00 AM were longer than those at 2:00 PM, 6:00 PM, and 10:00 AM (41.0±1.8, 48.2±2.7, and 55.0±3.3 vs. 35.4±1.0, 35.3±1.2, and 35.6±1.0, p<0.01). We compared plasma FPA levels and frequency of attacks under heparin treatment with those under no heparin treatment in these patients. The mean plasma FPA levels (ng/ml) at 2:00 PM, 6:00 PM, 10:00 PM, 2:00 AM, 6:00 AM, and 10:00 AM were 3.2±0.3, 3.6±0.4, 3.5±0.3, 3.3±0.4, 3.1±0.5, and 3.8±0.5 under heparin treatment and 3.0±0.4, 3.3±0.3, 4.0±0.5, 7.7±1.1, 9.2±1.3, and 3.8±0.6 under no heparin treatment. Although there were no differences in plasma FPA levels at 2:00 PM, 6:00 PM, 10:00 PM, and 10:00 AM between the two conditions, those at 2:00 AM and 6:00 AM were significantly lower under heparin treatment than under no heparin treatment (p<0.01), and plasma FPA levels became almost constant throughout the 24 hours after heparin administration (Figure 3, upper panel). The plasma FPA levels under no heparin treatment showed a circadian variation (p<0.01). On the other hand, the frequency of attacks during each 4-hour period was almost the same between under heparin and under no heparin therapy, and there was a significant circadian variation in both conditions (p<0.05) (Figure 3, lower panel).

Plasma Fibrinopeptide A Levels Before and After Ischemic Attacks

We performed blood samplings for FPA before and during or immediately after 11 attacks in addition to routine samplings every 4 hours in these seven patients not administered heparin. The plasma FPA levels increased significantly during or imme-
angina at 2:00 PM, 6:00 PM, 10:00 PM, 2:00 AM, 6:00 AM, and 10:00 AM are shown in Table 3. These values also demonstrated no significant circadian variation. The mean plasma PF4 levels were higher in patients with variant angina than in those with stable exertional angina at each sampling time (p<0.05).

**Discussion**

It has been shown that there is a circadian variation in the frequency of attacks of variant angina, with a peak frequency occurring in the early morning. The present study shows that there is also a circadian variation in the plasma FPA levels in parallel with that of the attacks in patients with variant angina, with a peak level occurring in the early morning. In contrast, there was no circadian variation in the plasma FPA levels in patients with stable exertional angina. The study also shows that plasma FPA levels were higher at each time of blood sampling in patients with variant angina than in those with stable exertional angina. Thus, there is an intimate relation between increased plasma FPA levels and the attacks in patients with variant angina. To elucidate further whether increased plasma FPA levels and thus increased thrombin activity are the cause or the result of the attacks, we examined the effects of heparin, an inhibitor of thrombin activation, on plasma FPA levels and the attacks in the patients with variant angina. Although heparin suppressed the elevation and circadian variation of plasma FPA levels, it did not suppress the attacks and their circadian variation in these patients. These results suggest that increased plasma FPA levels and thus increased thrombin activity are the result and not the cause of attacks. Furthermore, we examined plasma FPA levels before and during or immediately after the attacks. The plasma FPA levels increased significantly during or immediately after the attacks. Thus, the elevated plasma FPA levels and thus increased thrombin activity are the result and not the cause of attack or coronary spasm in patients with variant angina.

There is the possibility that the increased plasma FPA levels in patients with variant angina were due to the confounding effects of the 1 to 21 elastase product or the 1 to 23 plasmin product of fibrinogen was very low in the present study.

**Plasma β-Thromboglobulin Levels**

The mean plasma BTG levels (IU/ml) in patients with variant angina and in those with stable exertional angina at 2:00 PM, 6:00 PM, 10:00 PM, 2:00 AM, 6:00 AM, and 10:00 AM are shown in Table 3. These values demonstrated no significant circadian variation in both groups. The mean plasma BTG levels were higher in patients with variant angina than in those with stable exertional angina at each sampling time except at 6:00 AM (p<0.05).

**Plasma Platelet Factor 4 Levels**

The mean plasma PF4 levels (IU/ml) in patients with variant angina and in those with stable exertional
these values were within normal range. Thus, the possibility that the increased plasma FPA levels in patients with variant angina were due to the confounding effects of the 1 to 21 elastase product or the 1 to 23 plasmin product of fibrinogen was very low in the present study.

Neri Serneri and coworkers reported that there was a close association between plasma FPA levels and activity of spontaneous angina and that low dose heparin treatment significantly reduced both the plasma FPA levels and the clinical activity of angina. They suggested that the elevated plasma FPA levels and thus increased thrombin activity were the cause rather than the result of the attacks. This discrepancy was probably because of the difference in the patients studied. We studied patients with variant angina, and coronary spasm was documented during the attacks in all the patients who underwent coronary arteriography during an attack as shown in Table 1. Nine (47%) of the 19 patients who underwent coronary arteriography had normal coronary arteries and only seven patients (37%) had coronary arteries with more than 75% stenosis of luminal diameter (six had one-vessel disease and the remaining patient had two-vessel disease) in our study. Neri Serneri et al did not describe whether any of their patients had documented coronary spasm or what the coronary arteriographic findings were in their patients.

The plasma levels of BTG and PF4, on the other hand, did not show a significant circadian variation in either patients with variant angina or those with stable exertional angina, although the peak level of these values tended to occur at the same hour (2:00 AM) as that of FPA in the patients with variant angina. The plasma BTG and PF4 levels were increased in patients with variant angina as compared with those with stable exertional angina. However, the magnitude of the increases in these levels was less than that of FPA. The reason for the discrepancy between fibrin formation as assessed by FPA and platelet activation as assessed by BTG and PF4 in the present study cannot be readily explained. It may be because of the difference in sensitivity of fibrinogens and platelets to thrombin action or the different rates of clearance of these substances. Some investigators reported increases in BTG or PF4 in patients with unstable angina and acute myocardial infarction. Other investigators reported that there were no increases in BTG or PF4 in these patients. In all of these previous studies, coronary spasm was not documented. In the present study, we examined plasma FPA, BTG, and PF4 levels every 4 hours for 24 hours in patients with variant angina and in those with stable exertional angina.

Coronary thrombosis has been demonstrated in many patients with unstable angina and in those with acute myocardial infarction by using coronary arteriography or angioscopy or at autopsy. Plasma FPA levels are also reported to be increased in patients with unstable angina and in those with evolving myocardial infarction. Our study shows that plasma FPA levels and thus thrombin formation are also increased in patients with variant angina as a consequence of coronary spasm. Coronary spasm probably causes injury of coronary endothelium and thus may trigger the cascade of coagulation activation. Our study thus links coronary spasm with coronary thrombosis and ultimately with acute myocardial infarction. This study may also provide an important clue to the mechanisms of the circadian variation in the frequency of acute myocardial infarction or sudden cardiac death with a peak incidence in the morning.

On the other hand, the rate of conversion of plasminogen to plasmin, by tissue plasminogen activator and prourokinase, is enhanced by the presence of thrombin and fibrin. However, both the rate and extent of fibrinolysis are also regulated by the circulating inhibitors of plasmin. Thus, whether thrombin activation and fibrin formation precipitated by coronary spasm may actually lead to coronary obstruction resulting in unstable angina or acute myocardial infarction depends also on the activation of fibrinolytic system.

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