Increased Plasma Level of Endothelin-1 and Coronary Spasm Induction in Patients With Vasospastic Angina Pectoris

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To elucidate the pathogenic contribution of a potent vasoconstrictor, endothelin-1, to coronary artery spasm, we provoked spasm with intracoronary administration of acetylcholine or ergonovine and performed sensitive immunoassays of plasma levels of endothelin-1 and atrial natriuretic factor (ANF) in the peripheral vein and coronary sinus of patients with a tentative diagnosis of vasospastic angina (VSA, n=19). The validity of coronary sinus blood sampling was verified by simultaneous measurement of the ANF level. The plasma endothelin-1 levels in venous and coronary sinus blood of the spasm-provoked patients (n=12) were 1.71-fold and 2.16-fold higher, respectively, than those of nonprovoked cases (n=5, p<0.01). During left coronary spasm, the endothelin-1 level in coronary sinus transiently decreased from 2.27±0.14 to 1.76±0.14 pg/ml (p<0.01) and returned to the control level (1.98±0.20 pg/ml) after the spasm resolved, whereas the change was equivocal during right coronary spasm. In contrast, the patients in whom spasm was not provoked showed no changes and maintained low endothelin-1 levels both before and after the maximal provocation (0.90±0.13 versus 0.90±0.13 pg/ml). These findings imply that 1) low but significant concentrations of immunoreactive endothelin-1 circulate in the human systemic and coronary vascular beds, 2) an increased plasma endothelin-1 level may predispose but might not be sufficient per se to induction of the coronary spasm, 3) some inhibitory mechanism to endothelin-1 secretion may occur into the coronary circulation during the spasm, or 4) VSA might be classified to two subtypes or subtypes—a type (or stage) with a high endothelin-1 level responding to the drug provocation and another type (or stage) with a low endothelin-1 level that is nonresponsive to the provocation. (Circulation 1991;83:476–483)

Since the report of Prinzmetal et al1 on variant angina, the etiology of angina pectoris has been viewed as consisting of two general types—one due to organic stenosis of an atherosclerotic coronary artery and the other due to functional narrowing of a coronary artery secondary to transient, focal contraction of vascular smooth muscle cells.2 Both types of angina give rise to myocardial ischemia and its sequelae: cardiac dysfunction, acute atrioventricular block, or grave arrhythmias.2,3 However, the clinical implications, pathogenesis, and therapy of these types are fundamentally different.4 The precise mechanism of coronary artery spasm has not been clarified, although it has been speculated that a humoral factor is involved.3

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Recently, several vasoactive substances, including endothelin (ET),5,6 endothelium-derived relaxing factor (EDRF),7,8 atrial natriuretic factor (ANF),9 prostanoids, and/or several cytokines, have been proposed as endogenous substances regulating vascular

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smooth muscle tonus. All of these substances regulate smooth muscle contractility by changing intracellular Ca\(^{2+}\) concentration,\(^{10,11}\) which is essential for the regulation of cardiovascular tissue\(^{12-14}\) and may affect coronary blood flow. ET is the most potent vasoconstrictor; it was initially isolated from the supernatant of cultured porcine endothelial cells\(^4\) and then cloned from a complementary DNA (cDNA) library of human endothelial cells.\(^5\) Furthermore, ET has been found to have subpopulations: ET-1, ET-2, and ET-3.\(^{15}\) In the ET family, ET-1 possesses the most potent vasoconstricting activity in vitro, although it has not been established whether human ET-1 is actually expressed in endothelial cells in situ and secreted from endothelial cells into the coronary circulation. ET-1 is a promising candidate for the mechanism that causes coronary spasm. In contrast, both EDRF and ANF are vasorelaxant and might counteract any ET-1 action.

To evaluate the contribution of ET-1 to vasospastic angina, we performed a clinical study that included 1) coronary spasm provocation by intracoronary administration of acetylcholine (Ach) or ergonovine maleate (Er), 2) angiographic documentation of the spasm, 3) blood sampling from the peripheral vein and coronary sinus, and 4) use of recently developed sensitive immunoassays for ET-1 and ANF.\(^{16,17}\)

**Methods**

**Selection of Patients**

After excluding diseases of noncardiac origin, a tentative diagnosis of vasospastic angina (VSA) was made in patients who satisfied the criteria of clinical symptoms suggesting a temporary myocardial ischemia without physical or mental stress, ST segment elevation or depression on Holter electrocardiographic recordings coinciding with their chest discomfort, improvement of both the symptoms and the electrocardiographic findings with nitrate administration, and a prophylactic effect of calcium entry blockers (nifedipine or diltiazem) to the myocardial ischemia attack. Nineteen patients (17 men and two women; mean age, 51 ± 7 years) met the criteria and gave informed consent for the following study protocol. Two patients were excluded from the study because their coronary sinus blood was not precisely sampled.

Patients were divided into two groups according to the ability to provoke coronary artery spasm with the study protocols: provocation responsive (\(n=12\)) and nonresponsive (\(n=5\)). Clinical profile and risk factors associated with each group are summarized in Table 1.

**Study Design**

**Coronary angiography and sampling of coronary sinus blood.** All medications were withdrawn at least 1 week before the cardiac catheterization study, except for short-acting nitrates. After overnight sedation, all patients received routine pressure and volume studies. Before coronary angiography, a Goodale-Lubin catheter was introduced into the coronary sinus. Care was taken to not wedge the catheter tip during the coronary angiography procedure. Left and right coronary angiography was performed by administering a contrast agent through a Judkins or Sones catheter before, during, and after the occurrence of coronary spasm was suspected by chest discomfort and/or electrocardiographic recording.

To provoke coronary vasospasm, Ach (Daiichi Pharmacy, Tokyo) or Er (Takeda Pharmacy, Osaka) were given via graded intracoronary administration during 1 minute. Fundamentally, Ach was the first choice for spasm provocation, but Ach was replaced by Er when insertion of an additional catheter for cardiac pacing proved difficult in the older patients. Spasm provocation using both Ach and Er was not performed except in one patient because application of two provocation drugs to the same patient, coronary angiography performed twice, and blood taken from the coronary sinus four times would be time consuming and a burden for the patients. Furthermore, our preliminary data indicated that the degree of coronary spasm and its morphology were not different when both Ach and Er were administered to the same patient under the protocol described below (T. Aizawa et al, unpublished observations). In one patient, Ach was given first, followed by Er, to confirm a lack of response to administration of both agents.

With Ach administration, a pacing catheter was positioned in the right ventricle because Ach often causes sinus bradycardia or complete atrioventricular block. The dose was then increased from 25 to 50 or 100 \(\mu\)g at 3-minute intervals until chest discomfort or an electrocardiographic change was manifest. In the case of Er, the dose was increased from 20 to 40 or 60 \(\mu\)g at 3-minute intervals. After each coronary angiography, the Goodale-Lubin catheter was wedged into the coronary sinus, and the coronary blood was sampled three times—before, during, and after appearance of symptoms. When the spasm did not resolve spontaneously, nitroglycerin was administered via intracoronary catheter.

When a symptom or electrocardiographic change was not induced by even the highest drug dose, we considered the patient to be nonprovocative. In these cases, coronary sinus blood was sampled twice—before and after administration of the largest drug dose. The degree of coronary stenosis was evaluated by densitometry of cine angiography.

**Assay of endothelin-1 and atrial natriuretic factor.** Coronary blood (10 ml) was sampled in 300 IU/ml aprotinin (Bayer) and 2 mg/ml EDTA-\(\text{Na}_2\) to prevent possible degradation of these peptides by blood clotting and spun at 3,000g for 15 minutes. The plasma was divided into two portions and kept frozen at \(-20^\circ\text{C}\) until the assay of ET-1 or ANF. Plasma concentrations of ET-1 or ANF were measured within 2 weeks after the sampling. A pilot study indicated that both peptides were stable for as long as 3 months when kept frozen. ET-1 concentration in the blood was very low and difficult to assay even by radioimmunoassay. We
switched to the newly developed sandwich method described previously,\textsuperscript{16} using the monoclonal antibody AwETN40 against the N terminal and horseradish peroxidase–conjugated polyclonal antibody against synthetic heptapeptide of C terminal of human ET-1. The new method was very sensitive and measured as little as 0.4 pg per well of human ET-1.\textsuperscript{16}

Great care was taken to not mix venous blood of the systemic circulation with blood of the coronary sinus. The plasma concentration of ANF was assayed, as described previously,\textsuperscript{17} and is useful in verifying the exact blood sample from the coronary sinus because plasma ANF concentration is increased eightfold to 10-fold in coronary sinus blood.\textsuperscript{17} Assays of ET-1 and ANF require pretreatment with absorption column cartridge (Sep-Pak C-18, Waters, Mass.) in acetic acid to release bound peptide from the large amount of plasma proteins. Contrast material passed through the cartridge and did not interfere with the assay of ET-1 or ANF, even at the maximum concentration (3%, vol/vol) in blood. Furthermore, contrast material did not have a biological effect on the secretion of ET-1 or ANF, even after a bolus injection for ventriculography or angiography. Accordingly, volume expansion by the contrast medium, if any, could be neglected. Both measurements were stable, and interassay and intra-assay variabilities were less than 9%.

\textbf{Miscellaneous.} All reagents used for the assay of ET-1 and ANF were of analytical grade. Immunoassays were performed in duplicate, and the mean values were used. Assay data are given as mean±SEM and analyzed by Student’s nonpaired \( t \) test to compare the values between the two subgroups and by paired \( t \) test to examine the alteration along the provocation test in the same cases. Resultant probability values of less than 0.05 were considered significant.

\textbf{Results}

\textbf{Provocation of Coronary Vasospasm and Clinical Profiles}

In 19 entry patients with a tentative diagnosis of VSA, full protocols including coronary angiography

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
 & Provocation responsive (\( n=12 \)) & Nonresponsive (\( n=5 \)) & \( p \) \\
\hline
Age (yr) & 59.8±2.79 & 51.0±3.65 & <0.01 \\
Sex (male/female) & 10/2 & 5/0 & NS \\
Patients with history of myocardial infarction (\( n \)) & 1 & 0 & NS \\
Blood pressure & & & \\
Systolic/diastolic (mm Hg) & 123±4/73±2 & 139±7/82±6 & <0.01 \\
 Patients with hypertension (>160/100 mm Hg) (\( n \)) & 0 & 0 & NS \\
Diabetes mellitus & & & \\
Fasting blood glucose level (mg/dl) & 107±11 & 105±19 & NS \\
 Patients with diabetes mellitus history (\( n \)) & 3 & 1 & NS \\
Plasma lipid levels & & & \\
Total cholesterol (mg/dl) & 187±11 & 155±13 & <0.01 \\
Triglycerides (mg/dl) & 168±18 & 134±19 & <0.01 \\
 High density lipoprotein (mg/dl) & 44±4 & 47±7 & NS \\
 Patients with hyperlipidemia (\( n \)) & 7 & 1 & NS \\
Gout & & & \\
Uric acid level in serum (mg/dl) & 5.9±0.4 & 6.4±0.6 & NS \\
 Patients with gout (\( n \)) & 0 & 0 & NS \\
Exercise test & & & \\
Patients with positive electrocardiographic results (\( n \)) & 6 & 2 & NS \\
Tolerance to exercise test by Bruce protocol (min) & 8.4±0.7 & 9.0±1.2 & NS \\
 Patients with transient defect in thallium-201 scintigraphy (\( n \)) & 7 & 1 & NS \\
 Patients with chest symptoms during provocation (\( n \)) & 7 & 1 & NS \\
Patients with positive electrocardiographic findings suggestive of myocardial ischemia during provocation (\( n \)) & 12 & 0 & <0.01 \\
\hline
\end{tabular}
\caption{Clinical Profiles and Risk Factors in Patient Subgroup With Tentative Diagnosis of Vasospastic Angina}
\end{table}
TABLE 2. Results of Coronary Angiography Before and After Provocation in Patients With Tentative Diagnosis of Vasospastic Angina

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Provocation dose</th>
<th>CAG results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Agent</td>
<td>µg</td>
</tr>
<tr>
<td>Spasm provoked only in left coronary artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>69</td>
<td>Ach</td>
<td>100</td>
</tr>
<tr>
<td>M</td>
<td>53</td>
<td>Ach</td>
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<td>M</td>
<td>51</td>
<td>Ach</td>
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</tr>
<tr>
<td>M</td>
<td>62</td>
<td>Ach</td>
<td>25</td>
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<tr>
<td>M</td>
<td>66</td>
<td>Ach</td>
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<tr>
<td>M</td>
<td>68</td>
<td>Ach</td>
<td>50</td>
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<td>M</td>
<td>51</td>
<td>Er</td>
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<tr>
<td>M</td>
<td>51</td>
<td>Er</td>
<td>20</td>
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<tr>
<td>Spasm provoked in right coronary artery or both coronary arteries</td>
<td></td>
<td></td>
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<tr>
<td>M</td>
<td>68</td>
<td>Ach</td>
<td>50</td>
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<tr>
<td>F</td>
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</tr>
<tr>
<td>M</td>
<td>59</td>
<td>Er</td>
<td>60</td>
</tr>
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</table>

CAG, coronary angiography; Ach, acetylcholine; Er, ergonovine maleate.

and exact blood sampling from the coronary sinus was carried out in 17. No patient had a serious complication except for the dislodging of Goodale-Lubin catheter from the coronary sinus during catheterization and coronary angiography procedures in two patients. The data of these patients were excluded from the study.

The results of spasm provocation are summarized in Table 2. Of 17 patients, 12 (10 men and two women) responded to the provocation test (Table 1). During control coronary angiography, VSA-induced patients had no significant stenosis of more than 75%, but there was mild narrowing ranging from 25% to 60% in 12 arteries before the provocation. After an intracoronary administration of Ach (eight cases) and Er (four cases), a transient coronary stenosis was documented in 15 and nine arteries, respectively. The degree of narrowing was always severe with 90% or 100% stenosis revealed. Most arteries that responded to the provocation showed a segmental narrowing localized in one artery, except for eight arteries that demonstrated diffuse stenosis spanning over two arteries. Two patients demonstrated a double response in which both left and right coronary arteries showed spasms in their proximal portions with 100 µg Ach or 40 µg Er. In those patients, the electrocardiogram showed ST segment depression in leads V	extsubscript{4}, V	extsubscript{5}, and V	extsubscript{6} during left coronary spasm and ST segment elevation in leads II, III, and aVF during right coronary spasm. Furthermore, it should be mentioned that many arteries responding to the provocation exhibited organic lesions with variable stenosis, but severe (90–99%) local stenosis was induced in six arteries that revealed no organic lesions before the provocation.

Five patients did not show significant narrowing, even with a maximum dose of Ach (100 µg) in two cases, Er (60 µg) in two cases, or both Ach (100 µg) and Er (60 µg) in a case. In these patients, the electrocardiographic recordings revealed no changes throughout cardiac catheterization. Although all 12 patients who responded to the provocation exhibited
ST segment changes by electrocardiography during the spasm, only seven complained of chest discomfort. In the nonresponsive group, no patient demonstrated an electrocardiographic change, and one patient complained of chest pain without an accompanying electrocardiographic change (Table 1). Only one patient needed intracoronary administration of nitroglycerin to resolve the long-lasting spasm, and other patients demonstrated transient spasms that ceased spontaneously within a few minutes.

Plasma Endothelin-1 Level in Venous Blood and Coronary Sinus Blood Before Provocation

As summarized in Figure 1, venous blood sampled before provocation revealed a plasma ET-1 level in the spasm-provoked cases higher than that in the nonprovoked cases by a factor of 1.71 [2.39±0.32 pg/ml (n=12) versus 1.40±0.05 pg/ml (n=5), p<0.01]. The plasma ET-1 level in the nonprovoked cases was similar to that of age-matched normal men (1.51±0.07 pg/ml, n=40) or women (1.34±0.08 pg/ml, n=36).

Coronary sinus blood in VSA-provoked patients also contained 2.18-fold more ET-1 than in non-VSA-provoked patients (1.99±0.14 versus 0.92±0.13 pg/ml, p<0.01). Furthermore, in the non–spasm-provoked group, the venous blood contained more ET-1 than did the coronary sinus blood (p<0.05). These results were in good agreement with the fact that ET-1 is taken up in the pulmonary circulation and the arterial concentration was the smallest in circulation. However, there was no significant difference in plasma ET-1 levels between venous blood and coronary sinus blood in the spasm-provoked cases.

Change of Plasma Endothelin-1 Level in Coronary Sinus Blood During and After Coronary Spasm

Figure 2 illustrates the results of our study. Because not all right coronary arterial blood returns to the coronary sinus, the spasm-provoked group was divided into two subgroups: a group who demonstrated a spasm in only the left coronary artery and a group who demonstrated right coronary artery spasm or spasms of both right and left coronary arteries. During left coronary spasm, the ET-1 level in coronary sinus blood transiently decreased from 2.27±0.14 to 1.76±0.14 pg/ml (p<0.01) and then returned to the control level (1.98±0.20 pg/ml, not significant versus before or during the spasm) after the spasm was resolved spontaneously or by the intracoronary administration of nitroglycerin in one patient.

The patients with right coronary spasm or both right and left coronary spasms showed no appreciable change of the ET-1 level during the provocation (1.58±0.11, 1.78±0.38, or 2.01±0.25 pg/ml at before, during, or after the spasm, respectively). Although the plasma ET-1 level seemed to increase during the provocation, inspection of each result revealed increment or reduction during the spasm. Thus, the authors would retain the conclusion concerning the change of plasma ET-1 level during right coronary spasm because the number of cases was small (n=3).

In contrast, the patients who did not respond to the provocation test demonstrated no change of ET-1 level by the provocation (0.90±0.13 versus 0.90±0.13 pg/ml). ET-1 levels before and after the provocation were much lower than those in the spasm-provoked cases (40% or 46% before or after the provocation, respectively; p<0.01).
Plasma Concentration of Atrial Natriuretic Factor in Coronary Sinus Blood

To verify appropriate sampling of coronary sinus blood rather than right atrial blood, the plasma ANF level proved an excellent indicator because its level in the coronary sinus is sevenfold to 10-fold that in systemic venous blood. The results also indicated a distinct increment of plasma ANF level (from 63.0±10.5 to 437±128 pg/ml in coronary spasm-provoked cases and 62.7±14.7 to 494±108 pg/ml in non-coronary spasm-provoked cases; p<0.001 in both cases). There was no significant difference in each plasma level between VSA-provoked and non-VSA-provoked groups. Whenever the catheter tip was dislodged from coronary sinus, ANF levels were less than 100 pg/ml; these samples were excluded from the study.

The provocation test did not significantly alter plasma ANF level. Although four cases showed doubled levels during the provocation, the other cases demonstrated reduced levels. Furthermore, these changes were not dependent on positive or negative results of the provocation test (data not shown).

Relation of Coronary Spasm Provocation to Patient Background or Risk Factors

When patients with tentative diagnosis of VSA were divided into provocation-responsive and non-responsive groups (Table 1), the former group included older patients (p<0.01) with lower systolic (p<0.01) and diastolic (p<0.01) blood pressures or higher plasma levels of total cholesterol (p<0.01) and triglycerides (p<0.01) than the latter group, although the incidence of hyperlipidemia was not significantly different between the groups.

There also was no significant difference between the two groups in sex ratio, previous history of myocardial infarction, incidence of hypertension or diabetes mellitus (both of which were controlled in all cases), fasting blood glucose level, plasma level of high density lipo-protein or uric acid, incidence of hyperlipidemia, tolerance time to exercise testing with Bruce protocol, or incidence of a transient defect in thallium-201 scintigraphy during exercise testing.

Discussion

The physiological process of ET-1 secretion and its metabolic fate are not clear. Vascular smooth muscle cells have a specific receptor for ET-1 that is distinctively different from voltage-dependent Ca²⁺ channel. It is possible that ET-1 is preferentially secreted abluminally and that most of the ET-1 secreted is taken up by the underlying smooth muscle cells. However, the results of the present study indicate that a portion of the ET-1 was actually excreted into both coronary and systemic circulations, as was the case in acute myocardial infarction. Plasma ET-1 levels in peripheral venous and coronary sinus blood were higher in patients with VSA than in those without VSA. In vitro and animal studies have indicated that ET-1 causes a potent and long-lasting contraction of vascular smooth muscle. We expected the ET-1 level to also increase with coronary vasospasm if locally produced ET-1 directly contributes to the pathogenesis of VSA. Contrary to our expectation, the plasma concentration of ET-1 in coronary sinus blood was transiently and partially decreased during spasm provoked by Ach or Er, whereas the levels did not change in the nonprovoked patients. Furthermore, the measurement of plasma ANF level was extremely useful in identifying a precise sampling site for coronary sinus blood (instead of the right atrium).

Most patients with VSA present a systemic angiostenic diathesis often noted by resistance to the passing of a catheter. If present, part of this tendency to general vasospasm might be associated with the plasma ET-1 level in the systemic circulation. Even if the increased plasma ET-1 level is assumed to be a preconditioning for causing the coronary spasm, the direct trigger for initiating the spasm is unknown. The fact that Ach or Er provoked spasm in only some of the patients with a history of VSA may be related to the differential effects of these provocation agents on the release of EDRFs in these patients. Ludmer et al. suggested that vasospasm with Ach in certain patients may be related to an endothelium dysfunction. Indeed, a decreased level of endothelium-derived nitric oxide or, more likely, S-nitrosothiol may be associated with increased levels of ET-1.

The imbalance of these factors and additional perturbations caused by, for example, respiratory alkalosis in hyperventilation or neural influences would result in spasm. Another possibility is a potentiating effect of low threshold and subthreshold concentrations of ET-1 on the responsiveness of vascular smooth muscle cells to other vasoconstrictor substances, such as norepinephrine and serotonin. The results of the present study indicate that the pathogenesis of VSA cannot be solely attributed to the ET-1 level and suggest that it may instead be multifactorial.

It is tempting to speculate that even in the spasm-provoked patients, some feedback mechanism is attenuating the coronary spasm with ET-1 because plasma ET-1 levels in coronary sinus were transiently reduced during the spasm. In the 12 patients who showed coronary vasospasm, Ach and Er provoked typical coronary spasm in eight and four cases, respectively. Ach has dual actions on vascular smooth muscle cells—a direct vasoconstricting action when endothelium is removed and an indirect vasorelaxation action mediated by EDRF. In the patients who exhibited coronary spasm to Ach, either the EDRF released from endothelial cells by Ach stimulation might not be sufficient for vasorelaxation of the underlying vascular smooth muscle cells, or smooth muscle cells in the vasospastic region could be more sensitive to Ach and/or less sensitive to EDRF than those in other regions, because vascular smooth
muscle cells have been shown to be heterogeneous in response to vasoactive substances.

The cause of transient reduction of ET-1 concentration during the coronary spasm is not known. Decrease of ET-1 synthesis in endothelial cells would not be a likely cause because the reduction took place within several minutes after the provocation. ET-1 secretion from endothelial cells into coronary circulation might be modified during spasm. On the mechanism of ET secretion, we propose three hypotheses. First, ET-1 is continuously excreted from coronary endothelial cells, as occurred before and after relief of the spasm (Figure 2). Active contraction of smooth muscle cells by Ach or Er in the spastic region would reduce coronary perfusion pressure and coronary flow rate downstream to the spasm. Because ET-1 secretion has been shown to be increased by shear stress, ET-1 secretion during transiently reduced shear stress might be attenuated (Figure 2B). Resolution of the spasm would then restore ET-1 secretion in spasm-provoked patients (Figure 2). Second, the uptake or binding of ET-1 by underlying smooth muscle cells might be regulated by coronary blood flow because clearance rate of ET is sufficiently fast. The coronary flow reduction during spasm would increase the transit time of blood stream and uptake rate of ET-1, and the resultant plasma concentration of ET-1 might be reduced. Finally, this hypothesis is based on an increasing coronary flow in myocardial segments adjacent to the ischemic segments during the spasm due to increased contractile performance and enhanced levels of catecholamines. The coronary sinus catheter might have sampled blood from the hyperperfused coronary vascular bed.

Without use of plasma ET-1 level, we overestimated the ET-1 concentration during spasm because right atrial blood included a higher concentration of ET-1 than did coronary sinus blood (data not shown). Coronary sinus blood is a mixture of both provoked and nonprovoked coronary vascular beds, and the local ET-1 concentration in the spastic region without dilution might therefore be much lower than that given in the present study. Superselective sampling of blood would validate the above scheme more clearly.

A direct effect of the Provocation drugs, Ach or Er, on the reduction of ET-1 secretion does not seem likely because these drugs did not modify the ET-1 concentration in nonprovoked patients (Figure 2). Absolute plasma concentrations of ET-1 were low (pg/ml or pM). However, the local interstitial concentration surrounding vascular smooth muscle cells might be sufficient to control the tonus of vascular smooth muscle, considering the potent action of ET-1 at the subnanomolar level. Furthermore, the present results of higher plasma ET-1 levels in the lower systolic and diastolic blood pressure group (Table 1) and a study in patients with acute myocardial infarction by Miyauchi et al strongly suggest that plasma ET-1 level is not primarily correlated with hypertension.

It is tempting to speculate that even in the spasm-provoked patients, some feedback mechanism attenuates the coronary spasm with ET-1 because plasma ET-1 levels in coronary sinus were transiently reduced during spasm. In the 12 patients who showed coronary vasospasm, Ach and Er provoked typical coronary spasm in eight and four cases, respectively. However, Ach or Er did not always cause vasospasm, even in the patients in whom spasm was strongly suggested by a transient myocardial ischemia by clinical symptoms, electrocardiographic recording, 201TI myocardial scintigraphy, and/or improvement of symptoms with the administration of nitrate. These findings indicate that VSA might be classified into two subtypes or subtypes—a type, or stage, with a high ET-1 level responding to the drug provocation and another type, or stage, with a low ET-1 level that is nonresponsive to provocation. The number of patients in each group was insufficient to be conclusive, and discrete differences in background or risk factors in these subpopulations were difficult to determine exactly. A more-detailed analysis in a larger study population is necessary to reach firm conclusions regarding the pathogenesis of VSA.

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**KEY WORDS** • endothelin-1 • vasospastic angina • atrial natriuretic factor • coronary circulation
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