Coronary Hyperreactivity to Adrenergic Stimulation and Increased Nocturnal Vagal Tone Trigger Coronary Vasospasm

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The relationship between autonomic nervous system activity (ANA) and coronary vasoreactivity during transient myocardial ischemia was determined in patients with vasospastic angina (VA). ANA was measured by power spectral analysis of heart rate variability and humoral factors following intravenous infusion of insulin in 24 patients with VA and 6 control patients. Nine (38%) of the VA patients had significant ST segment depression (STD), and 4 of these patients had symptomatic STD. The frequency of anginal episodes in the 9 patients with VA and STD was significantly greater than that in the 15 VA patients without STD (3.4 ± 3.1 vs 0.5 ± 0.8 episodes/week, $p < 0.05$). The increase in the LF/HF ratio 30 min after insulin injection in patients with STD was significantly greater than that in patients without STD ($34 \pm 31\%$ vs $4 \pm 34\%$, $p < 0.05$). All of the patients with VA and STD had significant coronary vasospasm in response to the infusion of $\leq 20 \mu\text{g}$ of acetylcholine, higher levels of nocturnal parasympathetic activity, and greater norepinephrine production in response to insulin stimulation than the VA patients without STD. These findings suggest that increased vagal tone and hyperreactivity to adrenergic stimulation may trigger vasospasm in patients with VA. (*Jpn Circ J* 1998; 62: 721–726)

Key Words: Autonomic nervous system activity; Coronary vasospasm; Vasospastic angina

Transient myocardial ischemia (TMI) in patients with variant angina occurs more frequently during the period from midnight to the early morning. It has been reported that autonomic nervous system activity and humoral factors play important roles in the development of TMI in patients with variant angina.¹ However, the responsible pathophysiologic mechanism remains unknown,^{2,3} as do the stimuli that trigger vasospasm in patients with variant angina. Several investigators have reported that either sympathetic or parasympathetic tone, as measured by heart rate variability (HRV), increases acutely immediately before the onset of TMI in patients with variant angina.^{4,5} However, the mechanisms responsible for the changes in autonomic tone and the time course for changes in autonomic activity are still unclear.

In the present study, coronary vasomotor tone was augmented by increasing sympathetic nervous activity via the administration of insulin^{6,7} in order to assess the changes in autonomic tone before and during TMI 'at rest'. In addition, we measured the time course for changes in the heart rate, blood pressure and humoral factors in response to sympathetic activation, and assessed autonomic tone by HRV analysis.

Methods

Patients

We studied 26 patients with known vasospastic angina

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(VA) and 6 control patients. Patients with VA had spontaneous episodes of chest pain during sleep with accompanying ischemic ST segment elevation (ST-E) or depression (ST-D). In addition, patients with VA had angiographic evidence of 50% or more vasoconstriction induced by acetylcholine infusion in one or more segments of the epicardial arteries with associated ischemic ST segment changes. Age-matched control patients had atypical chest pain without ischemic ECG changes or acetylcholine-induced vasospasm. Patients with arrhythmic activity accounting for more than 0.5% of the total beats in 1 day or patients taking medications affecting autonomic tone were excluded. Antianginal agents were discontinued at least 48 h before the study. Following the study, 2 patients had hypoglycemic symptoms and were excluded. Therefore, the results from 24 patients with VA (11 men, 8 women; mean age: 60 ± 6 years) and 6 control patients (2 men, 3 women; mean age: 63 ± 5 years) were included in the analysis.

Study Protocol and Assays

The Nippon Medical School Ethics Committee approved this clinical study, and informed consent was obtained from all patients. Patients underwent 24-h Holter monitoring starting the night before the study. Thirty minutes prior to the infusion of insulin, the patients were placed in the supine position. An intravenous catheter was placed in the left median antecubital vein to infuse insulin and to withdraw blood. Humulin-Regular (Eli Lilly and Company, Indianapolis, IN, USA) was infused at a dose of 0.1 IU/kg and at a rate of 1 U/min beginning at 7.00 h. The plasma glucose concentration was measured every 30 min, and serum potassium and norepinephrine concentration were measured every 15 min. The ECG was monitored continu-

ously. The study was discontinued if severe symptoms of hypoglycemia or anginal pain that was 30% or more of the patient's most severe anginal pain occurred. Plasma norepinephrine was assayed by an autoanalyzer (HLC-725CA, Tohso Chemical Co, Tokyo, Japan) using a high performance liquid chromatographic method with an interassay coefficient of variation of 2.5%.

Data Analysis

Heart rate and blood pressure data were obtained by Holter ECG recording and noninvasive ambulatory blood pressure monitoring (model ABPM630, Nihon Colin Co, Tokyo, Japan). The standard V₅ lead and a modified inferior lead were used for 24-h Holter monitoring. The recording tape was analyzed using the Marquette Holter system (Marquette model 8500 & 8000/T, Marquette Ltd, Milwaukee, WI, USA). The noninvasive blood pressure monitoring system was placed on the upper arm 15 min before the beginning of the study and acquired data every 5 minutes. ST segment deviation was considered to represent TMI if there was 1 mm or more of ST-D with a horizontal or downsloping pattern or 1 mm or more of ST-E 60 msec after the J point lasting at least 60 sec. The time of onset of ST segment deviation was determined from the real-time ECG based on ST segment trends. Autonomic nervous system activity was evaluated by the frequency domain analysis of HRV using data from the Holter monitor ECG and calculated using a personal computer (PC-9821, Nihon Electronics Co, Tokyo, Japan). Power spectral analysis of HRV was performed on 5 min of consecutive RR intervals. The data were processed by the maximum entropy method (MEM; MemCalc⁸, Suwa Trust, Tokyo, Japan). The resulting power spectrum was separated into a low-frequency component (LF, 0.04–0.15 Hz) and a high frequency component (HF, 0.15–0.40 Hz). RR interval data containing irregular beats were analyzed using the complement method. The HF component, an indicator of parasympathetic activity, and the LF/HF ratio, an indicator of sympathovagal balance, were calculated every 5 min during the insulin infusion and every 60 min beginning at 1.00–5.00 h (ie, during sleep) before the study.

Cardiac Catheterization

Calcium channel blocking agents and long-acting nitrates were discontinued at least 48 h before cardiac catheterization. After a standard protocol for left-sided cardiac catheterization and coronary angiography was performed, the coronary vasomotor response to intracoronary acetylcholine infusion was determined. Incremental doses of acetylcholine chloride (Dai-ichi Pharmaceutical Co, Tokyo, Japan) in 5 ml of saline were infused, first at 20 µg (5 ml/min), then at 20 µg (5 ml/20 sec), followed by 50 and 100 µg (5 ml/min). Coronary angiography was performed 1 min after each acetylcholine injection. If acetylcholine-induced vasospasm occurred, no further doses of acetylcholine were administered. Coronary arteriographs were obtained when acetylcholine-induced vasospasm (>50% vasoconstriction in one or more segments of the epicardial coronary arteries) occurred with associated ischemic ECG changes (>1 mm ST-D or ST-E) occurred, or 3 min after each acetylcholine injection, if no vasospasm occurred.

Statistical Analysis

We compared data from patients with VA who had

either with ST segment deviation (ST(+)) or did not ST segment deviation (ST(-)). All results are expressed as the mean±SD. A two-tailed, unpaired Student's t-test and the Fisher exact probability test were used to compare group mean values. Group differences for categorical data were tested by Chi-squared analysis employing Yates correction. Humoral factor responses to insulin infusion were compared between groups using a paired Student's t test for repeated measures. Values of $p < 0.05$ were considered statistically significant.

Results

Patient Characteristics

There were no significant differences in mean age, gender makeup, insulin doses, or coronary risk factors between the VA group and the control group (Table 1). The mean frequency of occurrence of spontaneous chest pain during the 1 week prior to the study was 2.9 ± 3.1 episodes. Eighteen (75%) of the patients with VA had no significant stenoses noted during coronary arteriography.

Nine (38%) of the patients with VA had ST-D after the infusion of insulin. Four of these patients had symptomatic ST-D. The mean time to onset of ST-D after the infusion of insulin was 44 ± 10 min. The mean doses of insulin infused in the ST(+) group was significantly lower than in the ST(-) group (5.6 ± 0.9 IU vs 6.3 ± 0.8 IU, $p < 0.05$). However, there were no significant differences in the mean age, gender makeup, or number of coronary risk factors between these 2 subgroups.

Plasma Glucose and Serum Potassium Concentration

Analysis of the temporal changes in plasma glucose concentration for both the VA and control groups showed that the nadir was reached 30 min after the infusion of insulin. With respect to the relationship between glucose concentration and the development of ST-D, there was no significant difference in the plasma glucose concentration before insulin infusion between the ST(+) group and the ST(-) group. However, the plasma glucose concentration 30 min after insulin infusion was significantly lower in the ST(+) group than in the ST(-) group (Table 1; $p < 0.05$).

The maximum decrease in the serum potassium concentration in VA and the control groups occurred 30 min after insulin infusion, and then gradually increased to the baseline concentration 30 min later in all 3 groups (Table 1).

Plasma Norepinephrine

The mean plasma norepinephrine concentration 30 min after insulin infusion in the ST(+) group was significantly greater than in the ST(-) and control groups (314 ± 140 pg/ml vs 134 ± 62 pg/ml, 150 ± 46 pg/ml respectively; $p < 0.05$, Fig 1). Furthermore, the norepinephrine concentration in the ST(+) group increased significantly compared with the value before the infusion of insulin in the same group ($p < 0.05$). The mean plasma norepinephrine concentration in the ST(-) group did not increase significantly and was similar to that in the control group.

Hemodynamics

The mean heart rate (Fig 2, upper panel) in the ST(-) and control groups did not change after the administration of insulin. In contrast, in the ST(+) group, the heart rate increased from 64 ± 10 /min before insulin infusion to 69 ± 9 /min 45 min after infusion, although the increase was not

Table 1 Baseline Characteristics of the Study Population

Variable	Total	VA		Control Patients
		ST(+)	ST(-)	
Number of patients	24	9	15	6
Age (yr)	59±9	63±7	56±10	63±4
Gender (M:F)	16:8	6:3	10:5	3:3
Anginal episodes (episodes/week)	2.9±3.1	3.4±3.1	0.5±0.7	
Quantity of In infused (IU)	6.0±0.9	5.6±0.9	6.3±0.8*	5.5±1.1
Patients with angina	4(17)	4(44)	0(0)	
Time to onset of ST-segment Deviation (min)		44±10		
Coronary risk factors				
Diabetes mellitus	1(4)	0(0)	1(7)	1(20)
Hypertension	8(33)	3(33)	5(33)	3(50)
Hyperlipidemia	11(46)	5(56)	6(40)	1(17)
Smoking	17(71)	7(78)	10(67)	2(33)
No. of diseased coronary arteries				
0 vessels	18(75)	6(67)	12(80)	6(100)
1 vessel	5(21)	3(33)	2(13)	0(0)
2 vessels	1(4)	0(0)	1(7)	0(0)
3 vessels	0(0)	0(0)	0(0)	0(0)
Plasma glucose concentration (mmol/L)				
Before In infusion	4.9±0.4	4.8±0.4	5.0±0.3	5.5±0.7
At 30min after	3.3±0.4‡	3.0±0.3*‡	3.4±0.4‡	3.6±0.6‡
Serum potassium concentration (mmol/L)				
Before In infusion	4.2±0.3	4.4±0.4	4.2±0.3	4.3±0.3
At 30min after	3.9±0.3‡	4.0±0.3‡	4.0±0.3‡	3.9±0.4‡

Data are presented as the mean±SD or number (%) of patients. VA, patients with variant angina; ST(+), VA patients with ST segment deviation; ST(-), VA patients without ST segment deviation; In, insulin; * $p<0.05$, ST(+) vs ST(-); ‡ $p<0.01$, before insulin infusion vs 30 min after insulin infusion.

Fig 1. Time course for changes in plasma norepinephrine concentration. ST(+), patients with ST segment depression (n=9); ST(-), patients without ST segment depression (n=15); controls, control patients (n=6); ** $p<0.01$, ST(+) vs ST(-); * $p<0.05$, before insulin infusion vs 30 min after.

statistically significant. The mean systolic blood pressures tended to increase over time in the ST(+) group only (Fig 2, lower panel). However, the mean diastolic blood pressure was almost the same in all 3 groups.

Autonomic Nervous System Activity

In the ST(+) group, the HF component of HRV gradually decreased during the 60 min after insulin infusion, but this did not reach statistical significance (Fig 3, upper panel), whereas the LF component in the ST(+) group gradually increased (Fig 3, middle panel). In contrast, the changes in the HF and LF components in the ST(-) group were similar to those in the control group. There was no

Fig 2. Time course for changes in heart rate and blood pressure. Upper panel: heart rate; lower panel: systolic and diastolic blood pressures. SBP, systolic blood pressure; DBP, diastolic blood pressure; other abbreviations as in Fig 1.

Fig 3. Time course for changes in autonomic nervous activity. Upper panel: high frequency (HF) component; middle panel: low frequency (LF) component; lower panel: LF/HF ratio. Abbreviations as in Fig 1. * $p < 0.05$, ST(+) vs ST(-); † $p < 0.05$, before insulin infusion vs 30, 45, 60, and 75 min after insulin infusion in the ST(+) group.

significant difference in the LF/HF ratio between the ST(+) and the ST(-) groups before and after insulin infusion, except 15 min after insulin infusion (Fig 3, lower panel). However, the change in the LF/HF ratio in the ST(+) group 30 min after insulin infusion was significantly greater than that in the ST(-) group ($34 \pm 31\%$ vs $4 \pm 34\%$, respectively, $p < 0.05$). The changes in the LF/HF ratio in the ST(-) group were similar to those in the control group.

Coronary Angiography

All of the patients in the ST(+) group experienced significant vasospasm in response to $20 \mu\text{g}$ of acetylcholine. In contrast, 10 (63%) of 16 patients in the ST(-) group experienced significant vasospasm in response to $20 \mu\text{g}$ of acetylcholine. In patients with VA and stenotic coronary arteries, the site of vasospasm was the same as the site of stenosis. In 7 of the 9 patients in the ST(+) group, the region with ST segment depression during insulin infusion coincided with the site of acetylcholine-induced vasospasm and the region with spontaneous ST segment elevation on Holter or standard 12 lead ECG.

Nocturnal HRV

Analysis of the Holter ECG obtained during sleep (1.00–5.00 h) immediately before insulin infusion revealed

Fig 4. Nocturnal time course for changes in autonomic nervous activity. Upper panel: HF; lower panel: LF/HF ratio. Abbreviations as in Fig 3.

that the HF component in the ST(+) group was greater in the early morning hours than in the ST(-) and control groups (Fig 4). The changes in the HF component in the ST(-) group were similar to those in the control group. With respect to the LF/HF ratio, the changes in the ST(+) group were similar to those in the ST(-) and the control groups.

Discussion

Induction of Ischemia

In the present study, changes in the catecholamine concentrations in response to insulin were significantly higher in the ST(+) group than in the ST(-) and control groups. Furthermore, significant ST segment depression occurred only in patients with increased plasma norepinephrine concentration and associated changes in either blood pressure or heart rate. We assumed that the insulin loading performed in the present study transiently activated the sympathetic nervous system via α -adrenergic receptors thereby increasing coronary arterial vasoconstrictor tone.⁹ Previous studies have demonstrated a relationship between enhanced insulin responsiveness and acetylcholine-induced vasoconstriction in patients with vasospastic angina.¹⁰ There are several methods that can be used to induce vasospasm (ie, hyperventilation, smoking stimulation, vagomimetic agents, α -adrenergic blockade, and exercise). However, in order to study the mechanism and time course of autonomic nervous system activation during spontaneous nocturnal myocardial ischemia, the patient should be resting and methods that do not directly influence autonomic nervous activity are required. Using the euglycemic-

hyperinsulinemic clamp method, gradual stimulation of the sympathetic nervous system could cause α -adrenergic stimulation of the coronary arteries leading to vasodilation. Therefore, we chose to use the insulin loading method, without glucose infusion, to study the time course of sympathetic nervous system activation. Although hypoglycemia can occur during insulin loading, only 2 patients in our study developed hypoglycemic symptoms or mild chest discomfort and there were no severe hypoglycemic complications.

Time Changes in Autonomic Nervous Activity

The present study demonstrated that in the ST(+) group there was a mild decrease in the HF component of HRV indicating a decrease in vagal activity. In addition, a gradual increase in the LF component and the LF/HF ratio indicated an increase in sympathetic activity that preceded the development of ECG evidence of myocardial ischemia. These findings suggest either that the autonomic changes are part of the mechanism that triggers increased coronary vasomotor tones or that the changes facilitate or predispose to the development of vasospasm by other vasoconstrictor agents. Our results are consistent with those of a previous study which reported that increases in the LF/HF ratio and decreases in the HF component preceded spontaneous ST-E₅. In fact, almost all of the patients in the ST(+) group in our study had dramatic insulin-induced norepinephrine release and an increased nocturnal HF component. These changes result in an increased frequency of anginal episodes. In addition, the coronary arteries in these patients reacted to low-dose intracoronary acetylcholine injection. We hypothesize that nocturnal coronary spasm occurs in these patients during acute reductions in the HF component with concomitant increases in the LF component or LF/HF ratio.

Many hypotheses have been proposed to explain the increased susceptibility to coronary spasm in patients with variant angina. However, both the basic factors predisposing to vasoconstriction and the stimulus that triggers spontaneous spasm in the hyperreactive coronary artery remain unknown.¹¹ Several investigators have proposed a role for vagal activation in coronary vasospasm.^{12,13} However, some investigators have suggested that adrenergic activation, not vagal activation, is responsible, because of upregulation of vascular adrenergic receptors during low sympathetic activity during sleep.¹⁴⁻¹⁸ Based on our results, the trend toward a decrease in the HF component before the onset of ischemia suggests that vagal withdrawal may be a factor involved in spontaneous coronary vasospasm in patients with variant angina.

Nocturnal Level of Parasympathetic Nervous Activity

Although a decrease in the nocturnal HF value in patients with coronary artery disease has been reported previously,¹⁹ the severity of coronary artery disease in this study population was mild, and the value for the HF component in the ST(-) group from midnight to early morning was similar to that in the control group.²⁰ However, it is unclear why the level of parasympathetic nervous activity in the ST(+) group was higher than in the ST(-) group. In the present study, antianginal agents that could increase parasympathetic nervous activity were discontinued at least 48 h before the study. In addition, there were no significant differences in the frequency of diabetes mellitus, hypertension, smoking and the severity of coronary

artery disease, which could influence parasympathetic nervous activity, in our subgroups. Because there was no significant difference in the mean heart rate between the ST(+) and the ST(-) groups (data not presented), we hypothesize that the mechanism of increased parasympathetic nervous activity during sleep involves direct activation of the central nervous system (CNS). This hypothesis is supported by reports that anginal attacks at rest occur in patients following cardiac transplantation and/or cardiac denervation,²¹ both of which modify the CNS.

The nocturnal changes in the HF component in the ST(+) group peaked at 3.00 h and thereafter fell sharply. The cause of the rapid decrease in parasympathetic nervous activity is unclear. There were no anginal episodes from 3.00–5.00 h in the ST(+) group. One possible cause of the reduction in the HF component is that 4 of the 9 patients in the ST(+) group woke up around 6.00 h and began their daily activities. From 4.00–5.00 h, the LF/HF ratio gradually increased prior to the patients waking up. It has been reported that activation of the sympathetic nervous system cause the heart rate and systolic blood pressure to gradually increase before people wake up.²²

It has been reported by Yoshio et al that the HF and LF components of HRV rapidly increase immediately prior to ST segment elevation during sleep.⁴ Although it is possible that increased parasympathetic nervous activity during sleep triggers vasospasm, only 7 episodes of vasospasm were analyzed in that study. In addition, at least 3 of the 7 episodes were not accompanied by significant increases in the HF component prior to ST segment elevation. Spectral analysis of HRV was performed for longer periods than in the present study and using a nonstandard range for the HF component (0.22–0.32 Hz). In contrast to that study, we were unable to detect temporal changes in autonomic tone in the present study.

Mechanism Responsible for Increased Sympathetic Nervous System Activity

The mechanism responsible for the effect of insulin on the sympathetic nervous activity is not clear. However, possible mechanisms include: (1) decreased plasma glucose concentration causing counterregulation; (2) baroreflex-mediated increases in sympathetic tone caused by an insulin-mediated increase in skeletal muscle blood flow and resultant decrease in blood pressure; and (3) activation of the CNS. With respect to the first mechanism, hypoglycemia occurred both in patients with VA and in the control group in response to insulin, but the degree of hypoglycemia 30 min after the insulin infusion in the ST(+) group was greater than in the ST(-) group. In addition, significant increases in the concentration of norepinephrine occurred in the ST(+) group, whereas the increase in norepinephrine concentration was minimal in the ST(-) group. The second possible mechanism involves reflex increases in sympathetic tone caused by vasodilation and a slight decrease in diastolic blood pressure. After the infusion of insulin, diastolic blood pressure decreased slightly in the ST(-) and control groups, which could increase baroreceptor activity. However, diastolic blood pressure in the ST(+) group did not change. Further, it has been reported that patients with vasospastic angina have a high incidence of impaired glucose tolerance, which is responsible for severe coronary vasospasm.²³ We hypothesize that this baroreflex increases coronary arterial vasomotor tone via α -adrenergic receptor stimulation. The third possible mechanism in-

volves a direct CNS insulin action.^{24–26} Thus, while insulin infusion produces substantial increases in the LF/HF ratio, the mechanism responsible for this is not clear.

Study Limitations

In the present study, we attempted to increase sympathetic nervous system activity through insulin-induced hypoglycemia. Nine (38%) of the patients with VA experienced significant ST segment depression. Patients without ST segment depression consisted of 2 subgroups: one with and one without increases in the plasma norepinephrine concentration. There are several possible reasons why there were no significant ST segment changes in these patients. First, activation of the cardiac sympathetic nervous system may not be sufficient to cause ST segment deviation. Second, the cardiac sympathetic nervous output, even if it is maximum, is not able to trigger vasospasm. Third, the activity of the α -adrenoreceptor system is greater than that of the β -adrenoreceptor, resulting in augmentation of coronary blood flow through the endothelial release of NO.²⁷ Finally, no conclusions can be drawn from the present study about the role of sympathetic nervous system activity in the pathogenesis of vasoconstriction, because there is no accurate HRV index for sympathetic nervous system activity. Instead, we used the LF/HF ratio, which is an index of sympathovagal balance.

Conclusion

Patients with VA who have high levels of nocturnal parasympathetic activity produce greater amount of norepinephrine in response to insulin stimulation than patients with VA and normal nocturnal parasympathetic activity. These findings suggest that patients with VA and frequent night time ischemic episodes have coronary hyperactivity to adrenergic stimulation and high levels of nocturnal vagal tone. These abnormalities may act as a trigger for spontaneous epicardial spasm.

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