Clinical Studies

The Role of Parasympathetic Nerve Activity in the Pathogenesis of Coronary Vasospasm

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SUMMARY

To evaluate the role of the autonomic nervous system, especially the parasympathetic nervous system, in the initiation mechanism of vasospastic angina pectoris (AP), the coefficient of R-R interval variation (CV) on the electrocardiogram (ECG) and plasma catecholamine concentration were measured in 25 patients with vasospastic AP, 10 patients with effort AP and 12 control subjects. CV which has been recognized as reflecting parasympathetic nervous system activity was calculated from 100 consecutive heart beats on the ECG and represented as the percentage of standard deviation of the R-R interval per mean R-R interval. Repeated measurements of plasma catecholamine concentration revealed higher values at any sampling point throughout a day in patients with vasospastic AP than those in control subjects. A distinctly higher CV was observed at night in the vasospastic AP group. This elevated CV was abolished by atropine sulfate (1.5 mg/day per os). Pilocarpine injection (1.3 mg/10 kg B.W. subcutaneously) induced a marked increase in CV that preceded the occurrence of chest pain and/or ischemic ECG changes in 5 patients with vasospastic AP. The increment in CV at 10 min after pilocarpine administration was greater in vasospastic AP than in control subjects (p<0.05). It is concluded that enhanced parasympathetic activity may play a role in the initiation of coronary vasospasm associated with sympathetic hyperactivity.

Additional Indexing Words:
Parasympathetic nerve activity R-R interval variation Coronary vasospasm Circadian rhythm of autonomic nervous system Pilocarpine Atropine Catecholamines

RECENT studies have indicated that the autonomic nervous system may play a role in the pathogenesis of ischemic episodes of vasospastic angina. Yasue and other investigators reported that parasympathomimetic agents such as methacholine and pilocarpine provoked anginal attacks in...
patients with vasospastic angina, and postulated that autonomic dysfunction might be involved in the mechanism of coronary vasospasm.\textsuperscript{11,6} Tamada also indicated that an increased reflex response of the sympathetic nervous system was closely correlated with the initiation of anginal attacks under conditions of parasympathetic hyperactivity.\textsuperscript{7}

These observations suggest that underlying dysfunction of the autonomic nervous system might play an important role in the provocation of coronary artery vasospasm. However, there have been few reports concerning the role of the parasympathetic nervous system in the initiation mechanism of vasospastic angina because of the difficulty of assessing this.\textsuperscript{8,9} The coefficient of R-R interval variation (CV) on the ECG has recently been reported to be a better index of parasympathetic nerve activity compared with the biochemical approach of measuring plasma cyclic guanine monophosphate (c-GMP) concentrations.\textsuperscript{10,11} This study was performed to evaluate the role of the autonomic nervous system in the initiation of vasospastic angina by measuring plasma catecholamine concentration as a parameter of sympathetic nerve activity and by measuring CV on the ECG to assess parasympathetic nerve activity in control subjects and patients with angina pectoris, especially vasospastic angina.

**Materials and Methods**

*Subjects:*

The study was performed in 35 patients (33 males and 2 females) with an average age of 54.8±2.0 years (mean±SEM) and 12 age-matched control subjects without heart disease or diabetes mellitus, with an average age of 51.3±2.7 years (8 males and 4 females). Twenty-five patients (23 males, 2 females; mean age 53.6±1.6 years) with vasospastic angina were selected for the study. Patients with vasospastic angina exhibited ST segment elevation of 0.2 mV or more measured at a point 0.08 sec from the J point on ECG during an anginal attack at rest and/or were angiographically documented as having coronary artery spasm provoked by ergonovine maleate.

All patients reported angina predominantly at rest with frequent attacks in the early morning hours. Holter monitoring showed episodes of transient ST segment elevation in 15 patients; the remaining 10 had both ST segment depression and ST segment elevation alternating in the same leads during different episodes. The remaining 10 patients (10 males), with an average age of 57.7±2.7 years, had effort angina pectoris.

All patients were normotensive and in sinus rhythm and had no evidence of cardiac failure or abnormal glucose tolerance. No patient had
conduction disturbances or left ventricular hypertrophy on electrocardiogram that could prevent the interpretation of ST segment changes.

**Study protocol:**

Circadian variation of plasma catecholamine concentration was assessed by blood sampling every 4 hours. Blood samples were withdrawn from the antecubital vein through an indwelling scalp needle after the patients rested in the supine position for at least 30 min. The coefficient of R-R interval variation (CV) was measured every hour, before and after spontaneous attacks of vasospastic angina, with an Autonomic R-100 manufactured by ME Commercial Co. (Japan). CV was calculated by the following formula: \( CV = \frac{SD}{\text{mean R-R interval}} \times 100 \), wherein SD was the standard deviation of the R-R interval from 100 consecutive heart beats, mean R-R interval was the mean R-R interval of 100 consecutive heart beats and CV was represented as a percentage.

The pilocarpine loading test was done as follows. Subjects fasted in the morning, were kept at rest in bed for 30 min and then received a subcutaneous injection of 0.13 ml of 1% pilocarpine hydrochloride/10 kg of body weight at 10:00 A.M. Heart rate, blood pressure and CV were measured and blood samples were taken from the antecubital vein before and 15, 30, 60 and 90 min after pilocarpine administration.

Stimulated symptoms of parasympathetic nerve activity, including sweating and salivation, were also observed. When the patients developed chest pain with ischemic ECG changes during the test, nitroglycerin was promptly given sublingually. Plasma norepinephrine and epinephrine were determined using high performance liquid chromatography (HPLC) combined with a highly sensitive and specific electrochemical detector (VMD-501, Yanagimoto Co.).

The results are expressed as mean±1 standard error of the mean (SEM). Statistical analyses of data were made using Student’s t-test. A p value less than 0.05 was considered statistically significant.

**Results**

1. Circadian variation of plasma catecholamine concentration and the CV value

Plasma norepinephrine concentrations taken from 7 patients with vasospastic AP were higher than those of the control subjects at any point throughout 24 hours, and in particular were significantly elevated at 0:00 and 12:00 (p<0.05) (Fig. 1).
Plasma epinephrine concentrations taken from patients with vasospastic AP showed almost the same tendency as plasma norepinephrine concentrations and were significantly higher at 4:00 and 12:00 (p<0.02) (Fig. 1). Though the CV value is known to be lower in elderly people, there were no differences in age among the control, vasospastic and effort angina groups.

Fig. 1. Circadian variation of plasma catecholamine in control subjects and patients with vasospastic AP. Values are given as mean±1 SEM. ** p<0.02, * p<0.05 vs. control subjects. AP=angina pectoris. Hatched zone shows the lights-out period.

Fig. 2. Circadian rhythm of R-R interval variation and heart rate in control subjects. Hatched zone shows the lights-out period. HR=heart rate.
The CV value in control subjects was $4.27 \pm 0.45\%$ just before lights-out at 21:00, gradually declined to a minimum of $2.40 \pm 0.73\%$ at 4:00, and then increased and reached $3.23 \pm 0.56\%$ around 6:00 when they awoke (Fig. 2). There was no difference in CV between vasospastic AP and control subjects during the daytime, but a distinctly higher CV at night was observed in vasospastic AP as compared to that of control subjects (Fig. 3). This higher CV was abolished by atropine sulfate (1.5 mg/day per os) and stabilized at $1.0 \pm 0.1\%$ (data not shown). CV in patients with effort AP showed the

![Graph showing circadian rhythm of R-R interval variation in control subjects and patients with vasospastic AP.](image1)

**Fig. 3.** Circadian rhythm of R-R interval variation in control subjects and patients with vasospastic AP. *p<0.05 vs. control subjects. Hatched zone shows the lights-out period.

![Graph showing circadian rhythm of R-R interval variation in control subjects and patients with effort AP.](image2)

**Fig. 4.** Circadian rhythm of R-R interval variation in control subjects and patients with effort AP. Hatched zone shows the lights-out period.
same tendency as that in control subjects (Fig. 4). In one representative case with vasospastic AP who had spontaneous attacks at night, a pronounced increase in CV was observed 2 hours before the occurrence of an anginal attack (Fig. 5).

2. Pilocarpine test

Chest pain and/or ischemic ECG changes induced by pilocarpine were found in 8 of 12 patients with vasospastic AP. These ischemic signs appeared from 7 to 25 min after the pilocarpine injection and did not relate to the extent of stimulated symptoms of parasympathetic nerve activity such as sweating and salivation. Plasma norepinephrine concentration at 15 min after pilocarpine administration was increased by 57% (from 226.2±25.2 pg/ml to 354.5±32.4 pg/ml) in control subjects and by 127% (from 252.3±25.6 pg/ml to 573±61.4 pg/ml) in patients with vasospastic AP, the increase in the latter group being significantly greater (p<0.005) (Fig. 6). In addition, a significant increase was also observed at 30 min in patients with vasospastic AP as compared with control subjects (p<0.05). Plasma epinephrine concentration in control subjects showed no change following pilocarpine, while in the vasospastic AP group this increased by 115% (from 95.4±20.0 pg/ml to 204.9±21.6 pg/ml) at 15 min (p<0.01 vs. control subjects) and 90% at 30 min (p<0.02 vs. control subjects) (Fig. 6). This increase was still maintained at 60 min after pilocarpine administration. With regard to hemodynamic responses to pilocarpine, there were no differences in heart rate, blood pressure and double product between vasospastic AP and control subjects (data not shown).
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Fig. 6. Effect of pilocarpine on the variation of plasma norepinephrine concentration in control subjects and patients with vasospastic AP. *** p<0.005, ** p<0.02, * p<0.05 vs. control subjects. AP=angina pectoris; B.W.=body weight; sc=subcutaneous injection.

Fig. 7. Effect of pilocarpine on R-R interval variation in control subjects and patients with vasospastic AP. * p<0.05 vs. control subjects.

CV increased by 30% from the baseline level of 3.61±0.51% and reached a maximum of 4.71±0.65% (p<0.05 vs. baseline) in control subjects at 15 min after pilocarpine administration. In vasospastic AP, on the other hand, CV started to increase several minutes before the occurrence of chest
pain in 5 patients and increased by 92% from the baseline of 3.06±0.30% to 5.21±0.36% at 10 min after pilocarpine administration (p<0.001 vs. baseline) (Fig. 7). CV at 10 min after pilocarpine administration was significantly higher in vasospastic AP than that in control subjects (p<0.05).

CV still maintained the high level of 4.44±0.37% at 15 min after pilocarpine administration (p<0.02 vs. baseline). In effort AP (Fig. 8), the val-
values of CV were 3.32±0.62%, 4.25±0.27% and 4.60±0.45%, respectively, before and at 10 and 15 min after pilocarpine administration (p<0.05 vs. baseline). In a representative case of vasospastic AP in whom an anginal attack was provoked by pilocarpine administration, CV was elevated by 110%, from the baseline level of 4.01% to 6.73%, 3 min before the occurrence of chest pain (Fig. 9).

**DISCUSSION**

The spontaneous attack of chest pain in patients with variant angina often occurs early in the morning, and nocturnal fluctuation of autonomic nerve activity is assumed to have some relationship to the occurrence of the anginal attack. Nowlin and Murao suggested that the anginal attack in vasospastic AP was closely related to the rapid eye movement (REM) sleep stage, which is considered to be the stage of autonomic storm. On the other hand, Clark and other investigators reported that anginal attacks at rest occurred in patients with cardiac autotransplantation and/or cardiac denervation. In patients with cardiac denervation, heart rate increased moderately on exercise through activation of the humoral regulation system, which was modified by the central nervous system. In the present study, plasma catecholamine concentrations at any point throughout a day were found to be higher in 7 patients with vasospastic AP than that in 5 control subjects. In particular, the plasma norepinephrine level was markedly elevated in patients with vasospastic AP at the period between 0:00 and 12:00. These results suggest that the nocturnal level of sympathetic nerve activity might be higher in patients with vasospastic AP as compared with control subjects. Although Robertson reported that there was no coronary A-V difference of plasma catecholamine concentrations in vasospastic AP before and during spontaneous attacks, Tamada recently observed that an increase in plasma catecholamine concentrations preceded the occurrence of chest pain and/or ischemic ECG changes in spontaneous and pilocarpine induced AP, and suggested that the higher sympathetic nerve responsiveness was probably the cause rather than the consequence of transient myocardial ischemia in vasospastic AP. Yasue proposed that coronary vasospasm could be provoked by stimulation of \( \alpha \)-adrenergic receptors in the large coronary arteries under conditions of parasympathetic nerve hyperactivity in vasospastic AP patients. Thus, it is possible that sympathetic nerve hyperactivity may be involved in the initiation mechanism of coronary vasospasm.

To evaluate the role of the autonomic nervous system in vasospastic
AP, it is absolutely necessary to assess not only sympathetic nerve activity but also parasympathetic nerve activity. However, there has not been an appropriate index for parasympathetic nerve activity. Kageyama recently suggested that the coefficient of R-R interval variation (CV) might possibly reflect parasympathetic nerve activity. In our previous report, CV ranged widely from 2% to 9% at rest in normal controls, but intravenous administration of atropine sulfate reduced the CV value, which stabilized at 1.0±0.1%, and no further fluctuation of CV was monitored. On the other hand, CV was not affected by propranolol. When norepinephrine was administered to normal subjects by intravenous drip infusion, heart rate decreased in parallel with an increase in blood pressure, by baroreflex-mediated parasympathetic nerve activation, resulting in an increased CV. Such an increase in CV by norepinephrine administration was effectively suppressed by treatment with atropine sulfate, following which both blood pressure and cardiac rate were markedly increased. We also found that no major influence on CV was exerted by moderate changes in heart rate and basal respiration states at rest. The above observations indicate that CV can be regarded as an index of parasympathetic nerve activity.

In humans, nocturnal parasympathetic nerve activity is generally thought to be higher than diurnal activity, as determined from clinical estimations of parasympathetic nerve activity based on heart rate and variation of cardiac beats. However, the concept has not yet been confirmed and is still controversial because of the difficulty of developing an appropriate approach to estimate parasympathetic nerve activity. In this study, the nocturnal CV level of control subjects was lower than its diurnal level.

Leichnetz recorded parasympathetic discharge electrophysiologically through electrodes implanted in the cervical vagal nerve in unanesthetized cats and observed a circadian rhythmicity in visceral nerve activity similar to that observed in the present study. Utsumi suggested that the nocturnal decrease in parasympathetic nerve activity was determined in humans by use of the pupillogram. Thus, it is suggested that the nocturnal level of parasympathetic nerve activity may be lower than the diurnal level in control subjects, and that a reduction in parasympathetic nerve activity may be proportionally smaller than that of sympathetic nerve activity at night. CV in vasospastic AP was maintained at a relatively higher level throughout the night until dawn than that in the control subjects. The results indicate the presence of enhanced parasympathetic nervous activity in patients with vasospastic AP during this period. In other observations, patients with coronary vasospasm have shown increases in plasma cGMP concentration at 30 to 60 min before the spontaneous anginal attack. These observations
indicate that hyperactivity of the parasympathetic nervous system might possibly be involved in the mechanism of coronary vasospasm.

To elucidate the pathophysiological significance of parasympathetic hyperreactivity in the provocation of anginal attacks in patients with vasospastic AP, pilocarpine was employed as a pharmacologic stimulus, however, pilocarpine actually induces not only a parasympathetic hyperactive state but also adreno-sympathetic activation including norepinephrine release from the postganglionic sympathetic nerve terminals, and epinephrine release from the adrenal glands. On the other hand, methacholine, which is considered to have only parasympathomimetic action, provokes intrinsic sympathetic nerve hyperactivity through the baroreflex. Therefore, it is clinically difficult to determine the isolated effect of parasympathetic nerve activity. Methacholine, atropine and epinephrine have been generally used for pharmacologic evaluations in clinical trials, but there is a limitation to the ability to isolate the pure effect of parasympathetic nerve activity in vivo. Therefore, further accumulation of clinical data concerning autonomic nerve activity will be needed to clarify the role of the autonomic nervous system in the initiation mechanism of coronary vasospasm.

CV in control subjects showed a significant increase at 15 min after pilocarpine administration and CV in effort AP revealed the same tendency as that in control subjects. Whereas CV in vasospastic AP showed significant increases at 5, 10 and 15 min after pilocarpine administration and returned to baseline at 30 min. In cases with vasospastic AP, CV was increased several minutes before the occurrence of an anginal attack. Thus, it was suggested that there was parasympathetic nerve hyperreactivity in patients with vasospastic AP as compared to control subjects.

Yasue and Horio recently observed that intracoronary acetylcholine infusion induced coronary vasospasm in patients with vasospastic AP, and that atropine sulfate successfully blocked this acetylcholine induced vasospasm. Vasospastic anginal attacks actually occur not so frequently in the daytime when sympathetic nerve activity is increased, but more frequently early in the morning when parasympathetic nerve activity is relatively high as observed above. The present study supports the hypothesis that the parasympathetic nervous system might be involved in the mechanism of coronary artery vasospasm.

Thus, it is concluded that enhanced parasympathetic nervous activity may play an important role in the pathogenesis of coronary vasospasm in patients with vasospastic AP.
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