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Frequency Domain Heart Rate Variability and Plasma Norepinephrine Level in the Coronary Sinus during Handgrip Exercise

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Summary

Background: Heart rate (HR) variability has been recognized as an important noninvasive index of autonomic nervous activities. However, the relationship between HR variability and cardiac circulating norepinephrine (NE), especially with respect to coronary ischemia, remains unclear.

Hypothesis: This study was undertaken to determine whether HR variability indices can reflect cardiac NE levels during handgrip exercise.

Methods: We simultaneously measured HR variability and cardiac NE overflow rate in 32 patients (30 men, 2 women) during a 6-min isometric handgrip exercise. Among the 32 subjects, 20 (19 men, 1 woman) had coronary artery disease (CAD) and 12 (control group; 11 men, 1 woman) did not.

Results: Hemodynamics and cardiac NE overflow rates among subjects at rest were not significantly different between the two groups. In the normal subjects, low-frequency (LF) spectra and LF/HF (high-frequency) ratios were not significantly changed during handgrip exercise, but HF spectra significantly increased from 10.1 ± 4.5 to 12.2 ± 7.0 ms ($p < 0.05$). In the subjects with CAD, LF and LF/HF spectra were significantly ($p < 0.05$ and 0.01, respectively) increased by handgrip exercise. High-frequency spectra were not significantly changed by handgrip exercise. In the normal subjects, a significant negative relation ($r = -0.76$, $p < 0.01$) was obtained between HF change and cardiac NE overflow rate, whereas this relationship was not significant in the subjects with CAD. The correlation between changes of LF/HF and cardiac NE overflow rate was significant in the normal ($r = 0.56$, $p < 0.05$) but not in subjects with CAD.

Conclusion: These results suggest that vagal modulation of HR variability is more prominent in normal coronary artery subjects than in CAD subjects during handgrip exercise. Heart rate variability indices may thus serve as adequate indicators of autonomic nerve activity in subjects with normal coronary arteries but not in those with CAD, probably due to decreased adaptation to physical stress during handgrip exercise.

Key words: heart rate variability, norepinephrine overflow rate, handgrip exercise

Introduction

Power spectral analysis, used to measure heart rate (HR) variability, has been recognized as an important noninvasive tool to assess autonomic nerve function[1–5] and to predict cardiac events and/or sudden cardiac death.[6–8] The low-frequency (LF) band is considered to be a measure of sympathetic activity with a minor parasympathetic component, the high-frequency (HF) band to be a measure of parasympathetic activity, and the ratio of the low- to the high-frequency bands (LF/HF) to be an index of sympathovagal balance.[1–4, 9] However, it remains unclear whether HR variability reflects the effects of cardiac circulating catecholamine during stress.

In the present study, therefore, we evaluate how changes of HR variability reflect cardiac catecholamine excretion. We also compare the relation of HR variability with changes of catecholamine overflow rates in patients with and without coronary artery stenosis (CAD), which is suggested to affect HR variability.[10, 11]

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Methods

Study Group

Thirty-two patients (30 men, 2 women) served as subjects in the present study. After providing informed consent, the patients were divided into two groups according to coronary arteriographic findings obtained at the outset of the study. The CAD group included 20 patients (19 men, 1 woman) who had ≥ 75% stenosis in the coronary arteries and/or a history of myocardial infarction. The normal coronary artery group consisted of 12 subjects (11 men, 1 woman) whose coronary angiographic findings were normal (NL). There were no significant differences between the two groups with respect to cardiothoracic ratio (CTR), blood pressure (BP), or serum total cholesterol (TC) levels (Table I).

Study Protocol

All patients were in a fasting state and had taken no antianginal agents (e.g., long-acting nitrates, calcium-channel blockers, or beta blockers) other than sublingual nitroglycerin for at least 5 days prior to the study. A 7F catheter introducer was inserted into the basilic vein of either the right or left upper limb and/or the right femoral vein. Under fluoroscopic guidance, a Ganz thermodilution catheter was then advanced into the coronary sinus (CS) until its tip was in a position suitable for blood sampling. A 7F pigtail catheter was positioned in the ascending aorta to record the aortic pressure precisely by means of an Acudynamic (Soreson Research Co., Salt Lake, Utah, USA) apparatus.[12] Changes in HR and in ST segments were recorded continuously throughout the procedure using two leads (V_5 and CM_5; central sternum to V_5) connected to a continuously recording Holter electrocardiographic (ECG) monitor, since it has been determined that most of the ischemic changes (90%) can be detected by these two leads.[13] Coronary sinus blood flow (CSBF) was measured at rest as a control. All hemodynamic measures were recorded on an eight-channel recorder (Nihonkoden RMC 1 00, Tokyo, Japan) at rest and during the isometric handgrip exercise. After determining the maximum voluntary contraction of the handgrip using a dynamometer for each patient, patients were asked to sustain a contraction of 30% of their maximum value while maintaining their original respiration rate as much as possible for a period of 6 min.

TABLE I Profiles of study subjects

	CAD (n = 20)	NL (n = 12)
Age	58 ± 12	62 ± 13
Sex (M/F)	19/1	11/1
CTR	52 ± 4	51 ± 4
BP	142 ± 12/90 ± 5	145 ± 7/92 ± 5
TC	198 ± 3	204 ± 4

Abbreviations: CAD = coronary artery disease, NL = normal coronary artery, CTR = cardiothoracic ratio (%), BP = blood pressure (mmHg), TC = total cholesterol (mg/dl), (mean ± standard deviation).

Blood Sampling and Calculation

Blood samples of approximately 10 ml each were obtained from the ascending aorta and CS, both while patients were at rest and immediately after the handgrip exercise. The blood was collected in heparinized tubes and immediately refrigerated. After centrifugation at 3,000 rpm for 15 min at 4°C, approximately 7 ml of plasma was transferred to a tube containing 4 mg of reduced glutathione (as a preservative) and stored at −80°C for subsequent analysis. Plasma norepinephrine (NE) was analyzed by liquid chromatography using an electrochemical detector. This method combined liquid extraction of NE from plasma with reversed phase chromatography and incorporated a cation exchange reagent.[15] The rate of endogenous NE overflow into the CS from the heart was calculated as[16]

$$\Delta NE \times CSBF \times 100\text{-}Ht/100,$$

where ΔNE is the difference between plasma NE concentration in the artery and CS blood, and Ht is hematocrit %. Coronary sinus blood flow was measured using a thermodilution technique and was calculated by the usual formula.[17] Lactate determinations were performed using an enzymatic method with a protein-free supernatant. The lactate extraction ratio (expressed as percent of lactate extraction) was calculated as

$$(La - Lcs) / La \times 100,$$

where La and Lcs represent lactate in the arterial blood and CS, respectively.

HR Variability Analysis

The bipolar leads of V_5 and CM_5 were attached to the precordium. Tape recordings were analyzed using a Marquette 8000 T Holter analyzing system (Marquette Electronics, Milwaukee, Wisconsin, USA). Although HR variability analysis using the time-domain method yields adequate information for the assessment of autonomic nerve function, it requires 24 h of Holter monitor recording. We therefore performed a spectral analysis of HR variability using computer software (version 5.8, 002A ; Marquette Electronics). All QRS labeling was manually edited by an experienced observer blinded to the clinical outcomes. Spectral indices of HR variability were computed by fast-Fourier transformation (FFT) on each 2 min segment of the recording with the application of a Hanning window to minimize spectral leakage. Data from ectopic beats and artifacts were excluded and replaced by maintaining the previous coupling interval levels. Segments containing more than 2% ectopic per total beats were excluded. We used the absolute values of FFT output on the amplitude response (in ms) to all frequencies for each of the 128 output bins along the horizontal axis. Assessment of the LF spectra was from 0.04 to 0.15 Hz, the HF spectra from 0.15 to 0.40 Hz, and the LF and

HF ratios were calculated. The LF and HF values were reported as their natural log (lg). These HR variability indices were measured both before and during the handgrip exercise.

Statistics

Results are expressed as means ± standard deviation. Measurements collected on the same subject before and during handgrip exercise were compared using the Student's *t*-test. To clarify the effects of the handgrip exercise we calculated the differences (Δ) in the exercise-induced changes from the basal state in NE release and HR variability, and an analysis of variance was used to compare between the two groups. The correlation between changes of cardiac NE release and HR variability indices was calculated. Differences were considered significant if $p < 0.05$.

Results

Changes in Hemodynamics

Measurements of HR, systolic (BPs) and diastolic (BPd) blood pressure, CSBF, cardiac NE release, and lactate extraction ratio (LER) obtained from each study group before handgrip testing (at rest) were compared with those obtained during the terminal phase of the handgrip test. In both groups, mean BPs (CAD, 185 ± 21; control, 192 ± 31 mmHg) and CSBF (CAD, 158 ± 104; control, 143 ± 95 ml/min) were significantly ($p < 0.05$) higher than equivalent measures obtained at rest (CAD BPs, 160 ± 23; NL BPs, 157 ± 20 mmHg; CAD CSBF, 90 ± 50, and NL CSBF, 125 ± 12 ml/min) (Table II). The calculated mean cardiac NE overflow rate in the CAD subjects was significantly higher during the handgrip exercise than at rest (57 ± 23 vs. 15 ± 17 pg/ml, ml/min, $p < 0.05$), but this rate did not change significantly in the control group. Heart rate, BPd, and LER measurements obtained at rest were not significantly different from those obtained during the test (Table II). Similarly, there were no significant differences in either group between the resting and handgrip measurements of all parameters, with the single exception of ST segments, which were decreased significantly by handgrip exercise in subjects with CAD but were unchanged by exercise in NL subjects.

Changes in Heart Rate Variability Indices

In each group, HR variability indices obtained at rest were compared with those obtained during handgrip exercise, and the ratio was compared between groups. There were no differences between the two groups with respect to HR variability parameters (LF, HF, LF/HF) at rest or during handgrip exercise (Table III).

In subjects with normal coronary arteries, LF and L/H were essentially unchanged during handgrip exercise [(LF, 17.0 ± 7.2 to 21.6 ± 7.0 ms; L/H, from 1.8 ± 1.3 to 2.2 ± 1.6 ms, not significant (NS)]. However, HF increased significantly ($p < 0.05$) from 10.1 ± 4.5 to 12.2 ± 7.0 ms. In the subjects with CAD, LF and LF/HF were significantly higher during handgrip exercise (LF, from 13.6 ± 8 to 18.8 + 12.3 ms, $p < 0.01$; LF/HF, 1.9 ± 1.1 to 2.6 ± 1.3, $p < 0.01$), but HF was relatively constant (from 7.9 ± 4.0 to 8.9 ± 6.3 ms, NS).

Correlations between Changes in Heart Rate Variability Indices and Changes in Cardiac Norepinephrine Overflow Rate

Correlations between changes in HR variability indices (HF, LF, and LF/HF) and changes in cardiac NE overflow rate during handgrip exercise were examined in the two groups.

TABLE II Changes in hemodynamic parameters by handgrip exercise

	Rest		HG	
	CAD (n = 20)	NL (n = 12)	CAD (n = 20)	NL (n = 12)
HR (beats/min)	67 ± 10	70 ± 10	77 ± 10[a]	81 ± 9.3[a]
BPs (mmHg)	160 ± 23	157 ± 20	185 ± 21[a]	192 ± 31[a]
BPd (mmHg)	72 ± 7.2	80 ± 14	87 ± 9.2	96 ± 17
CSBF (ml/min)	90 ± 50	125 ± 12	158 ± 104[a]	143 ± 95[a]
NEOF (pg/ml, ml/min)	15 ± 17	8.5 ± 14	57 ± 23[a]	31 ± 31
LER (%)	−0.4 ± 0.3	−0.7 ± 3.9	−0.5 ± 0.2	−1.8 ± 3.7
ST (mm)	−0.1 ± 0.1	−0.2 ± 0.3	−0.8 ± 0.3[a]	−0.1 ± 0.3

[a] $p < 0.05$ (rest vs. handgrip).

Abbreviations: HR = heart rate, BPs = systolic blood pressure, BPd = diastolic blood pressure, CSBF = coronary sinus blood flow, NEOF = cardiac norepinephrine overlow rate, LEF = lactate extraction ratio, ST = ST segment.

TABLE III Changes in heart rate variability indicies by handgrip exercise

	Rest		HG	
	CAD (n = 20)	NL (n = 12)	CAD (n = 20)	NL (n = 12)
LF (ms)	13.6 ± 8.0	17.0 ± 7.2[a]	18.8 ± 12.3[c]	21.6 ± 7
HF (ms)	7.9 ± 4.0	10.1 ± 4.5	8.9 ± 6.3	12.2 ± 7[b]
LF/HF (ms)	1.9 ± 1.1	2.0 ± 1.3	2.6 ± 1.3[c]	2.2 ± 1.6

[a] $p < 0.05$ (IHD vs. NL) at rest.

[b] $p < 0.05$ (rest vs. HG).

[c] $p < 0.01$ (rest vs. HG).

Abbreviations: LF = low frequency spectra, HF = high frequency spectra, CAD = coronary artery disease, NL = normal, IHD = ischemic heart disease, HG = handgrip.

Correlations between changes of NE and HR variability indices in the subjects with normal coronary arteries were significant with respect to LF ($r = -0.74$, $p<0.01$) (Fig. 1A; open circle), HF ($r = -0.76$, $p<0.01$) (Fig. 1B; open circle), and LH/HF ($r = 0.50$, $p<0.05$) (Fig. 1C; open circles). The correlation between changes in NE and HR variability indices in the group with CAD was not significant with respect to LF (Fig. 1A; closed circles) and the other parameters (Fig. 1B, C; closed circles).

Discussion

The present study explored the effects of handgrip exercise on HR variability and the relation between HR variability and changes in cardiac NE release. Spectral analysis of HR variability is now accepted as a noninvasive method of assessing cardiac neural modulation by allowing quantification of specific frequency components that are ascribed to the sympathetic and parasympathetic nervous system. The HF components are believed to be an index of parasympathetic nervous system activity. The LF components appear in part to be an index of sympathetic nervous system modification by respiration, and the LF/HF ratio is believed to reflect the state of sympathovagal balance.[1–4,18] Our findings suggest that the effects of handgrip exercise on HR variability may differ depending on the extent of coronary artery stenosis.

Changes in Heart Rate Variability Indices and Correlations of Cardiac Norepinephrine Overflow Rate in Subjects with Coronary Artery Disease and Normal Coronary Arteries

The handgrip exercise is associated with a number of cardiac and vascular responses, mediated primarily through the autonomic nervous system. As the handgrip exercise is initiated, there is an immediate decrease in vagal tone, creating sympathetic vasoconstriction in the capacitance vessels, which has been associated with increases in heart and respiratory rate.[19] In the early phase of the exercise, the cardiovascular system does not deliver enough blood to satisfy the increased metabolic needs of muscle, resulting in an oxygen deficit. At the steady state phase, however, an appropriate amount of blood is delivered to the skin and viscera. In the terminal phase of the handgrip exercise, the excitation of the sympathetic cardiac afferent fibers (via an excitatory sympathetic reflex) is thought to reflect primarily sympathetic efferent activity.[20] It is at this stage, which is regarded as a sympathetic state of the heart, that we measured the plasma NE level in the coronary sinus.

In the subjects with CAD, both LF and LF/HF values were significantly increased by handgrip exercise (Table III), whereas there were essentially no differences in either of these values in the subjects with normal coronary arteries. The difference between resting and handgrip HF was insignificant in the subjects with CAD, whereas in control subjects HF was significantly increased by handgrip exercise. Overall, these results suggest that vagal response to handgrip exercise was reduced in the subjects with CAD.

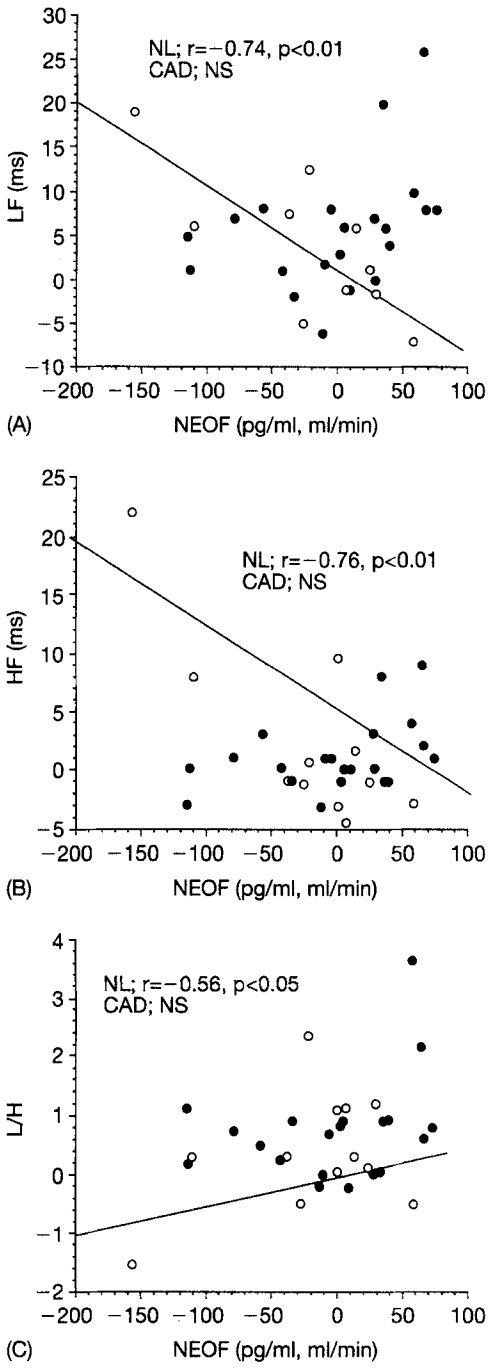

FIG. 1 Correlations between changes in heart rate variability indices (LF, HF, and LF/HF) and cardiac norepinephrine overflow rate (NEOF). (A) LF versus NEOF, (B) HF versus NEOF, (C) LF/HF versus NEOF). LF = low frequency, HF = high frequency, CAD = coronary artery disease, NS = not significant. Open circles indicate normal coronary subjects and closed circles indicate CAD subjects.

As shown in Figure 1A and B, both LF and HF were negatively related to cardiac NE overflow rate in the control subjects, whereas there were no such relationships in the subjects with CAD. These results might be explained by the limitation of HR variability indices to reflect sympathetic tone,

or by the difference between the autonomic tone response to exercise in subjects with and those without coronary artery stenosis. Ahmed *et al.*[21] reported that responses of HR viability changed to a differing degree in response to similar beta-adrenergic stimuli, such as exercise, isopreterenol infusion, epinephrine infusions, and the head-up tilt test. However, their data also showed that the frequency domain HR variability indices of LF/HF and HF showed similar responses to all these stimuli, suggesting that LF/HF and HF might reliably reflect changes in catecholamine release from the heart, but that LF is influenced by both vagal and sympathetic activity. Similar results were reported by Breuer *et al.*[22] Furthermore, in a study by Levy and Blattberg,[23] the cardiac NE release induced by handgrip exercise was enhanced by stimulation of cardiac sympathetic activity, and a concomitant increase in vagal tone was able to inhibit the spillover of NE into the coronary sinus by presynaptic inhibition of cardiac sympathetic nervous activity. Several studies [24–27] have investigated the relation between HR variability and spillover of NE into the coronary sinus. The results of these studies, however, often disagreed, probably because of differences in the etiology, severity of diseases studied, or in the expression of neural signals, since cardiac NE spillover is related to both sympathetic nerve firing and the electrochemical coupling of the neural signal in the heart, whereas HR variability represents multiple neural reflexes, including end-organ responses determined not only by nerve firing, electrochemical coupling, and cardiac adrenergic receptor sensitivity, but also by postsynaptic signal transaction.[28] Therefore, the presence of residual vagal activity resulting from inhibition of NE release would have complex effects on the actual activity of sympathetic nerve endings in patient characteristics such as coronary artery stenosis. High frequency in normal controls could reflect residual vagal tone, but that vagal tone could not appear harmoniously in patients with CAD, as was shown in Figure 1B, because there was no correlation between HF and spillover of NE in subjects with CAD. To address these factors, further studies with larger groups of subjects and different approaches will be required.

Methodology of Measuring Cardiac Norepinephrine Release

In the present study, we used a thermodilution technique to measure coronary blood flow. This method has been used in many laboratories, since it is regarded as the safest and most reasonable method to measure coronary blood flow. At least 94% of coronary outflow is drained from the coronary arteries,[29] and it is easy to measure the catecholamine content in blood samples taken from this area. However, in comparison with the recently developed Doppler flow wire technique, the thermodilution technique is less sensitive in measuring small changes in coronary flow generated by stimuli.

The Doppler flow wire technique, however, measures only the limited area around the flow wire itself and thus fails to reflect global cardiac blood flow.[30] Furthermore, although the Doppler flow wire technique requires instantaneous measurement of the diameter of the coronary artery in order to calculate blood flow, cineangiography can provide this measurement at intervals of 30 s at best, which may render the technique impracticable. Finally, the physiologic effect of cineangiography on the autonomic nervous system should be considered. Therefore, the present thermodilution technique would seem to be a reasonable method for measuring cardiac NE overflow rate.

Using the handgrip exercise, we can observe the effect of sympathetic tone of HR variability on cardiac NE release with fewer artifacts, since it is easier to sustain the original position of the thermodilution catheter over the course of several minutes of exercise than by other methods, such as pacing or an ergometer test. A pacing stress test rapidly simulates sympathetic activity, but does not serve as a full physiologic test because it increases only the HR. The bicycle ergometer test is reportedly useful as a physiologic test, but maintaining the thermodilution catheter in a steady position in a subject actively pedaling a bicycle is virtually impossible. Thus, the handgrip exercise used here constitutes a practical approach to simple stress testing.

Study Limitations

We recognize several limitations in the present study. First, we used a 2 min electrocardiographic recording to measure HR variability of spectral analysis. It is recommended that nonparametric spectral analysis is performed using at least 512 but preferably 1024 timepoints within a 5-min measurement period under physiologically stable conditions.[31] In this study, because we used 128 points for FFT by 2-min electrocardiographic spectral analysis of the 6-min isometric handgrip exercise, the resulting HR variability data provided short periods of FFT information.

A second limitation was that we measured cardiac NE overflow rate using a peripheral artery and coronary sinus blood by chromatography by an enzymatic transfer method instead of NE spillover to plasma by ^{3}H-NE infusion.[32, 33] ^{3}H-NE would probably have provided more accurate measurement, since a small fraction of the NE from sympathetic nerves spills over to plasma; thus, sympathetic nerve activity can be detected more precisely by the radioisotope method than by the present method. However, ^{3}H-NE infusion into peripheral blood cannot be used in routine catheterization laboratory testing because of the possibility of radiation contamination.

Despite these limitations, the present data suggest that HR variability during isometric handgrip exercise-induced cardiac NE release might vary between normal subjects and those with CAD due to the latter group's decreased sensitivity to physical stress adaptation.

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