Heart Rate Spectral Analysis, Cardiac Norepinephrine Spillover, and Muscle Sympathetic Nerve Activity During Human Sympathetic Nervous Activation and Failure

Bronwyn A. Kingwell, PhD; Jane M. Thompson, MB; David M. Kaye, MB, FRACP; Grant A. McPherson, MPharm, PhD; Garry L. Jennings, MD, FRACP; Murray D. Esler, MB, PhD, FRACP

Background Although heart rate variability (HRV) at 0.1 Hz has been proposed as a noninvasive clinical measure of cardiac sympathetic nerve firing, this premise has not been sufficiently validated by comparison with techniques such as microneurography and the measurement of norepinephrine spillover from the heart that more directly reflect presynaptic sympathetic activity.

Methods and Results We compared the three techniques under conditions of effective cardiac sympathetic denervation, pure autonomic failure (n=4), dopamine β-hydroxylase deficiency (n=1), and after cardiac transplantation (n=9) as well as in the context of sympathetic nervous activation in cardiac failure (n=15) and with aging (n=10). Age-matched comparisons were made in each case with healthy individuals drawn from a pool of 52 volunteers. In pure autonomic failure and early after transplantation, cardiac norepinephrine spillover was negligible, and HRV was low. Late after transplantation, however, cardiac norepinephrine spillover returned to normal levels, and HRV remained low. In comparison to younger subjects (18 to 35 years old), older individuals (60 to 75 years old) had higher muscle sympathetic nerve activity (young, 22.9±1.9; old, 31.3±5.8 bursts per minute; P<.05) and cardiac norepinephrine spillover (young, 14.3±2.5; old, 20.1±3.0 ng/min; P<.05). In contrast, total HRV was reduced by 89%, and at 0.1 Hz it was reduced by 93% (P<.05). Cardiac failure was also characterized by elevated cardiac norepinephrine spillover (cardiac failure patients, 59±4; healthy volunteers, 18±3 ng/min; P<.01) but reduced 0.1 Hz HRV (cardiac failure patients, 49±17; healthy volunteers, 243±4 ms²; P<.05).

Conclusions HRV at 0.1 Hz depends on factors in addition to cardiac sympathetic nerve firing rates, including multiple neural reflexes, cardiac adrenergic receptor sensitivity, postsynaptic signal transduction, and electrochemical coupling, and is not directly related to cardiac norepinephrine spillover, which is a more direct measure of the sympathetic nerve firing rate. (Circulation. 1994;90:234-240.)

Key Words • nerve • muscle • fast Fourier transformation • heart rate • norepinephrine

A reliable clinical test of cardiac sympathetic activity would have potential diagnostic applications, such as predicting serious ventricular arrhythmias and sudden cardiac death.1-6 Although cardiac sympathetic activity may be clinically important, its methods of measurement, such as in assessing cardiac norepinephrine (NE) overflow to plasma using isotope dilution, are invasive and unsuitable for widespread application or repeated measurement. More recently, there has been great interest among cardiologists and physiologists in the noninvasive assessment of spontaneous fluctuations in heart rate using spectral analysis as a potential measure of autonomic function, including cardiac sympathetic function.

Frequency domain analysis of heart rate typically characterizes low-frequency oscillations at approximately 0.1 Hz and high-frequency oscillations at the respiratory frequency (~0.2 to 0.3 Hz). Although the high-frequency oscillations appear to be mediated by the parasympathetic nervous system, the 0.1 Hz oscillations have a large sympathetic component. Sympathetic activation by mental stress3-5 and head-up tilt6 increases variability at 0.1 Hz, whereas long-term β-adrenergic blockade6 or bilateral stellectomy in dogs7 reduces 0.1 Hz variability. Data such as these have stimulated some investigators to use 0.1 Hz heart rate variability (HRV) as a measure of cardiac sympathetic activity.6-12 However, although the evidence for a substantial sympathetic contribution to 0.1 Hz power is unequivocal, no evidence has been provided of a strictly quantitative relation between HRV at 0.1 Hz and other methods that reflect presynaptic sympathetic activity. An understanding of the relation between HRV and nerve firing rate is particularly important when comparisons are made between patient populations in whom end-organ responsiveness may vary. For such comparisons, presynaptic indexes of sympathetic nerve firing rates, such as cardiac NE spillover, would not be expected to correlate with HRV at 0.1 Hz, which incorporates a measure of end-organ responsiveness to give an integrated index of sympathetic activity.

In the present study, we compared HRV at 0.1 Hz with presynaptic indexes of sympathetic activity, including the rate of spillover of the sympathetic neurotransmitter.
mitter, NE from the heart measured using a radiotracemaker technique, and sympathetic nerve firing rates measured in skeletal muscle postganglionic fibers by microneurography. We applied these three methodologies (Fig 1) in clinical conditions in which cardiac sympathetic activity is known to be functionally reduced, after cardiac transplantation, in patients with pure autonomic failure (PAF) and with autonomic dysfunction attributable to dopamine β-hydroxylase (DBH) deficiency and, conversely, in the context of sympathetic nervous activation, in patients with cardiac failure, and in healthy subjects in relation to aging.

Methods

General Procedures

This study was approved by the Ethics Review Committee of the Alfred Hospital, and all subjects gave their written informed consent for participation. Studies were performed with participants in the supine position after a standardized light breakfast, with caffeinated beverages and alcohol withheld on the morning of the study. HRV at 0.1 Hz was compared with the rate of spillover of NE from the heart to plasma and with muscle sympathetic nerve activity (MSNA) recorded using microneurography (no microneurography in patients with cardiac failure).

Study Subjects

Healthy Volunteers

Patient groups were compared with age-matched participants drawn from a pool of 52 healthy individuals with an age range of 18 to 75 years who were recruited by advertisement from the general community.

PAF. We studied two men and two women (age 62±6 years) with PAF. All had a protracted history of symptomatic postural hypotension but no clinical features of a central nervous system defect of the Shy-Drager type. Standard noninvasive and invasive tests of autonomic reflex function confirmed the diagnosis in all four patients. In all there was biochemical evidence of almost total postganglionic sympathetic denervation of the heart. Fludrocortisone (0.2 to 0.4 mg/d) and the selective venoconstrictor dihydroergotamine (20 to 60 mg/d) for control of postural hypotension were administered as usual on the day of the study. We also studied one 14-year-old female patient with sympathetic nervous system failure attributable to DBH deficiency. This patient was characterised by a total body NE spillover rate that was less than 10% of normal, undetectable plasma epinephrine, markedly elevated plasma dopamine concentration (286 pg/mL; normal, <50 pg/mL), undetectable serum DBH, prominent sympathetic nerve firing recorded by microneurography, and severe postural hypotension.

Cardiac Transplantation

Nine orthotopic cardiac transplant recipients aged 50±3 years (mean±SEM) were compared with 30 healthy volunteers of similar age (53±3 years). Of the 9 transplant recipients, 4 had received their transplanted heart in the previous 18 months (mean, 24 weeks; range, 9 to 58 weeks), whereas the remaining 5 had been transplanted for more than 2 years (mean, 210 weeks; range, 116 weeks to 8 years). Cyclosporine was withheld on the morning of the study, and patients treated with antihypertensive medications ceased these drugs for 72 hours before study participation.

Cardiac Failure

Ten men and 5 women aged 49±2.7 years with severe heart failure were studied. For reference, comparisons were made with the same 30 healthy volunteers who served as the age-matched reference population for the heart transplantation group. The patients with heart failure were of New York
Heart Association functional class III or IV, with an average left ventricular ejection fraction (determined by radionuclide ventriculography) of 18 ± 1%, and were undergoing assessment for suitability as candidates for cardiac transplantation. Etiology of the heart failure was coronary artery disease and idiopathic dilated cardiomyopathy, in approximately equal proportions. Because of the severity of the cardiac failure, medication, which included frusemide, an angiotensin-converting enzyme inhibitor, warfarin, and digoxin in the majority of patients, was given as usual on the study day.

**Aging**

We compared 26 young healthy individuals aged 18 to 35 years (23 ± 1 years) with 10 healthy older subjects aged 60 to 75 years (68 ± 1 years).

**Spectral Analysis**

The ECG was recorded continuously for a 20-minute period from lead II and digitized on-line at 1000 Hz using a 386/25 IBM-compatible PC and a data-acquisition package, CVMs (McPherson Scientific, Australia), incorporating a 12-bit analog-to-digital converter (CIO-AD16-Jr, Computer Boards Inc). The data-acquisition system included a variable-threshold peak-detection technique from which the RR interval was determined. Data segments of 128-second duration were sampled at 2 Hz to create 256-point data sets. For each 20-minute recording, 15 data sets of 256 points overlapping by half were processed. The linear trend was removed from each data set to avoid its contribution to low-frequency power, and a Hanning window in the time domain was used to attenuate “spectral leakage.” Spectral analysis was performed using a direct fast Fourier transform. The frequency resolution was 0.0078 Hz, and the highest frequency evaluated was 0.5 Hz. The spectra obtained for different data sets were averaged to reduce variance and to sharpen reproducible spectral peaks. Power was calculated in the band range of 0.07 to 0.14 Hz (0.1 Hz or low-frequency power) and 0.14 to 0.5 Hz (respiratory frequency). Power in these bands may be expressed in either absolute units or as a percentage of total power (normalized power). We have chosen to use absolute low-frequency power values but also present total power measurements where relevant so that normalized power can be calculated.

**Cardiac NE Spillover**

The cardiac NE spillover rate, a biochemical index of the global sympathetic nerve firing rate in the heart (rather than just the sinoatrial node), was measured using the radiotracer method developed in our laboratory.13 In brief, the method involves the continuous intravenous infusion of a tracer dose of tritiated NE (0.35 μCi min⁻¹ m⁻² levo-[7⁻³H]NE; specific activity, 12 to 20 Ci mmol, New England Nuclear, Boston) to reach steady-state concentration in plasma. The rate of NE spillover from the heart was calculated according to the Fick principle, corrected for extraction of [³H]NE across the heart13,18,19.

\[
\text{Cardiac NE spillover} = \frac{(NE_{CS} - NE_A) + (NE_A \times NE_{EX})}{CSPF}
\]

where \(NE_{CS}\) is plasma NE concentration in the coronary sinus, \(NE_A\) is arterial plasma NE concentration, \(NE_{EX}\) is the fractional extraction of tritiated NE in passage through the heart, and CSPF is coronary sinus plasma flow (mL/min).

Arterial and coronary sinus samplings were performed using our previously published methods.13,18,19 The coronary sinus was catheterised using a 7F coronary sinus thermomodulation catheter (Webster laboratories, type CCS-7U-90B) introduced via an antecubital venous sheath. Coronary sinus plasma flows were derived from thermodilution-determined blood flows and
the hematocrit. Paired arterial and venous blood samples (5 mL) were transferred immediately to ice-chilled tubes containing an anticoagulant and antioxidant (EGTA and glutathione). Plasma was separated by centrifugation at 4°C, and samples were stored at −70°C until assayed. Endogenous NE plasma concentration was measured by high-performance liquid chromatography with electrochemical detection according to our previously published method.20

Microneurography

MSNA was recorded in selected subjects from the peroneal nerve posterior to the fibula head using a tungsten microelectrode (Titronics Medical Instruments) with an uninsulated tip diameter of approximately 1 mm. The electrode was inserted through the intact, unanesthetized skin using a stimulation voltage of less than 4 V to locate the muscle fasicle. The electrode was then manipulated until verification of a muscle sympathetic nerve recording was obtained using previously described techniques.14,21 Integrated nerve activity was analyzed manually and recorded as bursts per minute. It was not possible to perform single-fiber sympathetic nerve recording.

Statistical Analysis

Statistical comparisons of the patient groups with age-matched healthy subjects was performed using unpaired t tests.

Correlation analysis was performed using least-squares regression. All values given are mean±SEM, and the null hypothesis was rejected when P<.05.

Results

Models of Cardiac Sympathetic Denervation

PAF

Patients with PAF had negligible cardiac NE spillover relative to age-matched controls (1.2±0.9 versus 22.7±3.2 ng/min, P<.05), indicative of almost-complete cardiac postganglionic sympathetic denervation. Muscle sympathetic nerve firing was detectable in only one of the four patients with PAF, and then at the greatly reduced frequency of approximately 1 burst per minute compared with the control value of 35.3±3.6 bursts per minute. HRV was reduced to very low levels in all patients with PAF, and no heart rate oscillations were evident at 0.1 Hz (Fig 2). The patient with DβH-deficiency had a supranormal MSNA of 38 bursts per minute (compared with a normal value of 23±2 bursts per minute in young subjects) but low total body NE spillover (38 ng/min; age-matched healthy subjects, 523±52 ng/min). Cardiac NE spillover

Fig 3. Comparison of heart rate spectral analysis (top panels), muscle sympathetic nerve activity (b/min [bursts per minute]; middle panels), and whole body norepinephrine spillover (bottom panels) in a 14-year-old female patient with dopamine β-hydroxylase deficiency (right panels) with healthy age-matched subjects (left panels). Mean and SEM values are indicated.
was not measured. Consistent with the absence of DβH, HRV at 0.1 Hz was not present, although respiratory-related variability appeared normal (Fig 3).

**Cardiac Transplantation**

Early after transplantation (<18 months), patients exhibited both very low cardiac NE spillover (1.9±3.1 versus 23±3 ng/min, P<.05) and low HRV compared with healthy subjects (total power, 29±11 versus 1673±516 ms², P<.05). Spectral profiles also lacked the characteristic 0.1-Hz and respiratory-related peaks present in healthy individuals (Fig 2). Cardiac NE spillover had returned to near-normal levels (17.8±1.9 ng/min) in the patients studied more than 2 years after transplantation. Total HRV, however, was still markedly reduced with a total power of (70±30 ms²).

**Models of Sympathetic Activation**

**Cardiac Failure**

In patients with cardiac failure, the rate of spillover of NE from the heart was two to three times the value found in age-matched healthy controls (59±14 versus 18±3 ng/min, P<.05). In contrast, the total power of the heart rate spectrum was reduced to one third of normal in patients with heart failure, with the 0.1-Hz power being reduced from 243±44 to 49±17 ms² (P<.05, Fig 4).

**Aging**

Compared with young healthy subjects aged 18 to 35 years, older subjects aged 60 to 75 years had an elevated cardiac NE spillover rate (14.3±2.5 versus 20.1±3.0 ng/min, P<.05) and MSNA (22.9±1.9 versus 31.3±5.8 bursts per minute). Total HRV in the older group was reduced by 89%, and power at the 0.1-Hz low-frequency band was similarly reduced by 93% (P<.05).

**Relation Between Heart Rate Spectral Analysis Measurements and Cardiac NE Spillover at Rest in Healthy Subjects**

In 16 healthy subjects aged 18 to 75 years (mean, 30±4 years) in whom measurements were made simultaneously, there was no significant correlation between 0.1 Hz power expressed in either absolute units or normalized for total power and the rate of NE spillover from the heart to plasma (Fig 5). There was, however, a significant correlation between MSNA and cardiac NE spillover (linear correlation coefficient, .57).

**Discussion**

HRV is a useful new tool in neurophysiological research that has been used to provide insight into the autonomic regulation of the heart. Recent studies have used 0.1 Hz power as a surrogate measure of sympa-
Cardiac NE spillover is related to both sympathetic nerve firing and the electrochemical coupling of the neural signal in the heart and provides a cardiac-specific measure of sympathetic nerve firing rate. HRV represents an end-organ response determined by nerve firing and electrochemical coupling but also cardiac adrenergic receptor sensitivity, postsynaptic signal transduction, and multiple neural reflexes. When taken together, these three techniques can provide a comprehensive assessment of the sympathetic neuroeffector mechanism.

In this study, we examined a range of clinical conditions that demonstrate the specificity of each methodology for assessment of sympathetic nervous function. In the context of cardiac denervation as a result of PAF, loss of cardiac sympathetic nerve firing was translated into near-zero NE spillover from the heart and an associated reduction in 0.1 Hz HRV. Parasympathetic denervation also appeared to occur in this condition, and consequently respiratory-related HRV also was absent. Deficiency of the NE synthesizing enzyme DβH similarly produced lowered NE spillover and absence of 0.1 Hz HRV, whereas preservation of vagal function caused the respiratory-related parasympathetic oscillations to be retained. MSNA, however, was elevated above normal, but this was not translated into a functional response due to the absence of DβH uncoupling the electrochemical signal transduction. In this instance, both lowered NE spillover and HRV indicated a lack of functional response, and neither were related to rates of sympathetic firing.

Cardiac transplantation provided another example of cardiac denervation. Early after cardiac transplantation, NE spillover and spectral profiles were similar to those seen with PAF, although peripheral sympathetic nerve firing was normal.27 In patients who had been transplanted more than 2 years earlier, disparity between the NE spillover and HRV measurements became evident. Cardiac NE spillover was near-normal in the five patients who had been transplanted for more than 2 years, whereas HRV remained very low. Taken together, these results suggest that reinnervation late after transplantation may be patchy, particularly in the region of the sinoatrial node, or that there is a failure of the ingrowing nerves to reform the complex network of afferent and efferent connections that underlie the genesis of normal heart rate rhythms. Thus, HRV indicated a functional deficit despite near-normal cardiac NE spillover rates.27

In states of long-term sympathetic activation, such as cardiac failure and aging, the study of a single isolated aspect of sympathetic nervous function may misrepresent the true relation between sympathetic nerve firing and functional response. In each case, cardiac NE spillover was elevated, reflecting an elevation in sympathetic nerve firing.28 The increase in cardiac NE spillover rate, however, was not translated into an enhanced end-organ response. In fact, total HRV and variability at 0.1 Hz in both cases was markedly reduced when expressed as either absolute or normalized units. These findings may be reconciled when the β-adrenoceptor desensitization and impairment of postreceptor signal transduction known to occur with aging and in cardiac failure29,30 are considered. The elevated cardiac NE

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**Fig. 5.** Plots of relation between cardiac norepinephrine (NE) spillover and low-frequency power (LF; 0.1 Hz) expressed both in absolute units (top) and as a percentage of total power (middle). Bottom, Relation between muscle sympathetic nerve activity (MSNA) and cardiac NE spillover (r = .57, P < .05). Data were obtained at rest in 18 healthy subjects aged 18 to 75 years. Subjects aged 18 to 25 years are indicated by open circles, and those aged more than 30 years are indicated by closed circles.

**Table:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cardiac NE Spillover (ng/min)</th>
<th>Cardiac LF Power (ms²)</th>
<th>Cardiac MSNA (b/min)</th>
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<tr>
<td>18-25 years</td>
<td>25</td>
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<td>&gt;30 years</td>
<td>25</td>
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Note: LF Power is measured as a percentage of total power.
spillover indicated that the cardiac sympathetic nerve firing rate was high, whereas the HRV results show that this elevation in sympathetic activity was not well translated into a functional response.

These considerations indicate that 0.1-Hz power is not directly related to cardiac NE spillover rate, particularly in situations where there are differences in electrochemical, receptor sensitivity, postsynaptic signal transduction, neural reflexes, and parasympathetic function. Spectral analysis of HRV thus complements existing techniques through incorporating different aspects of the neuroeffector mechanism not assessed by microneurography or NE spillover measurements. The combination of the cardiac NE spillover technique and HRV will allow a more comprehensive assessment of both neuronal and postsynaptic aspects of the cardiac neuroeffector response.

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