DISCORDANCE BETWEEN MICRONEUROGRAPHIC AND HEART RATE SPECTRAL INDICES OF SYMPATHETIC ACTIVITY IN PULMONARY ARTERIAL HYPERTENSION

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Short Title: HRV and sympathetic activity in PAH

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ABSTRACT

Objectives: To determine, in patients with pulmonary arterial hypertension (PAH), whether there is a relationship: 1) between sympathetic nerve firing rate and spectral indices of sympathetic neural heart rate modulation; and 2) between heart rate variability (HRV) and right atrial pressure, a stimulus to sino-atrial node stretch.

Design: Characterisation of patients and healthy controls.

Setting: Teaching hospital-based study.

Patients: 9 PAH patients without elevated pulmonary capillary wedge pressure and 9 age-matched control subjects.

Interventions: Heart rate (HR) and muscle sympathetic nerve activity (MSNA) were recorded during 10 min of supine rest in both PAH patients studied after right heart catheterisation, and healthy volunteers. Coarse-graining spectral analysis determined HR spectral power.

Main outcome measures: 1) Low frequency (P_L) spectral component of HRV; 2) MSNA burst frequency; and in PAH patients: 3) right atrial pressure.

Results: MSNA burst frequency was higher in PAH patients (48 ± 24 and 29 ± 11 bursts/min, respectively; mean ± SD; p = 0.05), whereas total power (p = 0.01), its fractal (p < 0.01) and harmonic (p = 0.04) components, and P_L (p = 0.01) were all reduced. P_L related inversely to both MSNA burst frequency (r = -0.86, p = 0.005) and right atrial systolic pressure (r = -0.77, p = 0.04).

Conclusions: Thus in PAH (as in patients with left ventricular systolic dysfunction) loss of P_L relates inversely to gain in MSNA burst frequency. Diminished sympathetic neural heart rate modulation and increased right atrial stretch may combine to attenuate HRV, an adverse prognostic marker.
INTRODUCTION

Pulmonary arterial hypertension (PAH), a progressive and incurable disease of the pulmonary arterioles, progresses ultimately to right heart failure [1]. The participation of the autonomic nervous system in this process has yet to be elucidated.

Neurally-generated variations in heart rate can be identified by spectral analysis [2]. Total power (PT) across the heart rate variability (HRV) spectrum can be subdivided into two components: a non-harmonic (or fractal) component, and harmonic modulation. In healthy subjects, the latter spectrum exhibits distinct peaks within its high frequency (PH; 0.15 – 0.5 Hz), low frequency (PL; 0.05 to 0.15 Hz), and very low frequency (PVL; ≤ 0.05 Hz) bands [3]. Harmonic PH arises from vagal heart rate modulation, with respiration being its principal rhythmic stimulus, whereas PL is influenced primarily by sympathetic neural oscillations [2, 4-10]. However, in chronic heart failure (CHF), a condition characterized by increased central sympathetic outflow, total spectral power (PT) and PL are markedly reduced [6], and there is an inverse rather than positive correlation between one of its manifestations, muscle sympathetic nerve activity (MSNA) and PL [6]. Potential explanations for this loss of harmonic power include central attenuation of oscillations of efferent nerves modulating heart rate, reduced sino-atrial responsiveness to neurotransmitters, increased right atrial stretch, and tachycardia driven heart rate invariance [6, 11, 12].

By definition, patients with PAH have normal left ventricular systolic function [1], but for reasons yet to be elucidated their sympathetic nerve firing rate also is increased [13], and PT and PL are attenuated [14]. These observations suggest an inverse relationship in PAH, similar to that seen in CHF, between sympathetic nerve firing rate and spectral indices of sympathetic neural heart rate modulation. However, there are as yet no published tests of this hypothesis involving PAH patients with documented normal left-sided cardiac filling pressures, in whom MSNA and power spectrum estimates of sympathetic modulation of heart rate were acquired simultaneously. Our secondary hypothesis was that PT would also relate inversely to MSNA. A further objective was to determine whether these two indices of heart rate variability correlate with right atrial pressure, which is both a stimulus to sino-atrial node stretch, and in PAH patients, an important predictor of survival [15, 16]. The presence of sympathetic excitation and diminished HRV in both CHF and PAH patients introduces the possibility that their prognostic consequences [4] may be similar in these two conditions.

METHODS AND MATERIALS

Participants

Newly diagnosed, untreated, PAH patients underwent echocardiography and right heart cardiac catheterization to exclude all with echocardiographic evidence for reduced left ventricular systolic function and a pulmonary capillary wedge pressure ≥ 15 mmHg [1], and to obtain hemodynamic data to relate to HRV indices. As those with connective tissue diseases are known to share similar clinical presentations, pathologic changes, and response to therapy as patients with idiopathic PAH [17], nine patients (3 males and 6 females; mean age: 47 ± 13 years) with either idiopathic (n = 6) or connective tissue related PAH (n = 3; 2 scleroderma and 1 lupus) were studied. Of these, four were receiving treatment for non-PAH related conditions (Losec, Methotrexate, Nexium, Plaquinil, Lorazepam, Prednisone, Ramipril, Synthroid, Crestor, Pravacid). Nine age-matched healthy, un-medicated, non-smoking volunteers (6 males and 3
females; mean age: 45 ± 11 years) comprised a reference population. This protocol was approved by our institutional (University Health Network, Toronto) Research Ethics Board. All participants provided written informed consent.

**Experimental Protocol**

Autonomic data were acquired in the morning in a quiet, temperature-controlled room, following a light caffeine-free breakfast. Participants first voided their bladder to minimize the effects of its distension on blood pressure (BP) and MSNA [18]. Blood pressure was determined from the brachial artery by an automated non-invasive device (Dinamap Pro 100, Critikon, Tampa, FL). Lead II of the electrocardiogram was recorded continuously to derive heart rate. Breathing patterns were recorded by a pneumobelt connected to a pressure transducer. Multi-unit recordings of post-ganglionic MSNA were obtained with a unipolar tungsten electrode inserted selectively into a muscle-nerve fascicle of the right fibular nerve in accordance with previously described and published procedures [3, 6]. As with our previously published protocols, data were acquired simultaneously during 10 minutes of supine rest and spontaneous breathing [6].

Signal output was inscribed by a Gould Viper recorder (Gould Instrument Systems, Madison, WI), sampled at a frequency of 200 Hz (with the exception of the electrocardiogram: 1,000 Hz) and following conversion from analogue to digital format stored on a PC desktop for subsequent off-line analysis [3, 6]. Ensemble averages of 256 beat sequences were taken from a minimum time series of 400 to 500 beats. Ectopic beats were edited, and replaced via linear interpolation from adjacent cardiac cycles. Only tracings with ≤ 5% ectopy-corrected beats were accepted for analysis, and as a consequence, HRV data from one PAH patient was excluded. To replicate our CHF protocol [6], pulse (R-R) intervals were analyzed using coarse graining spectral analysis (CGSA) [10], which first divided total spectral power (PT) into its non-harmonic (1/f) or fractal (PF) and its harmonic (PHHP) components, from which harmonic power integrated across the low-frequency (PL) and high-frequency (PH) ranges was calculated [6]. PH normalized to total spectral power (PH/PT) was derived to estimate parasympathetic heart rate modulation [6, 10]. The ratio between PL and PH, a consensus estimate of sympato-vagal balance [2, 4], was also calculated. Average MSNA burst frequency (bursts/min) and burst incidence (bursts/100 cardiac cycles) were determined using the LabVIEW based software system (National Instruments, Austin, TX) with burst identification verified by an experienced investigator.

**Statistical analysis**

Data are presented as mean ± standard deviation (SD). Between-group differences in descriptive, hemodynamic, HRV and MSNA variables were assessed by unpaired t-tests or Mann-Whitney Rank Sum tests. Linear regression was used to determine correlations between variables of interest. If these did not adopt Gaussian distribution, data were transformed to a log_{10} scale; if the data remained non-normally distributed, a square root transformation was performed. If the assumption of normal distribution could not be met, Spearman rank correlations were used [19]. All data were analyzed using Sigma Stat™ for Windows (version 3.5; Jandel Scientific Corp., San Rafel, CA), and an alpha level of ≤ 0.05 was considered statistically significant.
RESULTS

At cardiac catheterization (Table 1) PAH patients exhibited an average pulmonary artery mean pressure > 25 mmHg, pulmonary vascular resistance > 240 dyne·sec·cm⁻⁵, and a pulmonary capillary wedge mean pressure within the normal range [1]. Mean right atrial pressure was at the upper limit of normal at 7 ± 2 mmHg, with its “a” wave pressure measured at 10 ± 3 mmHg. Left ventricular ejection fraction was > 50%.

Table 1. Hemodynamic characteristics of patients with pulmonary arterial hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary Artery Mean Pressure (mmHg)</td>
<td>63 ± 13</td>
</tr>
<tr>
<td>Pulmonary Capillary Wedge Mean Pressure (mmHg)</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>Transpulmonary Pressure (mmHg)</td>
<td>50 ± 14</td>
</tr>
<tr>
<td>Right Atrial Mean Pressure (mmHg)</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Cardiac Output (l·min⁻¹)</td>
<td>4.0 ± 1.8</td>
</tr>
<tr>
<td>Pulmonary Vascular Resistance (dyne·sec·cm⁻⁵)</td>
<td>1049 ± 598</td>
</tr>
</tbody>
</table>

‡n = 8, §n = 9

Those with PAH did not differ from healthy subjects with respect to mean age (p = 0.73), and brachial artery systolic (p = 0.73) or diastolic BP (p = 0.34), but had higher mean resting heart rate (p = 0.03). Resting MSNA burst frequency was also greater (p = 0.05), but burst incidence was not (p = 0.26) due to the higher heart rate (Table 2).

Table 2. Comparison of study variables between healthy subjects and patients with pulmonary arterial hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Subjects</th>
<th>PAH Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45 ± 11</td>
<td>47 ± 13</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>118 ± 15</td>
<td>116 ± 10</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73 ± 8</td>
<td>77 ± 5</td>
</tr>
<tr>
<td>PT (ms²)</td>
<td>917 ± 632</td>
<td>221 ± 343**</td>
</tr>
<tr>
<td>PF (ms²)</td>
<td>683 ± 512</td>
<td>139 ± 200**</td>
</tr>
<tr>
<td>PH (ms²)</td>
<td>234 ± 230</td>
<td>81 ± 148*</td>
</tr>
<tr>
<td>PL (ms²)</td>
<td>145 ± 158</td>
<td>19 ± 31**</td>
</tr>
<tr>
<td>PH/PT</td>
<td>77 ± 80</td>
<td>13 ± 23</td>
</tr>
<tr>
<td>P_I/P_H</td>
<td>11.5 ± 19</td>
<td>2.7 ± 5*</td>
</tr>
<tr>
<td>P_H/P_T</td>
<td>0.2 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>66 ± 6</td>
<td>85 ± 23*</td>
</tr>
</tbody>
</table>

MSNA
- bursts/min      | 29 ± 11          | 48 ± 24*     |
- bursts/100 beats| 45 ± 17          | 56 ± 25      |

Values are Mean ± SD. *p ≤ 0.05; ** p ≤ 0.01
Total heart rate spectral power and its fractal (Pr) and harmonic components (P_{HH}) were significantly reduced in PAH compared with healthy subjects (p = 0.01, <0.01, and 0.04, respectively). Similarly, absolute power in the low frequency band (PL; p = 0.01) and the P_L/P_T ratio (p = 0.04) were also reduced. High frequency (P_H) spectral power and P_H/P_T tended to be lower, but not significantly different between groups (p = 0.07 and 0.50, respectively) (Table 2).

Linear regression analysis revealed an inverse relationship between MSNA burst frequency and P_T (r = - 0.89, p = 0.003) and P_L (r = - 0.86, p = 0.005) in PAH patients, but not in healthy subjects (Figures 1 and 2). Significant inverse relationships were also observed between MSNA burst frequency and P_L (r = -0.88, p < 0.01), P_{HH} (r = -0.86, p < 0.01), and P_H (r = -0.83, p = 0.01) in patients with PAH, but not in healthy subjects (r = 0.22, p = 0.56; r = -0.35; p = 0.36, and r = -0.27, p = 0.48; respectively). MSNA burst frequency was unrelated to either P_L/P_H or P_H/P_T in both groups (p > 0.5 for all). Linear regression revealed similar relationships between MSNA and HRV spectral indices when MSNA was normalized for heart rate (burst incidence) in both groups, with the exception of P_H in PAH patients (data not presented).

There were significant inverse relationships between the right atrial “a” wave pressure and P_T (r = -0.80, p = 0.03), P_L (r = -0.75, p = 0.05), P_{HH} (r = -0.88, p < 0.01), P_L (r = -0.77, p = 0.04), and P_H (r = -0.81, p = 0.03).

**DISCUSSION**

Although there are as yet few studies of autonomic function in PAH, the published evidence thus far suggests similarity to disturbances of neurogenic control of the circulation characteristic of left heart failure due to systolic dysfunction [20]. If so, these may have prognostic implications in PAH comparable to those now well established for heart failure patients [4].

Fauchier and colleagues [14], using Fast Fourier transformation (which does not distinguish between harmonic and non-harmonic power) documented diminished HRV (P_T, P_L, and P_H) over a 24-hour recording period in 10 patients with idiopathic PAH, as compared with 15 age-matched controls. Velez-Roa and colleagues [13] reported significantly higher MSNA burst frequency and MSNA burst incidence in 17 PAH patients compared to 12 control subjects matched for age, sex and body mass index (BMI). This is the first report describing total harmonic and non-harmonic components of HRV in patients with PAH and relating these findings to simultaneously measured MSNA. In addition components of HRV were compared to right atrial pressure. Mean right atrial pressure in the present population was similar to the average value recorded at the time of diagnosis of PAH (8 mmHg) in a French registry comprising 674 patients [21].

Reductions in both non-harmonic (P_T) and harmonic (P_{HH}) spectral power, as compared with age-matched healthy subjects, contributed to the attenuated P_T in these PAH patients (Table 2). There was a significant decrease in absolute power in the low frequency band of P_{HH} and also in the P_L/P_H ratio. With the exception of the non-significant trend towards lower high frequency power (possibly due to the number of PAH subjects studied), these alterations in HRV are remarkably similar to those reported previously for CHF [6]. These observations suggest that the higher heart rates of these PAH subjects result primarily from augmented sympathetic, rather than diminished vagal, modulation of sino-atrial discharge.

MSNA burst frequency was increased in these subjects with PAH. There was an inverse rather than a positive relationship between this direct measure of sympathetic outflow to skeletal muscle and the indirect (HRV) estimates of sympathetic modulation of heart rate. There were
similar inverse relationships between MSNA and both total ($P_T$) and harmonic power ($P_{HP}$). These findings parallel those of Notarius and colleagues [6], who identified inverse rather than direct correlations between MSNA burst frequency and $P_L$, $P_T$, and $P_T$ in 35 patients with CHF due to left ventricular systolic dysfunction, but not in 34 age-matched healthy subjects. As with the present series in PAH, these authors also noted that MSNA burst frequency in CHF did not correlate significantly with either $P_L/P_H$ or $P_H/P_T$ [6].

In CHF, attenuated baroreflex control of heart rate and the loss of modulation of autonomic outflow, decreased responsiveness of the sino-atrial node to neurotransmitters, and/or impaired parasympathetic ganglionic neurotransmission are associated with decreased HRV [6, 22]. Whether similar neural mechanisms attenuate HRV in PAH has yet to be established. Mechanical stretch of the sino-atrial node, as would occur with increased right atrial pressure, also has been shown to decrease HRV [11]. The significant inverse relationships, in the present study, between right atrial “a” wave pressures as a marker of sino-atrial stretch during atrial systole, and $P_T$, $P_{HP}$, $P_L$, and $P_H$ suggests that atrial stretch may act to suppress HRV in PAH. Higher heart rates, as present in our PAH cohort, are also associated with decreased HRV [23]. Our observed reductions in fractal power may have resulted from loss of autonomic nervous system modulation of fractal HRV dynamics [24, 25], and alterations in respiratory patterns and/or chemoreflex contributions [26, 27] in addition to increased sino-atrial stretch.

Mechanisms responsible for sympathetic excitation in PAH have yet to be characterized in full. A substantial component of the increase in MSNA in PAH appears to be mediated by the arterial chemoreceptor reflex pathway [13]. Systolic blood pressure and ventricular systolic function, two stimuli to baroreceptor nerve firing, were similar in the two subject groups (Table 2). In CHF, an increase in left atrial pressure also appears to activate a normally quiescent sympathoexcitatory reflex [4, 20, 28]. The present findings suggest that a selective increase in right sided cardiac pressures may also be capable of eliciting similar reflex increases in sympathetic outflow to the heart and skeletal muscle.

There is considerable interest in inferring vagal and sympathetic modulation of sino-atrial discharge in health and disease by the application of proprietary algorithms to R-R signals acquired under resting conditions. This is based on the assumption that $P_L$ provides clinically informative insight into the magnitude of central sympathetic outflow. However, in health, observation of responses to perturbations in central sympathetic or parasympathetic nerve firing is generally more informative than is acquisition of resting values if the intent is to relate power spectra in specific frequency bands to specific autonomic influences [2, 5, 7-9] and even in the setting of a vasodilator infusion, which elicits concordant increases in both muscle sympathetic nerve activity (MSNA) and low frequency spectral power ($P_L$) [7, 9] absolute values for $P_L$ fall. This response is also observed if central vagal outflow is increased by low dose atropine [5]. In diseases such as heart failure, characterized by chronic increases in neurally released and circulating catecholamines and decreases in tonic and reflex vagal tone, absolute values for total spectral power and power in the high and low frequency bands are all attenuated [3, 4, 6, 22]. The present finding that $P_L$ also correlates inversely with a direct measure of sympathetic outflow in PAH provides a guide to the interpretation of HRV data in such patients at a single point in time.

In summary, both neural (diminished amplitude of sympathetic neural heart rate modulation) and tensile (increased right atrial stretch) forces may combine to attenuate HRV in PAH. The present findings suggest several clinical implications. Approximately 8% of patients with PAH die suddenly; the majority suffer from progressive right heart failure [29]. Although
the prognostic value of low HRV in PAH patients has yet to be determined, reduced HRV does predict long-term mortality in patients following a myocardial infarction [30], depressed P_T is a potent predictor of mortality in CHF [31], and diminished P_L increases the risk of sudden cardiac death in patients with moderate to severe CHF [32-34]. The hypothesis that reduced HRV and sympathetic nervous system excitation in PAH patients increase their likelihood of sudden death or accelerate the progression of right heart failure merits prospective evaluation, as does the concept that HRV spectral power might become in this population a useful non-invasive surrogate for elevated right atrial pressure, a key hemodynamic predictor of their survival [15, 16]. Also unknown is whether P_L, P_T or MSNA can be restored by PAH-specific pharmacological therapy.
FUNDING

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COMPETING INTERESTS

J.S.S. has received honoraria from Actelion Pharmaceuticals Ltd. and Encysive for Continuing Medical Education (CME) presentations. He has also received travel support for educational meetings and honoraria for advisory board meetings from Actelion Pharmaceuticals Ltd. and GlaxoSmithKline (GSK). J.T.G. has received funds from Actelion Pharmaceuticals Ltd. for an investigator initiated trial, in addition to consultant and speakers honoraria from Actelion Pharmaceuticals Ltd., GSK, Encysive, United Therapeutics and Pfizer. Neither investigator participated directly in the acquisition or analysis of main outcome measures. No other authors have competing interests as defined by Heart.
FIGURE LEGENDS

Figure 1. Relationships between muscle sympathetic burst frequency (MSNA) and total spectral power. There was a significant inverse relationship between MSNA burst frequency and sqrtPT in PAH patients (●; n = 8) (r = -0.89, p = 0.003), but not in healthy subjects (○; n = 9) (r = 0.03, p = 0.94).

Figure 2. Relationships between muscle sympathetic burst frequency (MSNA) and log10PL. There was a significant inverse relationship between MSNA burst frequency and log10PL in PAH patients (●; n = 8) (r = -0.86, p = 0.005), but not in healthy subjects (○; n = 9) (r = -0.18, p = 0.63).
REFERENCES


Figure 1. Relationships between MSNA burst frequency and total spectral power. There was a significant inverse relationship between MSNA burst frequency and $\sqrt{P_T}$ in PAH patients (●; n=8) ($r = -0.89$, $p = 0.003$), but not in healthy subjects (○; n=9) ($r = 0.03$, $p = 0.94$).
Figure 2. Relationships between MSNA burst frequency and $\log_{10} P_L$
There was a significant inverse relationship between MSNA burst frequency and $\log_{10} P_L$
in PAH patients (○; $n=8$) ($r = -0.86$, $p = 0.005$), but not in healthy subjects (○; $n=9$) ($r = -0.18$, $p = 0.63$).