R-R variability detects increases in vagal modulation with phentolamine infusion

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The use of R-R variability to estimate the state of the autonomic nervous system has become increasingly useful in a number of clinical situations. Decreased R-R variability has been shown to predict mortality in patients after myocardial infarction (4, 13). R-R variability measures of vagal modulation have been used to evaluate the effects of drugs on the autonomic nervous system (6, 7, 11). R-R variability is also being used to further elucidate the pathophysiology of autonomic dysfunction in a number of diseases, such as diabetes (3, 8), congestive heart failure (20), and vasovagal syncope (6).

The analysis of R-R variability can be used to quantitate changes in autonomic function and adds significantly to the information provided by heart rate, which by itself is an inaccurate measurement of autonomic function. Heart rate reflects the intrinsic sinus node rate plus the sum of the influences of the sympathetic and parasympathetic nervous system without providing independent information about either system. Power spectral analysis of the cyclic fluctuations in R-R intervals, however, does provide additional information about the separate influences of the sympathetic and parasympathetic nervous systems. A number of studies have elucidated the physiological significance of these R-R variability measurements (1, 5, 15, 19).

Rapid cyclical changes in R-R intervals at the respiratory frequency [high-frequency (HF) power] have been shown to provide a pure index of parasympathetic modulation (1).

A number of recent studies have begun to identify limitations on the use of HF power to estimate the state of the parasympathetic nervous system (6, 9, 14). One study by Goldberger et al. (9) infused phentolamine into normal subjects, which caused an increase in blood pressure and a baroreflex-mediated prolongation of R-R intervals (which suggests an increase in vagal activity). Paradoxically, HF power decreased at the highest doses of phentolamine (suggesting a decrease in vagal modulation) despite an increase in R-R intervals. Goldberger et al. (9) explained these findings with the hypothesis that phentolamine infusion increased parasympathetic nervous system activity to the point of producing nearly constant vagal stimulation of the sinus node. This, they hypothesized, had the effect of decreasing the cyclical oscillations of vagal nerve firing on the sinus node that produce the cyclical oscillations in R-R intervals, which are detected as HF power using power spectral analysis of R-R variability.

This study was designed to further investigate the effects of baroreceptor activation with phentolamine infusion on R-R variability. If the theory postulated by Goldberger et al. (9) is correct, then phentolamine infusion should increase vagal modulation and HF power if phentolamine is given at a time when vagal nerve activity is reduced (such as during head-up tilt). Higher doses of phentolamine should continue to increase HF power until vagal nerve activity has increased to the point of constant, rather than cyclic, stimulation of the sinus node.

**METHODS**

Experimental protocol. The protocol was approved by the Institutional Review Board of the Columbia Presbyterian Medical Center. Studies were performed in a quiet room where blood pressure was continuously monitored noninvasively from a finger by infrared photoplethysmography (Finapres BP Monitor model 2300, Ohmeda, Englewood, CO) and recorded on a computer (17). The electrocardiogram was monitored on an oscilloscope and continuously recorded on a Holter recorder during the study. Fifteen minutes after an intravenous line was placed, baseline measurements were taken in the supine position.

There were four phases to the protocol. Each phase consisted of a 10-min period when the subject was supine followed by a 10-min period when the subject was tilted head-up to 60°. Phase 1, in the drug-free state, provided two baseline measurements for all future comparisons: a baseline measurement in the supine position (0°), and a baseline measurement at 60° of head-up tilt. After 10 min at 60°, subjects were returned to the horizontal position, and the
Phenylephrine infusion was started at a dose of 0.4 µg·kg⁻¹·min⁻¹ through the intravenous line (phase 2). Subjects remained in the horizontal position for 10 min during this dose of phenylephrine infusion and were then tilted head-up 60° for 10 min. After 10 min at 60°, subjects were returned to the horizontal position, and the dose of phenylephrine was increased to 0.8 µg·kg⁻¹·min⁻¹. This sequence was repeated for phase 3 (0.8 µg·kg⁻¹·min⁻¹) and phase 4 (1.2 µg·kg⁻¹·min⁻¹).

All measurements were made during the last 5 min of each period at 0° (supine) and each period at 60° (head-up). The data from the first 5 min of each period were discarded to allow for equilibration after a change of position and/or a change in the dose of phenylephrine infusion. This protocol was designed with four separate head-up tilts to ensure that all measurements made at 60° are made in the same condition (i.e., 5 min after the subject was tilted head-up 60°). The event button on the Holter recorder was used to mark the beginning and end of each stage in the protocol.

Study subjects. Ten normal, healthy adult volunteers taking no medications were recruited for this study and signed informed consent. There were two women and eight men, aged 29 ± 3 (means ± SD) yr (range 26–36 yr). One subject did not complete the protocol and was excluded from the analysis.

Analysis of Holter electrocardiogram recordings. All Holter tapes (~2 h in duration) were analyzed with a Marquette 8000 scanner running version 5.8 of the Marquette analysis program to identify and label each QRS complex. After the computer had automatically detected and labeled each QRS complex, a frequency histogram of the normal R-R (N-N) intervals was displayed, and the electrocardiograms of the intervals in both tails of the N-N distribution were reviewed by a technician. After editing was completed, the labeled QRS data stream was moved by means of a high-speed interface to a Sun 4/75 microcomputer, where a second stage of editing was performed using algorithms developed at Columbia University to find and correct any remaining errors in QRS labeling that adversely affect measurement of R-R variability.

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Analysis of success of N-N intervals. We computed the standard deviation of N-N intervals (SDNN), the root-mean-squared difference (RMSSD), and the percentage of differences between adjacent N-N intervals >50 ms (PNN50) for the final 5-min segment of each 10-min segment of the study.

Power spectral analysis of N-N intervals. Power spectral analysis of R-R variability can be used to estimate the contributions of the sympathetic and parasympathetic modulation of the sinus node. We computed R-R interval power spectra on the final 5-min segment of each 10-min segment of the study. The methods used for spectral analysis have been described previously (2, 5). A continuous function was derived from the discrete N-N intervals, filtered, and then sampled at 1,024 samples per 5-min segment to produce a time series for spectral analyses. The average N-N interval was subtracted from the time series, and a fast Fourier transform was performed to resolve the frequency components of cyclic activity in the time series of N-N intervals. Because the average N-N interval was subtracted from the time series of N-N intervals, changes in average N-N interval between different treatment periods should not affect the frequency-domain analyses.

Total power between 0.003 and 0.40 Hz was calculated. This approximates the total variance of the signal for a 5-min interval. Power in two bands of this power spectrum were quantified as the following: 0.15–0.40 Hz (HF power) and 0.04–0.15 Hz [low-frequency (LF) power]. HF power is a pure parasympathetic signal reflecting respiratory sinus arrhythmia (1, 18), whereas LF power reflects both sympathetic and parasympathetic modulation of R-R intervals and is strongly influenced by baroreflex activity (1, 16, 18). In addition, the ratio of LF power to HF power was calculated as an index of autonomic balance; increases in LF/HF power indicate sympathetic predominance (16).

Statistical analysis. Each subject served as his/her own control. Power spectral values (LF power, HF power, and total power) were transformed to their natural logarithms for statistical analysis because of their positively skewed distributions. The logarithmic transformations succeeded in producing approximately normal distributions and thus allowed the use of parametric statistics. To overcome the arbitrary choice of numerator and denominator in the LF-to-HF ratio, it too was log transformed. This is necessary because the critical value of t in a paired t-test of the LF-to-HF ratio is different from the critical value of t in a paired t-test of the reciprocal, the HF-to-LF ratio. The results of a statistical analysis of the logarithm of the LF-to-HF ratio will equal, in absolute value, the results of an analysis of the logarithm of the HF-to-LF ratio. After logarithmic transformation the arbitrary choice of numerator and denominator in the LF-to-HF ratio does not affect the statistical analysis.

Comparisons between treatments were made on the log-transformed power spectral values using a repeated measures analysis of variance (ANOVA). Relative (percent) change between baseline and treatment for all log-transformed power spectral values was calculated in the following manner: the difference between the log-transformed power spectral values for each treatment was calculated for each subject. The mean difference for the group was calculated and then exponentiated, which resulted in the mean relative change in a power spectral value for the group.

RESULTS

Effect of phenylephrine on supine subjects. The infusion of increasing doses of phenylephrine in supine subjects was associated with progressive increases in systolic blood pressure and N-N intervals. The highest dose of phenylephrine (1.2 µg·kg⁻¹·min⁻¹) was associated with an increase in systolic blood pressure from 118 ± 4 mmHg at baseline to 129 ± 5 mmHg (ANOVA F = 7.62, P < 0.005), and an increase in N-N intervals from 881 ± 44 ms at baseline to 1,274 ± 69 ms (ANOVA F = 79.52, P < 0.0001). The infusion of phenylephrine was associated with an increase in the broad-band measure of R-R variability, SDNN, from 50.6 ± 5.5 ms at baseline to 96.7 ± 10.7 ms at the highest dose of phenylephrine (ANOVA F = 27.59, P < 0.0001).

All three specific indexes of parasympathetic nervous system activity (HF power, RMSSD, and PNN50) progressively increased with increasing doses of phenylephrine (Fig. 1). With the comparison of baseline values to the values at the highest dose of phenylephrine, there was a 568% increase in HF power [95% confidence interval (CI), 293–1,036%, P < 0.001], a 227% increase in RMSSD (95% CI, 184–269%, P < 0.0001), and a 267% increase in PNN50 (95% CI, 231–365%, P < 0.0001). This increase in parasympathetic nervous system activity, as measured by HF power, was observed in every subject (Fig. 2).

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An example of the effect of phenylephrine on one subject is shown in Fig. 3. This example demonstrates that phenylephrine was associated not only with an increase in the mean R-R interval but also with an increase in the amplitude of the fluctuation occurring at the respiratory frequency (measured between 0.15 and 0.40 Hz, corresponding to respiratory rates of 9–24 breaths/min), thus accounting for the increase in HF power.

The infusion of increasing doses of phenylephrine in supine subjects was also associated with a 77% increase in LF power that was not statistically significant (ANOVA F = 3.81, P = 0.06). The much greater increases in HF power compared with these smaller increases in LF power resulted in a marked decrease in the LF-to-HF ratio; comparing baseline values to the values at the highest dose of phenylephrine, there was a 74% decrease in the LF-to-HF ratio (95% CI, −83 to −59%, P < 0.001). This decrease in the LF-to-HF ratio is consistent with a shift in sympathovagal balance toward a parasympathetic predominance.

DISCUSSION

Effect of phenylephrine on vagal measures. The data from this study clearly demonstrate that phenylephrine infusions produced baroreflex-mediated increases in both time- and frequency-domain measures of R-R variability known to reflect parasympathetic nervous system activity. Increasing doses of phenylephrine produced increases in blood pressure, increases in R-R intervals, and a fivefold increase in HF power. This marked increase in HF power was also observed in the other two indexes of parasympathetic nervous system activity, RMSSD, and PNN50. This effect of phenylephrine on parasympathetic nervous system activity was observed in every subject (Fig. 2).

These findings contrast with those of Goldberger et al. (9), who reported that phenylephrine infusion was unexpectedly associated with a decrease in HF power and RMSSD in every one of their 10 normal subjects despite the observed expected increases in R-R intervals. There are three potential explanations for the striking difference in the response of HF power and...
RMSSD to phenylephrine in these two studies: differences in the subjects, differences in the method of dosing, and differences in the algorithms used to calculate RMSSD and HF power.

Table 1. Comparison with study by Goldberger et al.

<table>
<thead>
<tr>
<th></th>
<th>This Study</th>
<th>Goldberger et al. (9)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>29 ± 3</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>Baseline R-R interval, ms</td>
<td>881 ± 44</td>
<td>1,034 ± 109</td>
</tr>
<tr>
<td>R-R interval at maximum dose of phenylephrine, ms</td>
<td>1,274 ± 69</td>
<td>1,335 ± 140</td>
</tr>
<tr>
<td>Baseline MBP, mmHg</td>
<td>74 ± 6</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>MBP at maximum dose of phenylephrine, mmHg</td>
<td>84 ± 9</td>
<td>109 ± 8</td>
</tr>
<tr>
<td>Maximal dose of phenylephrine used, µg·kg⁻¹·min⁻¹</td>
<td>1.2 (Maximum dose)</td>
<td>1.1 ± 1.6 (Average maximal dose)</td>
</tr>
<tr>
<td>Baseline SDNN (means ± SD)</td>
<td>50.6 ± 16.6</td>
<td>71.1 ± 25.8</td>
</tr>
<tr>
<td>Baseline RMSSD (means ± SD)</td>
<td>33.9 ± 10.9</td>
<td>72.4 ± 42.4</td>
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MBP, mean blood pressure. SDNN, standard deviation of N-N interval; RMSSD, root-mean-square successive difference.

A comparison of the baseline characteristics of the subjects and the methodologies used is listed in Table 1. There are some important differences in some of the baseline characteristics of the subjects in the two studies. Goldberger et al. (9) described their subjects as normal healthy volunteers (their average R-R intervals were 1,034 ms); these subjects, however, had higher values for SDNN and MSSD than the subjects described in this study, suggesting that their subjects had greater resting parasympathetic nervous system activity. Goldberger et al. (9) as well as Malik and Camm (14) have suggested that increases in vagal nerve activity in subjects with greater resting vagal modulation of the sinus node may saturate the parasympathetic effect on the sinus node, thereby eliminating the cyclic respiratory modulation of R-R intervals, resulting in a decrease in HF power. Whereas the difference in resting vagal modulation in this sample and the sample studied by Goldberger et al. (9) allows for this theoretical possibility, the data from this study do not support this hypothesis. In this study, even the subjects with the greatest baseline vagal modulation had significant increases in HF power, RMSSD, and PNN50 with...
supine phenylephrine infusion. Furthermore, the increase in HF power during phenylephrine infusion in the head-up tilt position was similar to the increase in HF power during phenylephrine infusion in the supine position. If Goldberger’s saturation theory were correct, one would expect a greater increase in HF power during phenylephrine infusion into subjects tilted head-up: given that vagal modulation of the sinus node is reduced when a subject is upright, phenylephrine infusion potentially could have produced larger increases in HF power before reaching the purported threshold. Whereas the possibility still exists that there is a subset of individuals with high resting vagal tone (such as highly trained endurance athletes) in whom an increase in vagal activity produces a decrease in cyclic vagal modulation of R-R intervals, the physiological characteristics and the relative size of this subset of individuals require further investigation and more detailed description. (It is important to note, however, that Goldberger’s subjects were not described as highly trained endurance athletes.)

The method for phenylephrine dosing was different in the two studies: we used fixed doses of phenylephrine, whereas Goldberger titrated the dose of phenylephrine to achieve a specified increase in mean blood pressure. Despite this different approach to dosing, the average dose of phenylephrine used in the Goldberger study was nearly identical to the maximum dose used in this study. Whereas some of the subjects in the Goldberger study may have received higher doses of phenylephrine, at least half of their subjects received <1.2 µg·kg⁻¹·min⁻¹ of phenylephrine (the maximal dose used in this study); yet, all of their subjects demonstrated a decrease in HF power, and all of our subjects demonstrated an increase in HF power at the highest dose. Similarly, the effects of phenylephrine on R-R intervals and blood pressure were similar in the two studies. Whereas the increase in mean blood pressure was larger in the Goldberger study (24 vs. 10 mmHg), the increase in R-R intervals was slightly larger in this study (365 vs. 301 ms in the Goldberger study). In a subsequent study by Goldberger et al. (10), lower doses of phenylephrine were used (0.3 and 0.6 µg·kg⁻¹·min⁻¹), but even these lower doses of phenylephrine caused a drop in HF power in all of their subjects.

The small differences in method of dosing and subjects raise questions about differences in the two algorithms used in the two studies to calculate HF power and RMSSD. The algorithms used for calculating HF power are complex, and a comparison is beyond the scope of this paper. The determination of RMSSD, however, is straightforward once ectopic and nonsinus beats are removed. Notably, the mean value for RMSSD reported by Goldberger et al. (9) is high, 72.4 ms, compared with values for RMSSD reported in other studies of young normal subjects that range from 29 ± 15 ms (21) to 41 ± 14 (12) and 54 ± 22 ms (7). The ultimate reconciliation of the algorithm used in this paper and the algorithm used by Goldberger et al. (9) requires that both sets of data be analyzed by both algorithms.

Effect of phenylephrine on sympathovagal balance. Baroreceptor activation with phenylephrine produced only small and statistically insignificant increases in LF power. LF power is known to be influenced by both sympathetic and parasympathetic nervous system activity. One would expect, therefore, that the effect of baroreceptor activation with phenylephrine on LF power would reflect the opposing effects of an increase in parasympathetic nervous system activity and a decrease in sympathetic nervous system activity. The overall shift in sympathovagal balance toward a parasympathetic predominance is reflected in the decrease in the LF-to-HF ratio.

Effect of phenylephrine on autonomic changes associated with head-up tilt. In this study, head-up tilt was associated with a decrease in R-R intervals, a decrease in HF power, and an increase in the LF-to-HF ratio. These changes are consistent with the expected decrease in parasympathetic nervous system activity and the shift in sympathovagal balance toward a sympathetic predominance that have been published previously. Whereas phenylephrine infusion had the effect of shifting the supine autonomic state toward a parasympathetic predominance, it did not affect the typical shift in autonomic balance that occurs with head-up tilt. At every dose of phenylephrine, we observed similar decreases in R-R interval, decreases in HF power, and increases in the LF-to-HF ratio associated with head-up tilt.

In conclusion, increasing doses of phenylephrine infused into normal subjects produced increases in blood pressure, increases in R-R intervals, and increases in RMSSD, PNN50, and HF power consistent with the expected increase in parasympathetic nervous system activity. Vagal measures of R-R variability track the increase in parasympathetic nervous system activity during baroreceptor activation with phenylephrine.

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