Human sinus arrhythmia: inconsistencies of a teleological hypothesis

Y. C. Tzeng, P. Y. W. Sin, and D. C. Galletly
Physiological Rhythms Unit, Department of Surgery & Anaesthesia, University of Otago, Wellington, New Zealand

Submitted 11 July 2008; accepted in final form 26 October 2008

Respiratory sinus arrhythmia (RSA) manifests in most mammalian species as shortening and lengthening of beat-to-beat cardiac cycle intervals throughout the respiratory cycle and accounts for the high frequency peak in the heart rate (HR) variability (HRV) power spectrum. RSA is mediated by phasic afferent inputs, including arterial baroreceptor and chemoreceptor afferent inputs, and reflex pathways associated with pulmonary and atrial stretch receptors, and intrinsic mechanical myocardial stretch. Although many studies have described the relationship between RSA and indexes of pulmonary gas exchange efficiency, measured as the average ventilatory equivalents of CO2 and O2, none have examined the relationship between RSA amplitude (modified by paced breathing) and indexes of pulmonary gas exchange efficiency. In human subjects, we found that normal amplitudes of RSA enhance pulmonary gas exchange efficiency via clustering of heart beats in inspiration, while high amplitudes of RSA enhanced by slow paced breathing cause more heart beats to cluster in inspiration. Therefore, normal amplitudes of RSA enhance pulmonary gas exchange efficiency via clustering of heart beats in inspiration.

METHODS

We studied 12 healthy male subjects at rest. The mean age of the subjects was 21 yr (range, 20–25), and all had abstained from caffeine-containing beverages for at least 4 h before the study. No subjects were receiving regular medication, and none had a known history of respiratory, cardiovascular, or endocrine disease. Ethical approval was obtained from the Central Regional Ethics Committee. Written informed consent was obtained from all volunteers, and the experiment was performed in accordance with the Declaration of Helsinki.

With the subjects lying in the supine position, we recorded the electrocardiogram (ECG lead CM5, Corometrics Neo-Trak 502), respiratory sinus arrhythmia; heart rate variability; ventilation perfusion matching; vagal modulation; pulmonary gas exchange

Address for reprint requests and other correspondence: Y. C. Tzeng, Dept. of Surgery & Anesthesia, Univ. of Otago, Wellington, PO Box 7343, Wellington, New Zealand (e-mail: shieak.tzeng@otago.ac.nz).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
respiratory flow (Hans Rudolph Pneumotach, Vacumed differential pressure transducer), and end-tidal PCO₂ (PetCO₂; Datex Instrumentation Division, Helsinki, Finland). For gas sampling, a tube was placed adjacent to each subject’s nostril. Data were acquired at 500 Hz/channel with a 16-bit I/O data acquisition board (PCI-6023E series; National Instruments). Subsequent offline analysis was performed using custom written software in LabView 7 (National Instruments) on a Macintosh 1 GHz PowerBook G4 computer.

Paced Respiration Protocol

The target breathing f was controlled in trained subjects by graphically displaying, in real time, a recurring digital sequence of 20 colored light-emitting diodes (LEDs) coupled with an auditory signal generated from a Macintosh MacBook computer. To maintain a constant inspiratory period-to-respiratory period (IE/II) ratio of 0.5, subjects were instructed to inhale when the first of a sequence of 10 blue LEDs appeared and to exhale when the 11th LED, which marks the beginning of a sequence of 10 red LEDs, appeared. Vt was not controlled to enable subjects to maintain minute ventilation (Ve) and normocapnia. Enabling subjects to adjust and increase Vt during slow breathing also served to maximize RSA, given that a higher Vt enhances RSA independent of f (14). Although no restrictions were made on Vt, subjects were trained to avoid hyperventilation. Following an initial 10-min stabilization period, subjects pace breathed at 12, 9, and 6 breaths/min (br/min). The paced rate sequence was randomized between subjects to remove potential order effects.

The choice to control IE/II was based on pilot data (n = 9), which showed that between the three breathing rates, IE/II was comparatively greater during 6 br/min breathing. The IE/II ratio was therefore fixed to 0.5 to ensure that changes in the distribution of heart beats associated with pace breathing were not due simply to alternations in breathing pattern. Although the influence of breathing control on RSA is generally thought to be negligible (20), more complex breathing protocols can alter RSA (19). However, our pilot analysis showed that IE/II control did not influence the relationship between RSA amplitude and Δphase (Fig. 1) against f, which suggests that our method of IE/II control did not unduly influence RSA parameters relevant to this study.

Data Analysis

From the raw ECG and respiratory flow signals, the R wave, inspiratory, and expiratory onset times were extracted. Vt was determined by integrating the respiratory airflow signal, and Ve was taken as the product of Vt and f. The ECG and respiratory flow recordings were manually checked for nonstationary behavior (e.g., ectopic beats, breathing interruptions), and where identified these were manually removed. In this study we used the ECG R wave (the electrical equivalent of ventricular depolarization) to represent the timing of each heart beat, recognizing that ventricular ejection will follow this time point.

Characterization of RSA. We applied a phase domain approach to characterize the way in which R wave-to-R wave (R-R) intervals varied throughout the respiratory cycle, i.e., the pattern of RSA. The algorithm used in this study was conceptually similar to previously described methods of RSA pattern extraction (11, 25) but modified for the purposes of this study. In brief, the preceding R-R interval times were plotted as a function of the respiratory phase. This was followed by cubic-spline interpolation of the R-R intervals in each respiratory period into n = 100 data points, giving the individual RSA pattern waveform for each breath as a function of respiratory phase. This procedure was repeated for each of the m respiratory cycles. The spline interpolated curves were then superimposed, and an average RSA pattern was calculated as

\[
\overline{X}_j = \frac{1}{m} \sum_{i=1}^{m} x_{ij}
\]

(where \(x_{ij}\) represents a sample of the \(i\)th interpolated point of the \(j\)th respiratory cycle and where \(i = 1, \ldots, n\) and \(j = 1, \ldots, m\)). Figure 1 shows the indexes that are derived from the average RSA pattern.

Assessment of heart beat distribution. Heart beat distribution was assessed by plotting histograms of R wave occurrence as a function of the respiratory phase. To determine whether higher levels of RSA were associated with greater clustering of heart beats in inspiration (HBinsp), we calculated and compared the proportion of R waves occurring in inspiration across each paced breathing f.

Statistical analysis. All values reported in this study are means (SD) given to two significant figures. Mean R-R interval, Vt, Ve, IE/II, and PetCO₂, were treated as parametric measures, whereas RSA amplitude, HBinsp, and Δphase were analyzed nonparametrically. The effects of f on cardiovascular and respiratory variables were evaluated with one-way repeated-measures ANOVA (RMANOVA) for parametric variables and Friedman’s test for nonparametric variables. Differences between parametric and nonparametric variables between the three breathing conditions were further assessed using Student’s paired t-test and Wilcoxon’s signed rank test where RMANOVA or the Friedman’s test was, respectively, significant. A Bonferroni correction was applied to all paired tests. Analyses were performed using SPSS 11 (SPSS, Chicago, IL), and α was set a priori at \(P < 0.05\).

RESULTS

The effects of paced breathing on mean R-R interval and ventilatory variables are summarized in Table 1. In this study, slowing down of f was associated with an increase in breath-to-breath Vt. However, there were no significant changes in Ve, mean R-R interval, or PetCO₂, across f. Subjects were able to comfortably maintain an approximate IE/II ratio of 0.5 across different f (RMANOVA; \(P = 0.45\)).

![Fig. 1. Stylistic representation of the respiratory sinus arrhythmia (RSA) pattern curve. RSA amplitude is taken as the difference between the maximum—minimum R wave-to-R wave (R-R) intervals. Phase = 0 corresponds to inspiratory onset. Phase of expiratory onset (E) is indicated by the solid vertical line and in this study was controlled at 0.5 of the respiratory period. The phase of RSA nadir corresponds to the point of minimum R-R interval throughout the respiratory cycle, and Δphase is calculated as the phase difference between E and RSA nadir. A negative Δphase indicates that RSA nadir occurs in inspiration, whereas a positive Δphase indicates that RSA nadir occurs in expiration.](http://ajpheart.physiology.org/)

AJP-Heart Circ Physiol • VOL 296 • JANUARY 2009 • www.ajpheart.org
Figure 2 shows a representative example of the R-R interval time series, R wave distribution histogram, and the RSA curve across different paced breathing for one subject. Although 6 br/min breathing was associated with an increase in RSA amplitude and phase shift of RSA nadir into inspiration, there was no visually apparent clustering of heart beats in inspiration.

Figure 3 shows the relationship between changes in f on RSA amplitude, HBinsp, and Δphase for all subjects. RSA amplitude was greatest during 6 br/min and lowest during 12 br/min breathing. Although the relationship between RSA and respiration is classically described as an inspiratory HR acceleration and expiratory HR deceleration, we found this relationship was only consistently valid during 9 and 12 br/min breathing. During 6 br/min breathing, HR acceleration consistently occurs before expiratory onset. Despite a significant gain in RSA amplitude and the occurrence of the RSA nadir in inspiration, HBinsp did not increase significantly with 6 br/min breathing.

In four subjects, 6 br/min breathing was associated with a reduction in HBinsp. The mean ΔHBinsp for all subjects was relatively small (~1.8%), consistent with RSA generally having a negligible effect clustering heart beats in inspiration.

DISCUSSION

The literature linking HRV to cardiovascular mortality is extensive, and the overwhelming consensus is that diminished HRV is associated with increased risk of sudden death from cardiovascular disease (15, 16). However, despite clear prognostic associations, there is still uncertainty over the information content revealed by indexes of HRV (17). In contrast with the paradigm that RSA is simply an epiphenomenon arising from independent cardiorespiratory processes, Hayano et al.’s (13) teleological hypothesis raises the possibility that loss of RSA may itself directly compromise the cardiorespiratory system margin for energy conservation. This may be clinically relevant in conditions associated with reduced RSA, such as severe left ventricular dysfunction and postmyocardial infarction, and the theory may partly explain the pathophysiological link between HRV and death.

It appears to be widely accepted that RSA optimizes pulmonary gas exchange efficiency through heart beats clustering during inspiration and scattering during expir-

Table 1. Effect of pace breathing on cardiorespiratory variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R interval, s</td>
<td>0.95 (SD 0.080)</td>
<td>0.94 (SD 0.10)</td>
<td>0.96 (SD 0.083)</td>
</tr>
<tr>
<td>Vt, liters</td>
<td>0.92 (SD 0.23)</td>
<td>0.64 (SD 0.14)*</td>
<td>0.49 (SD 0.10)*†</td>
</tr>
<tr>
<td>Vt, l/min</td>
<td>5.5 (SD 1.4)</td>
<td>5.7 (SD 1.2)</td>
<td>5.8 (SD 1.2)</td>
</tr>
<tr>
<td>PetCO₂, mmHg</td>
<td>42 (SD 4.8)</td>
<td>42 (SD 3.9)</td>
<td>42 (SD 3.4)</td>
</tr>
</tbody>
</table>

Values are means (SD). R-R interval, R wave-to-R wave cardiac period interval; Vt, tidal volume; Vt, minute ventilation; PetCO₂, end-tidal PCO₂. P values are for comparisons between different breathing conditions made using repeated-measures ANOVA. *Statistically different from 6 breaths/min (br/min); †significantly different from 9 br/min. Comparisons between pairs were made with the paired t-test, adjusted for multiple comparisons using the Bonferroni correction.

Fig. 2. Representative results from 1 subject showing the R-R interval time series (left) and RSA pattern curves superimposed on corresponding heart beat distribution histograms (right) during 12 (A), 9 (B), and 6 (C) breaths/min (br/min) pace breathing. Phase = 0 is onset of inspiration, and onset of expiration is indicated by the dashed vertical line. Only the first 100 s of the R-R interval time series is shown, whereas histograms are generated from the entire data epoch (~5 min). RSA is clearly apparent at all breathing frequencies (f), but the amplitude was clearly maximal during 6 br/min breathing. In this individual, RSA nadir clearly occurred in inspiration only during 6 br/min breathing. However, adopting a slow 6 br/min breathing rate does not result in any visually apparent redistribution of heart beats in inspiration.
tion to match the waxing and waning of alveolar ventilation (10, 12, 13, 29). However, we consider this an unproved assumption in humans, with no studies demonstrating a heart beat redistribution toward the inspiratory period at RSA amplitudes that are likely to be observed in resting human subjects. In relation to this, the current study has three major findings that are relevant to the interpretation of previous human research (10) and to the validity of Hayano et al.’s hypothesis in general.

First, it has been suggested that RSA improves respiratory efficiency by matching more heart beats during inspiration when alveolar oxygen tension is greatest. For this to occur, the phase of RSA associated with the fastest HR (shortest R-R interval) would need to occur in inspiration. However, it is arguable whether such a phase relationship between respiration and RSA is maintained across a range of f. Some studies suggest that the onset of HR deceleration (which corresponds to the RSA nadir in this study) is temporally fixed to expiratory onset (7), whereas others indicate that the phase relationship described by Hayano et al. (13) is true only during 6 br/min breathing (26). Our analyses support the latter view, showing that the occurrence of the RSA nadir in inspiration is consistently valid only during 6 br/min breathing (during 9 and 12 br/min breathing, RSA nadir tended to occur around expiratory onset). These results are inconsistent with the suggestion that the putative function of RSA is most relevant at rest (12, 29), given that normal resting f is around 10–15 br/min (25) rather than 6 br/min.

Second, in addition to changes in the RSA-respiration phase relationship, 6 br/min breathing was also associated with a ~1.6-fold average increase in RSA amplitude. Although both changes should favor a rise in HBinsp, we observed no significant changes in HBinsp across f (Figs. 3 and 4). With a similar breathing protocol, Giardino et al. (10) demonstrated in 10 volunteers significant associations between RSA amplitude and ventilatory equivalents for CO2 and O2 during pace breathing across a similar f range. We consider it unlikely that clear and significant relationships between RSA amplitude and pulmonary gas exchange efficiency are due to statistically inconspicuous changes in HBinsp in an otherwise healthy cohort of a similar sample size. Therefore, in our view, the logical conclusion is that the range of RSA amplitudes observed in paced breathing of human subjects is insufficient to generate significant degrees of heart beat clustering.

Finally, although it was not our intention to invalidate Hayano et al.’s original study in dogs (13), our results highlight the need for careful extrapolation of the data to humans. Hayano et al.’s simulation of RSA was achieved by electrical stimulation of the right cervical vagus nerve in time with expiration. The period of inspiration and expira-

Fig. 3. Effect of breathing frequency (f) on RSA amplitude (A), proportion of heart beats in inspiration (B; HBinsp), and the phase difference between expiratory onset and RSA nadir (C; Δphase) for individual subjects (left) and corresponding group averages (right). Comparisons across different breathing conditions with Friedman’s test were statistically significant for RSA amplitude (P < 0.01) but not for HBinsp (P = 0.34). RSA nadir tended to occur around expiratory onset during 9 and 12 br/min breathing but progressively shifts into inspiration during 6 br/min breathing (P < 0.01). *Significantly different from 6 br/min breathing; †significantly different from 9 br/min breathing. Comparisons between pairs were made with Wilcoxon’s signed ranked test and adjusted for multiple comparisons using the Bonferroni correction.
tion was set at 2 s, respectively, by diaphragmatic pacing, and current pulses to the vagus nerve were applied such that none, or only one heart beat, occurred during expiration and four or five heart beats occurred during inspiration (Fig. 5) (13). This design may have simulated physiologically plausible ranges of mean HR (60–75 beats/min) but generated RSA amplitudes that were, on average, sixfold greater than RSA amplitudes observed in this study during 6 br/min breathing. Our results suggest that such high amplitude RSA is unlikely to be observed in humans given that the amplitude of RSA at 6 br/min in young subjects represents a physiological maximum. Moreover, the pattern of RSA generated by the dog model produced a pattern of R wave distribution that scarcely resembled normal human physiology; in no subjects did we observe a pattern of heart beat distribution whereby virtually all heart beats occur in inspiration.

Taken together, our findings challenge the hypothesis that RSA optimizes pulmonary gas exchange efficiency by inspiratory heart beat clustering and suggest that some other mechanism(s) warrant consideration. One possibility relates to the effects of changes in intrathoracic pressure (ITP) associated with spontaneous ventilation on cardiac and pulmonary function. It is well known that falling ITP associated with spontaneous efforts lowers right atrial pressure and facilitates blood flow to the right ventricle because the rate of venous return changes inversely with right atrial pressure (4, 18, 27). It has been suggested that this bolus-increased venous return during inhalation may be transmitted to the pulmonary artery on subsequent beats, resulting in a matched increase in both pulmonary capillary blood flow and alveolar ventilation (21). This thoracic pump effect, which is distinct from RSA, may be greater during slower breathing and may confound the apparent relationship between RSA and pulmonary gas exchange efficiency (10).

Results of this study should be interpreted cognizant of the following limitations. First, we did not record beat-to-beat pulmonary capillary blood flow and, therefore, were unable to quantify the thoracic pump effect. We can only speculate that some process other than the clustering of heart beats may be responsible for previously reported associations between RSA and indexes of pulmonary gas exchange efficiency. Finally, although we have taken the necessary methodological precautions to ensure breathing control did not significantly alter RSA, the override of spontaneous respiratory rhythm interrupts other processes that may otherwise influence heart beat distribution throughout the respiratory cycle. One example is cardioventilatory coupling (23, 24), which is the tendency for HR and f to become aligned at whole number integer ratios under spontaneous breathing conditions. Therefore, the results of this study conducted under pace breathing conditions extend purely to the relationship between RSA and heart beat distribution.

In conclusion, we have examined the relationship between RSA and heart beat distribution in healthy subjects during paced breathing. Despite the significant gain in RSA amplitude and a negative Δphase relationship with 6 br/min breathing, our results do not support the hypothesis that RSA matches pulmonary capillary perfusion to alveolar ventilation in phase with inspiration since we observed no significant changes in R wave distribution that scarcely resembled normal human physiology; in no subjects did we observe a pattern of heart beat distribution whereby virtually all heart beats occur in inspiration.

Results of this study should be interpreted cognizant of the following limitations. First, we did not record beat-to-beat pulmonary capillary blood flow and, therefore, were unable to quantify the thoracic pump effect. We can only speculate that some process other than the clustering of heart beats may be responsible for previously reported associations between RSA and indexes of pulmonary gas exchange efficiency. Finally, although we have taken the necessary methodological precautions to ensure breathing control did not significantly alter RSA, the override of spontaneous respiratory rhythm interrupts other processes that may otherwise influence heart beat distribution throughout the respiratory cycle. One example is cardioventilatory coupling (23, 24), which is the tendency for HR and f to become aligned at whole number integer ratios under spontaneous breathing conditions. Therefore, the results of this study conducted under pace breathing conditions extend purely to the relationship between RSA and heart beat distribution.

Conclusion

In conclusion, we have examined the relationship between RSA and heart beat distribution in healthy subjects during paced breathing. Despite the significant gain in RSA amplitude and a negative Δphase relationship with 6 br/min breathing, our results do not support the hypothesis that RSA matches pulmonary capillary perfusion to alveolar ventilation in phase with inspiration since we observed no significant changes in

Fig. 5. A: schematic representation of ECG R wave occurrence as a function the respiratory cycle according to methodological descriptions from early dog experiments (Ref. 13). Inspiratory (I) and expiratory (E) onsets are indicated by gray vertical lines in A. Having 4 heart beats occur during inspiration and no heart beats occur during expiration gives a minimum R-R interval of 2/3 s, a maximum R-R interval of 2 s, and an equivalent RSA amplitude of 4/3 s. B: corresponding pattern of RSA. Note that the pattern of RSA scarcely resembles human RSA (Fig. 2).
HB_{insp}. Without clear evidence of heart beat clustering in inspiration, we can only conclude that any putative function of RSA in optimizing pulmonary gas exchange efficiency remains to be established.

GRANTS
This work was supported by New Zealand National Heart Foundation Grant 1284.

REFERENCES