Effects of hypercapnia and hypoxemia on respiratory sinus arrhythmia in conscious humans during spontaneous respiration

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Tzeng YC, Larsen PD, Galletly DC. Effects of hypercapnia and hypoxemia on respiratory sinus arrhythmia in conscious humans during spontaneous respiration. *Am J Physiol Heart Circ Physiol* 292: H2397–H2407, 2007. First published January 12, 2007; doi:10.1152/ajpheart.00817.2006.—Normally, at rest, the amplitude of respiratory sinus arrhythmia (RSA) appears to correlate with cardiac vagal tone. However, recent studies showed that, under stress, RSA dissociates from vagal tone, indicating that separate mechanisms might regulate phasic and tonic vagal activity. This dissociation has been linked to the hypothesis that RSA improves pulmonary gas exchange through preferential distribution of heartbeats in inspiration. We examined the effects of hypercapnia and mild hypoxemia on RSA-vagal dissociation in relation to heartbeat distribution throughout the respiratory cycle in 12 volunteers. We found that hypercapnia, but not hypoxemia, was associated with significant increases in heart rate (HR), tidal volume, and RSA amplitude. The RSA amplitude increase remained statistically significant after adjustment for respiratory rate, tidal volume, and HR. Moreover, the RSA amplitude increase was associated with a paradoxical rise in HR and decrease in low-frequency-to-high-frequency mean amplitude ratio derived from spectral analysis, which is consistent with RSA-vagal dissociation. Although hypercapnia was associated with a significant increase in the percentage of heartbeats during inspiration, this association was largely secondary to increases in the inspiratory period-to-respiratory period ratio, rather than RSA amplitude. Additional model analyses of RSA were consistent with the experimental data. Heartbeat distribution did not change during hypoxemia. These results support the concept of RSA-vagal dissociation during hypercapnia; however, the putative role of RSA in optimizing pulmonary perfusion matching requires further experimental validation.

Respiratory sinus arrhythmia (RSA) is a periodic high-frequency (HF) variation in heart rate (HR) due to the modulatory effects of respiration on vagal activity (1–3, 13, 17). The conventional belief is that the magnitude of RSA reflects the level of mean vagal outflow; therefore, estimation of RSA amplitude may provide an indirect measure of vagal tone (13, 28). However, it has been suggested that the apparent parallel relation between RSA amplitude and cardiac vagal tone is an indirect consequence. Indeed, several recent studies have shown that RSA and mean vagal tone may dissociate under stressful conditions, such as in response to hypercapnia or phenylephrine challenge (21, 22, 24, 42, 52).

The mechanism of this RSA-vagal dissociation is not well understood. Hayano and Yasuma (24) hypothesized that this RSA-vagal dissociation could be a result of two distinct control systems: one for RSA (phasic vagal activity) and the other for HR (tonic vagal activity). Furthermore, this dissociation has been linked to a putative role of RSA in matching alveolar ventilation to capillary perfusion throughout the respiratory cycle, i.e., “heartbeats clustering during inspiration and scattering during expiration” (24, 25). To achieve this, there would be a need for independent controlling systems for RSA and HR.

Supportive evidence from spontaneously breathing dogs and paced-breathing human subjects indicates that RSA-vagal dissociation occurs during hypercapnia, but not during hypoxemia (42, 52–54). This, however, remains to be verified during spontaneous breathing. Moreover, although heartbeat distribution throughout the respiratory cycle has been examined in relation to the tendency for heartbeat and inspiratory activity to become temporally aligned [cardioventilatory coupling (CVC)] (15, 33, 51), the role of RSA in heartbeat distribution is unclear. Therefore, the objective of the present study is to explore the effects of hypercapnia and hypoxemia on RSA-vagal dissociation, CVC, and placement of heartbeats throughout the respiratory cycle in spontaneously breathing humans.

**METHODS**

After approval from the Wellington Human Ethics Committee and written informed consent, 12 healthy volunteers (8 men and 4 women) were studied at rest in the supine position. The subjects’ mean age was 23 yr (range 18–30 yr), and all had fasted and abstained from caffeine-containing beverages for ≥4 h before the study. No subject was receiving regular medication, and none had evidence of respiratory, cardiovascular, or endocrine diseases. HR (ECG lead CM5; Neuro-Trak 502, Corometrics) and respiratory flow (Hans Rudolph Pneumotach and Vacumed differential pressure transducer) were recorded for all 12 subjects. End-tidal CO2 (PETCO2; Datex Instrumentation Division, Helsinki, Finland) and finger pulse oximetry (SpO2) were monitored throughout the study. For gas sampling, a tube was placed adjacent to the subject’s mouth.

Each subject lay comfortably in the supine position with head support. Subjects breathed through a tight-seal face mask connected to an anesthetic breathing circuit (6). Inhaled gas composition was controlled by adjustment of fresh gas flows of O2, medical air (21% O2 in N2), and O2-free N2. The breathing circuit was connected to a 4-liter reservoir bag. Three study conditions were achieved as follows:

**Control:** normocapnia-normoxia (normal PETCO2 and normal SpO2).

Medical air was administered at 6 l/min, and SpO2 and PETCO2 were allowed to remain at normal physiological levels.

**Hypercapnia:** hypercapnia-normoxia (50 mmHg PETCO2 and ≥97% SpO2). PETCO2 was elevated by rebreathing through the 4-liter reservoir bag, with the soda lime canisters excluded from the circuit, and ventilation-perfusion mismatch; heart rate variability

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decreasing the level of medical air inflow. To prevent development of hypoxemia, an amount of O2 just sufficient to maintain ≥97% SpO2 was introduced into the breathing circuit.

**Hypoxemia: normocapnia-hypoxemia (40 ± 1 mmHg PETCO2 and 90 ± 1% SpO2).** By adjustment of fresh gas flow of oxygen-free N2 in air, SpO2 was lowered to 90 ± 1%. This level of hypoxemia did not change PETCO2, from normal physiological levels.

Experiments were conducted in a quiet, air-conditioned (23–25°C) room. After an initial stabilization period (10 min), during which the subject breathed medical air with soda lime canisters included in the circuit, each of the gas compositions was inhaled for 15 min. Subjects were blinded to the gas composition, the order being determined according to a Latin-square crossover design. In all experiments, transitions between study gas compositions were readily completed within 5 min. ECG and respiratory flow were continuously recorded throughout the study period. Data were sampled at 500 Hz per channel with a multifunction input-output DAQ board (model PCI-6023E, National Instruments) and stored on hard disk for offline processing.

**Data Analysis**

From the raw ECG and respiratory flow data, the R wave and inspiratory (I) and expiratory (E) onset times were extracted. Tidal volume (VT) was obtained by integration of the respiratory flow signal. Minute ventilation (VE) was taken as the product of VT and respiratory rate (f). Analysis was performed on the entire data series, although average values during each given mixture were determined from the final 10 min of each 15-min period. All data analysis was readily completed within 5 min. ECG and respiratory flow were analyzed by calculation of the difference between the maximum and minimum values on the RSA pattern waveform.

**Spectral Analysis**

HR variability was examined by analysis of the power spectrum of R wave-to-R wave interval time series (R-R interval). The R-R interval time series was manually checked for the presence of artifacts, and spuriously detected or missed R waves were corrected by linear interpolation. The resulting R-R interval time series were high-pass filtered for removal of fluctuations below 0.015 Hz and low-pass filtered for exclusion of components above the Nyquist frequency (2 Hz). We then resampled the filtered signal at 4 Hz to provide equidistant data points. To obtain the dataset for spectral analysis from each recording epoch, we applied a series of windows of length n = 1,024 points, shifted by 512 points across the epoch. For each dataset, a Hanning window was applied before fast Fourier transform analysis. The spectral power was calculated for each subject for the integrated area under the power spectrum curve in the HF (0.15–0.40 Hz) and low-frequency (LF, 0.04–0.15 Hz) ranges.

The spectral power density within each frequency band was converted to its mean amplitude equivalent as follows:

\[
\text{mean amplitude (ms/Hz}^{1/2} = \sqrt{2 \times \text{power (ms}^2/\text{Hz)}}
\]

We also evaluated the ratio of LF to HF amplitude components (LFHF)(23).

**CVC**

Clinical and experimental studies show that CVC manifests as a constant temporal relation between inspiration and the immediately preceding heartbeat (RI–I interval) (16, 32, 51). Therefore, application of the proportional Shannon entropy (SHs) of the RI–I interval provides a statistical index of apparent CVC strength. Starting from the beginning of each RI–I interval time series, a 40-breath moving window was passed across the entire time series, and the corresponding RI–I interval within each window was placed into a 10-bin histogram. The outer histogram limits were set at 0 and the mean of the R-R intervals that had been encompassed by the 40 inspiratory onsets. From the bin occupancy of this histogram, SHs was calculated as

\[
SH = \sum_{b=1}^{N} Pb \times \log(Pb)
\]

where SHmax is maximum SH, P is actual histogram bin probability, and b is the bin number, and N is the number of histogram bins. Median values from successive moving windows over the entire R-I interval time series were used as a measure of R-I interval “randomness.” In theory, during perfect coupling, all R-I intervals will fall into a single histogram bin and give SHs = 0; in the absence of coupling, however, R-I intervals will occupy all histogram bins in equal proportions and give SHs = 1. We adopted a statistical threshold for significant CVC at threshold SH (SH1) = 0.93, as determined previously in human studies (51).

**Pattern of RSA**

A phase domain approach was applied to characterize the way in which the R-R interval varied throughout the respiratory cycle, i.e., the “pattern” of RSA. The algorithm used in this study is conceptually similar to previously described methods of RSA pattern extraction (8, 10, 19, 28). First, the preceding R-R interval times were plotted as a function of the respiratory phase. Then cubic spline interpolation of the R-R intervals in each respiratory period into n = 100 data points gave the individual RSA pattern waveform for each breath as a function of respiratory phase. This procedure was repeated for each of the m respiratory cycle. To account for long-term trends in HR throughout the experiment, the instantaneous R-R interval was normalized to the mean R-R interval over a given two-breath window (10). The spline-interpolated curves from all respiratory cycles were then superimposed.

The average RSA pattern was calculated from 80% of the respiratory cycles after removal of 20% of outliers. This was achieved by calculating an initial average RSA pattern (\(\bar{x}^0\)) and the phase of the RSA minima (\(\phi_{min}^{RSA}\)) of \(\bar{x}^0\), where \(\bar{x}^0 = (1/m)\sum_{j=1}^{m} x_j\), (\(x_j\) represents a sample of the \(j\)th interpolated point of the \(j\)th respiratory cycle, \(i = 1 \ldots n\), and \(j = 1 \ldots m\)). For each respiratory cycle, the variance of each individual cycle was compared with \(\bar{x}^0\), defined as \(V_j = \sum_{i=1}^{n} (x_{ij} - \bar{x})^2\), and phase difference \(\Delta \phi_j = \phi_0 - \phi_j\). All m values of \(V_j\) and \(\phi_{min}^{RSA}\) were then ranked, and a serial selection, which included 0.9m of the smallest values of \(V_j\) followed by 0.8m of the smallest values of \(\phi_{min}^{RSA}\), was performed. We chose to filter 20% of outliers, because previous systematic comparisons showed that inclusion of outliers (i.e., analyzing 100% of respiratory cycles) results in distortions of the RSA pattern curves due to nonstationary behavior, including HR arrhythmia, very long breaths, saliva swallowing, and breathing interruptions. In contrast, the filtered subset retains the individual RSA pattern curves that are more similar to the average RSA pattern in terms of variance and \(\phi_{min}^{RSA}\) (19). The filtered data were used to generate the final average RSA pattern for subsequent statistical analysis. Figure 1 shows a representative RSA curve before and after filtering, as well as the final average RSA pattern.

**Quantification of RSA**

Two measures were used to quantify the amplitude of RSA: a spectral-based estimate was used for consistency with previous studies (23), and a phase-domain estimate of RSA amplitude was obtained by calculation of the difference between the maximum and minimum values on the RSA pattern waveform.
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Fig. 1. Schematic representation of superimposed cubic spline-interpolated respiratory sinus arrhythmia (RSA) curves for all respiratory cycles in 1 spontaneously breathing subject (top). Filtered subset (middle) includes all RSA curves that were similar to the average RSA pattern in terms of variance and phase of RSA minima (φ_{RSA}). Final average RSA pattern (bottom) was derived from filtered subset. φ min (double-headed arrow) corresponds to the phase of the R wave that terminates the shortest R-R interval in the respiratory cycle. Difference between maximum and minimum values was taken as an estimate of RSA amplitude.

Modeling of RSA

We modeled the RSA pattern as a function of preceding R-R interval times to generate the R-wave time series. This enabled us to examine RSA on heartbeat distribution throughout the respiratory cycle. This model does not incorporate feedback processes that govern cardiorespiratory interactions, such as CVC (17, 51).

With the assumption that fluctuations in phasic vagal activity vary as a function of respiratory phase, the RSA pattern was modeled using the wave function

$$f(\phi) = \alpha \cdot \cos \phi + c$$

where φ is phase of respiration, α determines the amplitude of RSA, and c is a constant that was adjusted to maintain mean R-R interval at ~0.92–1.0 s. This wave function was chosen, because, for the majority of subjects, RSA pattern approximates a sinusoidal waveform (Fig. 1).

Respiratory time series consist of inspiratory onset times (I) and expiratory onset times (E). The subscript j denotes the jth respiratory cycle, where j = 1...m. Two different respiratory datasets were used for analysis.

**Dataset 1.** This I time series consists of equidistant points with added variability given by

$$I_{j+1} = I_j + II + \sigma \xi$$

where II is the predetermined mean respiratory period and ξ is the added Gaussian noise pattern of unit variance. Expiratory times were determined by taking the product of the II interval and a predetermined inspiratory period (IE/II)-to-respiratory period ratio (IE/II), as given by

$$E_j = (IE/II) \cdot (I_{j+1} - I_j) + I_j$$

This simplified respiratory time series preserves the overall distribution of respiratory periods but does not take into account breath-to-breath variability in IE/II or the effects of CVC inherent in physiological data.

**Dataset 2.** Dataset 2 consists of I and E times from experimentally recorded data. Therefore, this dataset preserves the breath-to-breath changes in IE/II during control, hypercapnia, and hypoxemia.

The function $f(\phi_j)$ represents the preceding R-R interval at any given phase in the respiratory cycle. Therefore, the time-based equivalent is given by the product of $f(\phi_j)$ and the respiratory period

$$f(i) = f(\phi_j) \cdot (I_{j+1} - I_j)$$

Because $f(i)$ defines the preceding R-R intervals at time $t$ for any given jth breath, this can be alternatively expressed as a function of R-wave times

$$R_{i+1} - R_i = f(R_{i+1})$$

where the subscript $i$ denotes the $i$th heartbeat. Rearranging Eq. 9 allows the generation of the R-wave time series, since

$$R_{i+1} = f(R_{i+1}) + R_i$$

where $i = 1...n$ and $n$ is the total number of generated heartbeats. The model operation is illustrated in Fig. 2.

![Fig. 2. Model-simulated R-R interval time series showing high-frequency (HF) oscillations of varying RSA amplitudes ($\alpha$).](image-url)
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Statistical Analysis

Values are means (SD) and are given to two significant figures. The effects of hypercapnia and hypoxemia were evaluated with a repeated-measures ANOVA. The effects of \( f \), VT, and HR on RSA amplitude were further assessed with an analysis of covariance, whereby comparisons between conditions (control, hypercapnia, and hypoxemia) were adjusted for changes in \( f \), VT, and HR. Differences in HR, HR variability, RSA amplitude, and respiratory variables between hypercapnia and hypoxemia against control levels were determined using Student’s paired \( t \)-test. Because \( SH_a \) values are nonparametric, differences in \( SH_a \), between hypercapnia and hypoxemia vs. control were examined using Wilcoxon’s signed rank test, where nonparametric analysis of variance with the Kruskal-Wallis test was significant. Significant differences were accepted at \( P < 0.05 \). Statistical analysis was performed using StatView 5 (SAS Institute, Cary, NC).

RESULTS

Response of HR, f, VT, and Ve to Hypercapnia and Hypoxemia

Responses of HR, \( f \), VT, and Ve during hypercapnia and hypoxemia are given in Table 1. Hypercapnia was associated with significantly increased HR, \( f \), and Ve, but \( f \) was not significantly altered. Mild hypoxemia was associated with significantly increased HR, but not \( f \), Ve, or \( f \).

Effect of Hypercapnia and Hypoxemia on CVC

During control breathing, 6 of 12 subjects achieved \( SH_a \) values that were below \( SH_T \), indicating statistically significant CVC. Similar proportions of subjects demonstrated statistically significant CVC during hypercapnia (6 of 12) and hypoxemia (5 of 12). There were no significant differences in \( SH_a \) values between the different conditions, indicating that, on average, there were no differences in CVC (Table 2).

Effect of Hypercapnia and Hypoxemia on Breathing Pattern, RSA Amplitude and Pattern, and Heartbeat Distribution Throughout the Respiratory Cycle

The effects of hypercapnia and hypoxemia on RSA are summarized in Table 2. Hypercapnia was associated with a significant lengthening of the inspiratory phase of the respiratory cycle (Fig. 3).

Spectral and phase domain estimates of RSA amplitude significantly increased during hypercapnia (Table 2). Analysis of covariance showed that the increase in RSA amplitude remained statistically significant after adjustment for changes in \( f \), VT, and HR. \( LF_{amp}/HF_{amp} \) significantly decreased during hypercapnia, but \( LF_{amp} \) was unchanged. In contrast, there was no significant change in \( IE/II \), RSA amplitude, \( LF_{amp} \), or \( LF_{amp}/HF_{amp} \) with mild hypoxemia.

In all subjects, the inspiratory phase was consistently associated with cardioacceleration (shortening of the R-R interval), and \( \Phi_{min} \) occurred in the early phase of expiration, after \( E \). The average phase difference between \( E \) and \( \Phi_{min} \) was 0.057 ± 0.071 in the control condition and did not change significantly during hypercapnia (0.063 ± 0.060) or hypoxemia (0.053 ± 0.057). However, across individuals, there were significant variations in RSA pattern morphology. In addition, the shape of the RSA pattern changed somewhat from control to hypercapnia, although these changes related primarily to increases in RSA amplitude. \( \Phi_{min} \) was significantly shifted to the right during hypercapnia (Table 2). In contrast, comparison of the RSA pattern during control and hypoxemia shows virtually identical curves (Fig. 3).

Table 4 shows the histograms of R-wave occurrence as a function of respiratory phase for the same examples of Fig. 3 during control, hypercapnia, and hypoxemia. Subject A showed clear clustering of heartbeats throughout the respiratory cycle during hypercapnia, but not during control or hypoxemia. This was consistent with preferential placement of heartbeats within the inspiratory portion of the respiratory cycle during hypercapnia. Preferential inspiratory phase clustering of heartbeats was not observed in subjects B and C, although they exhibited smaller RSA than subject A (Fig. 3).

The percentage of heartbeats during the inspiratory phase of the respiratory cycle (%HB_{insp}) was determined as an average for all subjects. During control breathing, 40 ± 2.3% of R waves occurred during the inspiratory phase, similar to %HB_{insp} during hypoxemia (41 ± 8.0%), but increased significantly to 45 ± 1.7% during hypercapnia. The independent effects of hypercapnia-induced changes in \( IE/II \) and hypercapnia-induced changes in RSA amplitude on %HB_{insp} were determined by applying mean \( IE/II \) during hypercapnia to the control R-wave times and then the hypercapnia R-wave times and calculating the percent gains in the number of inspiratory heartbeats. This process was repeated for all subjects, and the average values are summarized in Fig. 5, which shows the

Table 1. Effect of hypercapnia and hypoxemia on baseline cardiorespiratory variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Hypoxemia</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>61 (8.5)</td>
<td>68 (11)*</td>
<td>69 (10)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>( f ), breaths/min</td>
<td>13 (2.7)</td>
<td>14 (3.9)</td>
<td>12 (3.0)</td>
<td>NS</td>
</tr>
<tr>
<td>VT, ml</td>
<td>0.59 (0.17)</td>
<td>1.3 (0.52)*</td>
<td>0.65 (0.23)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ve, l/min</td>
<td>7.9 (2.9)</td>
<td>20 (7.8)*</td>
<td>8.1 (4.5)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means (SD). HR = heart rate; \( f \) = respiratory rate; VT = tidal volume; VE = minute ventilation; NS = not significant. Comparisons between control, hypercapnia, and hypoxemia were made using repeated-measures ANOVA.

\* \( P < 0.05 \) vs. control.
Model simulations were performed with respiratory datasets obtained using two different methodologies (see METHODS). Results obtained with dataset 1 are presented in Figs. 6–8. Figure 9 shows results obtained using dataset 2.

Figure 6 shows computer-generated heartbeat distribution histograms obtained with the model RSA pattern and illustrates the relation between RSA amplitude and heartbeat distribution as a function of the respiratory cycle. Simulations with relatively low levels of RSA (Fig. 6A), which correspond to RSA amplitudes observed during control and hypoxemia (Table 2), were associated with near-uniform heartbeat distribution. At higher levels of RSA (Fig. 6B), which correspond to average RSA amplitudes during hypercapnia, minor clustering of heartbeats was observed in the distribution histogram. Major clustering of heartbeats was observed only with very high levels of RSA (Fig. 6C), which were observed in only two subjects during hypercapnia.

To further illustrate the impact of increasing RSA amplitude on %HB\textsubscript{imp}, the difference in %HB\textsubscript{imp} (\(\Delta\%HB\textsubscript{imp}\)) between no RSA (\(\alpha = 0\)) and a high level of RSA (\(\alpha = 0.5\)) was determined as a function of IE/II (Fig. 7B). Figure 7B shows that \(\Delta\%HB\textsubscript{imp}\) values are positive for IE/II > 0.35; i.e., an increase in RSA amplitude is associated with an increase in %HB\textsubscript{imp}. However, for IE/II < 0.35, \(\Delta\%HB\textsubscript{imp}\) values are negative, consistent with a reduction in %HB\textsubscript{imp} with increasing RSA amplitude. Figure 7B also shows that the impact on %HB\textsubscript{imp} of a 0.5-unit increase in \(\alpha\) is maximal at IE/II = 0.6.
In contrast to the relation between %HB$_{insp}$ and RSA amplitude, we observed a strongly positive relation between %HB$_{insp}$ and IE/II, regardless of RSA amplitude (Fig. 8A).

Figure 8A shows %HB$_{insp}$ as a function of IE/II in the absence of RSA (H9251/H11005) and in the presence of high-amplitude RSA (H9251/H11005.5). In the absence of RSA, the relation appears linear, such that changes in IE/II were associated with proportional changes in %HB$_{insp}$. However, as RSA amplitude increases, the relation becomes “sigmoidal,” with the steepest portion of the curve corresponding to IE/II/H11005 = 0.4 – 0.5 (Fig. 8B), which encompasses the mean IE/II observed experimentally during hypercapnia.

We also repeated the analysis shown in Fig. 5 using human respiratory data obtained during control breathing and hypercapnia (dataset 2), coupled to model-generated R-wave times (Fig. 9). This analysis, which examined the relative contributions of IE/II and RSA amplitude changes to %HB$_{insp}$, was achieved by setting parameter $\alpha$ to approximate the experimentally observed mean values of RSA amplitude (Table 2). Thus the model predicts that, during control breathing, %HB$_{insp}$ = 39 ± 2.0%. With no change in RSA amplitude, an increase in IE/II associated with hypercapnia would significantly increase %HB$_{insp}$ with only a relatively minor further contribution from changes in RSA amplitude.

**DISCUSSION**

The present study describes the effects of hypercapnia and mild hypoxemia on CVC and breathing pattern, as well
as the magnitude and pattern of RSA, in conscious spontaneously breathing human volunteers. The phenomenon of RSA-vagal dissociation was validated and the distribution of heartbeats throughout the respiratory cycle was examined in relation to the hypothesis of RSA function of Hayano and Yasuma (24).

Hypercapnia was associated with significant increases in $V_T$, $V_e$, and RSA amplitude that were statistically significant after adjustment for $V_T$, $f$, and HR as covariates. As expected, the increase in RSA amplitude was associated with a significant decrease in $LF_{amp}/HF_{amp}$, which suggests, according to traditional views of spectral power distribution, a shift in autonomic balance toward a state of vagal dominance. However, rather than resulting in a bradycardia, this shift was associated with a significant tachycardia, which is consistent with a dissociation of RSA from vagal tone.

One potential explanation is the theory that the phasic modulation of vagal activity may occur independently of tonic vagal regulation (21, 26, 52, 54). According to this proposal, the augmentation of RSA during hypercapnia results from changes in respiratory drive and ventilatory mechanics, as well as direct CO$_2$ effects on vagal preganglionic neurons. Normally, during inspiration, vagal output is diminished by 1) suppression of cardiovagal motor neurons by medullary inspiratory neurons and 2) gating of vagal-excitatory inputs by slow adaptation of pulmonary stretch afferents. During hypercapnia, suppression of cardiovagal motor neurons by medullary inspiratory neurons will be greater because of increased

![Image](http://ajpheart.physiology.org/)

Fig. 6. RSA patterns and histograms of heartbeat distribution as a function of respiratory phase for different amplitudes of RSA generated from the model. RSA in A ($\alpha = 0.050$) is comparable to RSA amplitudes observed experimentally during control and hypoxemia. RSA in B ($\alpha = 0.11$) is comparable to mean RSA amplitudes during hypercapnia. RSA in C ($\alpha = 0.42$) simulates high-magnitude RSA, which was observed in only 2 subjects during hypercapnia, one of which is shown in Fig. 3A. A prominent peak in the corresponding heartbeat distribution histogram is shown in C. No clear histogram peaks are shown in A and B.
respiratory drive (20, 29, 47). The feedback from pulmonary stretch afferents will also be greater because of the rise in VT (2, 3, 12). These effects, combined with CO2-mediated stimulation of vagal preganglionic neurons and the relative absence of central gating of vagal excitatory inputs during expiration, may account for the increase in RSA amplitude (9, 38).

However, although results of this study support the dissociation of RSA from vagal tone during hypercapnia, there are significant differences from previous investigations. In spontaneously breathing dogs and human volunteers under volume- and rate-controlled breathing, no significant changes in HR were observed during hypercapnia (42, 54). In contrast, the present study showed that hypercapnia was associated with a significant increase in HR, which may relate to increased feedback stimulation of the heart by pulmonary stretch afferents secondary to changes in VT, alterations of sinoatrial node function by CO2, or a mechanical effect associated with hyperventilation (46). Although LFamp did not increase, it has been suggested that LF oscillations in HR reflect baroreflex sensitivity, rather than sympathetic tone (37). Therefore, our results do not exclude the possibility that the tachycardia observed during hypercapnia may be mediated, at least partially, by sympathetic excitation (44). If this is the case, our observations not only support RSA-vagal dissociation, but they would also challenge the view that RSA is restrained by sympathetic activity (48).

The present study is the first to examine RSA-vagal dissociation in conscious spontaneously breathing humans during mild hypoxemia. In contrast to previous canine studies that showed attenuation of RSA with hypoxemia (at 78% saturation) (54), no significant changes in RSA amplitude or LFamp/ HFamp were observed. HR showed a significant increase with hypoxemia, but no significant changes in VT, Ve, f or f were observed. Taken together, these results suggest that RSA is not augmented during hypoxemia in spontaneously breathing humans, and one possible explanation is the restraint of RSA by increased catecholamine levels, although this was not directly measured.

The respiratory response to hypoxemia in humans is small compared with the response to hypercapnia (11, 31, 41, 43, 49). This probably accounts for the absence of significant changes in VT, Ve, f and f in the present study. Although the possible outcomes of more profound levels of hypoxemia are unclear, it has previously been shown that a saturation level of 90% with use of the same anesthetic circuit corresponds to ~60 mmHg arterial PO2, which was considered to be the ethical limit of this study (7).

The present study shows that, in addition to the effect on RSA amplitude, hypercapnia and hypoxemia had differential effects on breathing and RSA pattern. When compared with control breathing, %HBinsp increased from 40 ± 2.3% to 45 ± 1.7% during hypercapnia, whereas no significant change was observed during hypoxemia (41 ± 8.0%). Phase characterization analysis showed that $\Delta_{\min}$ during hypercapnia was shifted significantly to the right, whereas the RSA pattern during hypoxemia remained essentially unchanged. During hypercapnia, this had the effect of maintaining $\Delta_{\min}$ in early expiration, despite the relative prolongation of inspiration.

According to Giardino et al. (18), Hayano and Yasuma (24), and Sasano et al. (42), RSA-vagal dissociation reflects the potential role of RSA as an intrinsic resting function of the cardiopulmonary system. This optimizes the efficiency of pulmonary gas exchange by matching cardiovascular and respira-
tory rhythms, such that more heartbeats occur during inspiration when alveolar ventilation is maximal and fewer heartbeats occur during expiration when alveolar ventilation is minimal. Two observations in the present study appear to limit this theory.

First, there was no augmentation of RSA during hypoxemia. In anesthetized dogs, simulation of artificial RSA by cervical vagus nerve stimulation during expiration was associated with a 10% reduction in the ratio of physiological dead space to VT, a 51% reduction in the fraction of intrapulmonary shunt, and a 4% increase in O2 uptake compared with animals with no RSA. Simulation of inverse RSA by vagus nerve stimulation during inspiration was associated with a 14% increase in the physiological dead space-to-VT ratio, a 64% increase in the fraction of intrapulmonary shunt, and 14% reduction in O2 uptake (25). Moreover, the absence of any significant changes in end-tidal, arterial, and mixed venous PCO2 between the three conditions appears to favor a role of RSA in improving O2 exchange, rather than CO2 elimination (52). However, this is not supported by our results, which showed marked RSA augmentation, presumably via stimulation of the phasic vagal controller during hypercapnia, but not during hypoxemia.

Second, it may be too simplistic to assume that temporal variations in HR necessarily account for putative improvements in ventilation-perfusion matching (18, 24, 25), because no consistent clustering of heartbeats was observed during control breathing or during hypoxemia. Although heartbeat clustering was observed during hypercapnia, it was clearly apparent in only 2 of the 10 subjects. Furthermore, although hypercapnia was associated with a significant rise in %HBinsp, we show that this is primarily due to an increase in IE/II, rather than the increase in RSA amplitude. In part, this is because ΔRSA occurs after expiratory onset during the expiratory phase, which suggests that the impact of an increase in RSA amplitude is greatest during early expiration, not inspiration, as is commonly assumed.

These experimental observations are further supported by model results, which indicate that although RSA can theoretically cause clustering of heartbeats in inspiration, this occurs only with very high RSA amplitudes, which are rarely observed experimentally. Moreover, Fig. 7 shows that, even with high-amplitude RSA, Δ%HBinsp is positive only with IE/II >0.35. This implies that, with IE/II values observed experimentally during control and hypoxemia, isolated changes in RSA amplitude may not necessarily result in significant gains in %HBinsp. In contrast, Figs. 8 and 9 show that changes in IE/II, which were commonly observed in association with hypercapnia, are accompanied by greater gains in %HBinsp. These results reinforce the experimental observation that increases in %HBinsp associated with hypercapnia result primarily from changes in IE/II, not RSA amplitude per se. More importantly, these results indicate that although RSA-vagal dissociation may potentially reflect an inherent physiological function of RSA, this hypothesis requires further experimental validation.

An important strength of the present study was the decision to conduct the experiments during spontaneous breathing, without fixed-paced or fixed-volume breathing. Although control of ventilation variables is helpful in separating the independent effects of VT and f on RSA amplitude and, therefore, isolating the effects of CO2 exposure, this is not without problems. Paced breathing has been associated with significant changes in cardiovascular and respiratory response to physiological perturbation (42, 46), which may complicate interpretation of data. This is apparent in the present study, where hypercapnia was associated with tachycardia, whereas previous studies carried out during paced breathing showed a bradycardic response (42).

Altering the state of the central respiratory pattern generator during hypercapnia and mild hypoxemia was not associated with any significant changes in SH. This indicates that CVC may be relatively insensitive to the state of the central respiratory pattern generator and that changes in RSA-vagal dissociation are independent of changes in CVC strength. In addition, it has been observed that, during periods of strong CVC, heartbeats occurred at positions in the respiratory cycle where they were maximally affected by RSA. This led to the hypothesis that CVC may contribute to the optimization of RSA (17). However, our results indicate that the increase in RSA amplitude during hypercapnia was unrelated to CVC strength.

This study should be considered in the context of the following experimental limitations and model assumptions. 1) Blood gas values of arterial PCO2, PO2, and pH were not measured, which limits our ability to determine the extent to which central and peripheral chemoreceptors were stimulated during hyperoxemia and hypercapnia. Thus, although the experimental data showed no evidence of RSA-vagal dissociation with ~90% O2 saturation, this may be due to weak stimulation of chemoreceptors. Similarly, blood gas values of CO2 and pH would provide more insight into the degree of peripheral and central chemostimulation associated with the observed changes in RSA and HR during hypercapnia. The effects of more profound levels of hypoxemia and the relative contribution of central and peripheral chemostimulation on RSA-vagal dissociation remain to be determined. 2) The modeling strategy assumes that the RSA pattern is sinusoidal. Results from this study and others indicate that, in some circumstances, the RSA pattern may deviate from the sinusoid waveform (5, 8, 19, 34, 50, 55). The relation between the morphology of RSA pattern and distribution on heartbeats was not explored in detail. 3) The model of RSA used in this study was simplistic and does not incorporate aspects of cardiorespiratory interactions that influence heartbeat timing. For example, it is known that CVC leads to preferential alignment of heartbeats throughout the respiratory cycle and is a major determinant of breath-to-breath respiratory period variability (17, 35). In addition, CVC and RSA may contribute to the synchronization of cardiorespiratory rhythm and, therefore, directly impact heartbeat distribution throughout the respiratory cycle (4, 17, 30, 39). The present RSA model does not incorporate CVC effects; therefore, conclusions of this study extend specifically only to the role of RSA on heartbeat distribution. 4) This study shares the common assumption of a physiological advantage from more heartbeats during inspiration (18, 24, 42).

However, the critical phases within the respiratory cycle associated with optimal pulmonary gas exchange in relation to heartbeat distribution may encompass, for example, only the latter portions of inspiration and/or the early portions of expiration. These issues extend beyond the scope of this study but require further investigation.

In conclusion, this study has examined the effects of hypercapnia and mild hypoxemia on CVC, RSA-vagal dissociation,
and the distribution of heartbeats throughout the respiratory cycle in spontaneously breathing humans. We found that RSA amplitude was increased during hypercapnia, but not during hypoxemia, independent of associated changes in f, VT, and HR, which is consistent with RSA-vagal dissociation observed in the spontaneously breathing dog and in humans during paced respiration (24, 42). However, although hypercapnia was associated with a significant increase in %Hb_{\text{insp}}, this was primarily due to the increase in IE/II, rather than the increase in RSA amplitude. Therefore, the hypothesis that RSA optimizes pulmonary gas exchange requires further study.

REFERENCES


