Contribution of the respiratory rhythm to sinus arrhythmia in normal unanesthetized subjects during positive-pressure mechanical hyperventilation

H. E. Cooper, T. H. Clutton-Brock, and M. J. Parkes. Contribution of the respiratory rhythm to sinus arrhythmia in normal unanesthetized subjects during positive-pressure mechanical hyperventilation. Am J Physiol Heart Circ Physiol 286: H402–H411, 2004. First published September 4, 2003; 10.1152/ajpheart.00504.2003.—The precise contribution of the CO₂-dependent respiratory rhythm to sinus arrhythmia in eupnea is unclear. The respiratory rhythm and sinus arrhythmia were measured in 12 normal, unanesthetized subjects in normocapnia and hypocapnia during mechanical hyperventilation with positive pressure. In normocapnia (41 ± 1 mmHg), the respiratory rhythm was always detectable from airway pressure and inspiratory electromyogram activity. The amplitude of sinus arrhythmia (138 ± 21 ms) during mechanical hyperventilation with positive pressure was not significantly different from that in eupnea. During the same mechanical hyperventilation pattern but in hypocapnia (24 ± 1 mmHg), the respiratory rhythm was undetectable and the amplitude of sinus arrhythmia was significantly reduced (to 40 ± 5 ms). These results show a greater contribution to sinus arrhythmia from the respiratory rhythm during hyperventilation caused by mechanical hyperventilation than previously indicated in normal subjects during hypocapnia caused by voluntary hyperventilation. We discuss whether the respiratory rhythm provides the principal contribution to sinus arrhythmia in eupnea.

Previous studies of mechanical hyperventilation in normal, awake subjects have been hindered by the fact that mechanical hyperventilation itself inhibits the respiratory rhythm in normocapnia (25, 35, 40, 43, 46, 65). Thus the respiratory rhythm apparently cannot be contributing to sinus arrhythmia during mechanical hyperventilation, although the fact that there is less sinus arrhythmia here than in eupnea (25, 35, 65) supports the idea that the respiratory rhythm contributes in eupnea.

We demonstrate a regimen of positive-pressure mechanical hyperventilation in normal unanesthetized subjects with the novel feature that the respiratory rhythm is detectable in normocapnia. Our aim therefore was to measure the contribution of the respiratory rhythm to sinus arrhythmia when normal unanesthetized subjects are not required to breathe voluntarily.

We deliberately do not describe sinus arrhythmia here as “respiratory” because of the potential confusion between mechanisms related to the respiratory rhythm or to chest inflation and its mechanical sequelae.

METHODS

The experiments conformed to the guiding principles (4) specified in the Declaration of Helsinki, were approved by the South Birmingham Health Authority Local Ethics Committee, and were carried out with informed consent of the subjects. Twelve normal, healthy subjects (aged 21–41 yr) who were not on any medication were instrumented when semirecumbent for noninvasive recording of blood pressure from a finger photoplethysmograph (Finapres 2300, Ohmeda, Englewood, CO). This technique records intra-arterial pressure patterns faithfully (33), and although there may be a small absolute difference with intra-arterial recordings (33, 51), this is acceptable, inasmuch as our analysis is primarily concerned with differences within subjects. ECG was recorded at a sampling rate of 1,600 Hz from chest electrodes with lead configuration II and electromyogram (EMG) activity from surface electrodes attached to the skin over the diaphragm and intercostal spaces (20, 39, 40, 43, 65, 72). Skin abrasion was performed before electrode attachment.

Control measurements during “eupnea.” The 12 subjects first rested for 15 min and listened to the radio through headphones. Their eupneic end-tidal PCO₂ (PETCO₂) was measured by expiration through an in-line capnograph (model 78354 A, Hewlett-Packard). PETCO₂ is an accepted measure of arterial PCO₂ (18) and would not underestimate it by >2 mmHg (56) over the inflation frequency range used in this study. The subjects then put on noseclips and breathed air through a three-way mouthpiece and flowmeter (BDRL Flowmeters, Birmingham, UK) for 20 min. Eupneic tidal volume and frequency were calculated for each subject from integrated inspiratory airflow. To maintain comparability with our hypocapnia...
experiments described below, only the last 2 min of spontaneous breathing were analyzed.

As further control experiments, two additional eupnea measurements were carried out with subjects connected for 20 min to the ventilator (Engstrom Erica) via a face mask and breathing 100% O2. Subjects were accustomed to mechanical ventilation in previous sessions. PETCO2 levels and airway pressure were measured in the face mask. During mechanical ventilation, the respiratory rhythm was detected noninvasively as described previously (20, 39, 40, 43, 65, 72) from the irregular rise and shape of the positive airway pressure waves (sometimes preceded by an initial negative deflection) and from the presence of rhythmic inspiratory muscle EMG activity.

First, the ventilator was set to “synchronized” mode (i.e., the negative pressure of each eupneic inspiration triggered the ventilator to open its gas valves to make O2 available). Sufficient inspiratory assist was applied only to overcome the resistance of the ventilator’s breathing circuit. Although positive end-expiratory pressure was set to zero, our pressure transducer indicated that a small amount of positive end-expiratory pressure (<1 cmH2O) remained in the system. To maintain comparability with our hypocapnia experiments described below, only data from the last 2 min were analyzed.

Second, the “synchronized intermittent mandatory (eupneic) ventilation” mode was used to apply pulmonary inflation with positive end-expiratory pressure to each subject’s mean eupneic frequency and volume. Nine subjects were mechanically ventilated in this manner for 20 min. During this mechanical ventilation at the eupneic frequency and volume, subjects allowed the ventilator to perform some of the work of breathing, and their metabolic rates fell, as shown by the need to add CO2 to the inspired gas to maintain PETCO2 at eupneic levels. (Because of the need to add CO2, our eupneic mechanical ventilation should strictly be termed “hyperventilation.”) It would, however, cause unnecessary confusion to refer to this as “hyperventilation at eupneic frequency and volume,” so, for simplicity, we continue to call this ventilation and use the term hyperventilation only for a frequency at 16 breaths/min with an inflation volume >1 l/min.) Only data from the last 2 min were analyzed.

Hyperventilation. Nine subjects voluntarily hyperventilated in air through the three-way mouthpiece in time to a metronome at a frequency of 16 breaths/min (0.27 Hz) for 20 min. Subjects kept their tidal volume at the same level used for their mechanical hyperventilation via visual feedback of integrated airflow from an oscilloscope. CO2 was added to the inspired gas to maintain PETCO2 at their eupneic level. Only the last 2 min were analyzed.

Twelve subjects were mechanically hyperventilated with 100% O2 under positive pressure in “synchronized intermittent mandatory hyperventilation” mode with a frequency of 16 breaths/min (0.27 Hz), with inflation volume increased sufficiently to lower PETCO2 to 24 mmHg. Subjects found this the most comfortable regime for mechanical hyperventilation. Normocapnia was achieved at the same inflation frequency and volume by addition of CO2 to the inspired gas. Subjects could not tolerate a rise of Pco2 above their eupneic level during positive-pressure mechanical hyperventilation in this mode. Because up to 18 min could be required to stabilize PETCO2 at the required hypocapnia level, all mechanical hyperventilation was applied for 20 min, and only the last 2 min were analyzed. Subjects were not told whether they were being hyperventilated in normocapnia or hypocapnia, and the order of the experiments was randomized. On completion of the experiments, subjects were asked whether they had been given normocapnia or hypocapnia, and none could reliably distinguish them.

Once the required period of mechanical hyperventilation was completed, we carefully observed the subjects to find a time when they appeared completely relaxed, unaware of events around them, and had not moved within the preceding 2 min. At this point, the ventilator was surreptitiously switched from mechanical hyperventilation (synchronized intermittent mandatory hyperventilation mode) to permit eupneic inspiration triggering access to ventilator gases (synchronized mode). Subjects could not see the ventilator controls, nor could they hear the switching, and the face mask was not removed. We measured the delay from the start of the last inflation to the start of the first spontaneous breath and the duration of every expiratory interval in the subsequent 1-min period.

Because of the novelty of mechanical ventilation, we expected subjects to remain awake during mechanical ventilation, so we did not confirm wakefulness by monitoring arousal from electroencephalographic activity or eye movement. Subjects also were not deprived of sleep before experimentation. Subjects’ eyes were open or closed during mechanical ventilation. They were not obviously asleep throughout the experiment, although we cannot exclude the possibility that when their eyes were closed, the subjects might sometimes have drifted into and out of light sleep.

Numerical analysis. Data were recorded onto a computer using the CED Spike2 data acquisition program. The systolic peaks were extracted from the blood pressure recording and converted to a continuous waveform. ECG was converted from R-R interval to a continuous line of instantaneous heart period.

Sinus arrhythmia in each subject was measured as the difference between the peak and the trough of the heart period waveform for all voluntary or ventilator inflation cycles in each 2-min recording period. [We have not reported absolute peak and trough values separately, because, without use of atropine and β-blockade (38), we cannot equate trough interval with inhibition of cardiac vagal motorneurons in inspiration.] This waveform was also sampled every 2.5% of the inflation cycle to measure the timing of the heart period trough relative to the start of inflation. The fluctuations in systolic blood pressure related to the inflation cycle were analyzed similarly. Sinus arrhythmia was additionally measured by spectral analysis of the heart period waveform, as we described previously (15). Mean blood pressure and heart period were also averaged for every heartbeat in these 2-min periods. Values are means ± SE.

Statistical analysis of parametric data was performed using one-way repeated-measures ANOVA by SPSS general linear modeling with application of the Huynh-Feldt correction for nonsphericity. To make use of all available data, separate ANOVAs were performed in the group of 9 subjects in all 6 conditions and in the group of 12 subjects in 4 conditions.

In analysis within nine subjects, significant F values within subjects in all six conditions were found for PETCO2 (F = 111, P < 0.001), sinus arrhythmia (F = 6.8, P < 0.001), and mean heart period (F = 3.2, P < 0.05) measurements (but not for mean blood pressure and timing measurements) of the mechanical ventilation. For the five measurements (tidal volume, frequency, minute ventilation, systolic pressure fluctuation, and its trough timing) made only in the three “control” conditions [eupnea, eupneic inspiration triggering access to ventilator gases, and synchronized (eupneic) ventilation], there were no significant F values for measurements within subjects.

In analysis within 12 subjects in the 4 conditions (eupnea, synchronized ventilation, and mechanical hyperventilation in normocapnia and hypocapnia), significant F values within subjects were found for PETCO2 (F = 122, P < 0.001), sinus arrhythmia (F = 14, P < 0.001), and mean blood pressure (F = 5.2, P < 0.01) measurements (but not for mean heart period and timing of the sinus arrhythmia trough). In the cases of mean blood pressure and heart period in the 9- or 12-subject groups, where the F value in certain conditions was significant in one group but not in another, we always considered the more valid probability level to be that from the larger group.

For each condition, the sources of this significance for five comparisons (the 4 normocapnia conditions vs eupnea and the hypocapnia vs normocapnia experiment) were then investigated using Student’s paired t-tests. Correction for multiple comparisons is not applicable, because strictly we were not considering a repetitive situation and testing whether the universal null hypothesis is true (52).
Student’s paired t-tests were also used to compare the breathing delays and longest expiratory intervals when the ventilator was switched from normocapnia or hypocapnia (ANOVA is not applicable for these because there are only 2 comparisons, or a comparison with only 3:75).

Statistical analysis for the nonparametric data power spectrum data was performed using one-way repeated-measures ANOVA with the Kruskal-Wallis test. In analysis of 12 subjects in all 6 conditions, a significant H value (19.7; P < 0.005) was found for power 0.03–0.5 Hz density within the appropriate inflation frequency range, and the sources of significance for the same five comparisons were then investigated by analyzing matched pair differences using Wilcoxon signed-rank test.

RESULTS

Sinus arrhythmia and respiratory rhythm during eupnea. Figure 1A shows substantial sinus arrhythmia (138 ± 21 ms, n = 12) all 12 subjects during eupnea (i.e., during normal chest inflation by negative pressure). The timing of the sinus arrhythmia trough within the inflation cycle was variable between subjects, because subjects breathed at different frequencies and because, in each subject, neither their eupneic frequency nor their inflation volume was necessarily regular. Their mean PETCO2 was 41 ± 1 mmHg. Averaged mean blood pressure was 86 ± 2 mmHg (Table 1), but a better measure of baroreceptor input is obtained from fluctuations in systolic pressure over the inflation cycle. Figure 2A shows fluctuations in systolic pressure with inflation in all subjects. Mean heart period was 1,014 ± 52 ms (Table 1). Figure 3A shows the respiratory rhythm and sinus arrhythmia in subject 1 during eupnea. Subjects breathed with a mean tidal volume of 612 ± 59 ml, a mean frequency of 11.9 ± 1.2 breaths/min (0.2 ± 0 Hz), and a mean minute ventilation of 6.8 ± 0.1 l/min.

Sinus arrhythmia was not significantly different (Fig. 1B) in the 12 subjects when they were connected to the ventilator set on synchronized mode, i.e., when the negative pressure of each breath was used to trigger access to ventilator gases (mean tidal volume 650 ± 50 ml and frequency 11.4 ± 0.8 breaths/min). There was less variability between subjects in the timing of the sinus arrhythmia trough within the inflation cycle (Fig. 1B), indicating that, under these conditions, subjects maintained more regular eupneic frequency and inflation volumes. There were no significant differences from eupnea in PETCO2 (41 ± 1 mmHg), mean blood pressure (Table 1), the size or timing of the fluctuations in systolic blood pressure within the inflation cycle (Fig. 2B), or mean heart period (Table 1). Figure 3B shows the respiratory rhythm in subject 1 during connection in synchronized mode.

Sinus arrhythmia was not significantly different from eupnea (Fig. 1C) in the nine subjects when they were mechanically ventilated with positive pressure [synchronized intermittent mandatory (eupneic) ventilation mode] at their eupneic frequency and volume. Here the timing of the sinus arrhythmia trough in the inflation cycle is relatively constant, because all subjects had a constant frequency and inflation volume. There were no significant differences from eupnea in mean PETCO2 (42 ± 1 mmHg), mean blood pressure (Table 1), or the size and timing of the fluctuation in systolic blood pressure with the inflation cycle (Fig. 2C). Mean heart period was significantly shorter (by 134 ms, Table 1) than in eupnea. The respiratory rhythm continued in all subjects during positive-pressure ventilation, although our measures of respiratory rhythm do not have sufficient sensitivity to determine whether it remains exactly the same as in eupnea. All subjects continued to inspire during mechanical inflation. Figure 3C shows this respiratory rhythm in subject 1.

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Voluntary hyperventilation in normocapnia. Sinus arrhythmia (Fig. 4A) was not significantly different from eupnea (Fig. 1A) in the nine subjects during normocapnic voluntary hyperventilation in air through the three-way mouthpiece at the frequency (16 breaths/min), inflation volume (1 ± 0 liter), and PETCO₂ (41 ± 1 mmHg) used for mechanical hyperventilation. Although voluntary hyperventilation changes respiratory pattern and drive, previous studies in which each was changed separately in normocapnia indicate that voluntarily increasing frequency reduces sinus arrhythmia, whereas voluntarily increasing inflation volume increases it (5, 21, 25, 28, 29, 31, 38, 49, 60, 65, 68). The obvious conclusion from such previous work is that voluntarily changing both together by the appropriate amounts could have no net effect on sinus arrhythmia. We now demonstrate that this is the case when frequency is voluntarily increased above eupneic levels by 22% and volume by 45%. It is important to establish that this hyperventilation pattern itself has no effect on sinus arrhythmia, because we use the same pattern for mechanical hyperventilation with positive pressure.

Although all subjects voluntarily hyperventilated at the same frequency and at a constant volume, the timing of the sinus arrhythmia trough (Fig. 4A) was slightly more variable than when all were mechanically hyperventilated (Fig. 4B) at the same frequency and volume (although it is still less variable than in eupnea; Fig. 1A). This slightly increased variability reflects how accurately and consistently each subject’s breathing followed the metronome and oscilloscope.

As frequency is voluntarily increased above eupneic levels, timing changes are seen (5, 21, 26, 31, 38, 58, 60, 65, 68). The lack of a significant effect of our small voluntary increase in frequency on the timing of the heart period trough (Fig. 4A) within the ventilatory cycle compared with eupnea (Fig. 1A) may be due to its being masked by variance between and within subjects.

During voluntary hyperventilation, mean blood pressure was not significantly different from that in eupnea (Table 1), but mean heart period was significantly shorter (by 119 ms, Table 1); this is consistent with the greater discomfort and arousal during voluntarily hyperventilation to maintain breathing within the required parameters.

Mechanical hyperventilation in normocapnia. Sinus arrhythmia (Fig. 4B) was not significantly different from eupnea (Fig. 1A) when the 12 subjects were mechanically hyperventilated in normocapnia (41 ± 1 mmHg) with positive pressure (synchronized intermittent mandatory hyperventilation mode) at 16 breaths/min and 1 ± 0 liter. Similarly, there was no significant effect on mean heart period (Table 1), although averaged mean blood pressure was significantly higher by 10 mmHg (Table 1). The respiratory rhythm always continued during our positive-pressure hyperventilation. It was always detectable from airway pressure and EMG activity, but again our measures of respiratory rhythm are not sufficiently sensitive to determine whether it remains exactly the same as in eupnea. All subjects continued to inspire during mechanical inflation. Figure 5B shows this respiratory rhythm in subject 1.

When the ventilator was surreptitiously switched from mechanical hyperventilation (synchronized intermittent mandatory hyperventilation mode) in normocapnia to permit eupneic inspiration triggering access to ventilator gases (synchronized mode), the mean delay from the start of the last inflation to the start of first spontaneous inspiration was 4.8 ± 0.4 s (n = 12). This delay was significantly longer (P < 0.05, Student’s paired t-test) than expected if the subjects had been accurately following the ventilator in normocapnia, when the next expected inflation should have occurred at 3.75 ± 0.00 s. The mean longest expiratory interval over the subsequent 1-min period was 7 ± 1 s (n = 12).

Mechanical hyperventilation in hypocapnia. When the 12 subjects were mechanically hyperventilated with positive pressure at the same frequency and volume but without addition of CO₂, their mean PETCO₂ was 24 ± 1 mmHg (P < 0.001, ANOVA with Student’s paired t-test) and the respiratory rhythm was undetectable. Figure 5C shows its absence in subject 1. A reduction of their respiratory rhythm by hypocapnia is further indicated by the fact that when the ventilator was surreptitiously switched during hypocapnia to permit spontaneous breathing, the mean delay to the first breath (11.3 ± 2.5 s, n = 12) and the mean longest expiratory interval over the first minute of breathing (12 ± 2 s, n = 12) were significantly longer (P < 0.05, Student’s paired t-test) than those after the switch from normocapnia.

Table 1. Heart period, average mean blood pressure, and spectral power at 0.03–0.5 Hz in all experiments

<table>
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<tr>
<th></th>
<th>Eupnea (n=12)</th>
<th>Positive-Pressure MV at Eupneic Frequency and Volume in Normocapnia (n=9)</th>
<th>Voluntary Hyperventilation in Normocapnia (n=9)</th>
<th>Positive-Pressure Mechanical Hyperventilation in Normocapnia (n=12)</th>
<th>Hypocapnia (n=12)</th>
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<tr>
<td>Heart period, ms</td>
<td>1.01±0.52</td>
<td>977±39²</td>
<td>948±23³</td>
<td>962±10¹</td>
<td>964±25²</td>
</tr>
<tr>
<td>Avg mean blood pressure, mmHg</td>
<td>86±2.2</td>
<td>85±3²</td>
<td>89±5³</td>
<td>95±3³</td>
<td>97±5²</td>
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*Values are means ± SE unless otherwise noted. Comparisons with fewer subjects (n=9) are shown in parentheses. MV, mechanical ventilation. ²Values are medians, with range in brackets. ³Not significant (P>0.05); ⁴P < 0.05; ⁵P < 0.01 vs. eupnea. ⁶Not significant (P>0.05); ⁷P < 0.005 vs. positive-pressure mechanical hyperventilation in normocapnia (ANOVA with Student’s paired t-test for heart period and mean blood pressure and Kruskal-Wallis ANOVA with Wilcoxon signed-rank test for matched-pair differences for spectral power).
Sinus arrhythmia was significantly reduced (by 98 ms) to 40 ± 5 ms (Fig. 4C) during mechanical hyperventilation in hypocapnia. Spectral analysis of heart period variability confirms that hypocapnia had the only significant effect on sinus arrhythmia (Table 1). Hypocapnia had no significant effect on averaged mean blood pressure or mean heart period (Table 1).

We also considered investigating whether sinus arrhythmia was increased by hypercapnia during mechanical hyperventilation, but subjects could not tolerate such hypercapnia with our mechanical ventilation regimen.
Fig. 4. Sinus arrhythmia in all subjects during hyperventilation. Heart period averaged every 2.5% of the inflation cycle is shown over all inflation cycles for each subject during voluntary hyperventilation \( (n = 9) \) in A, during positive-pressure mechanical hyperventilation in normocapnia \( (n = 12) \) in B, and in hypocapnia \( (n = 12) \) in C. In A–C, breath frequency was 16 min \(^{-1}\), so percent inflation cycle is of a mean breath period of 3.75 ± 0.00 s. Sinus arrhythmia is mean sinus arrhythmia for 12 (or 9) subjects, where each subject’s sinus arrhythmia is calculated as the difference between their maximum and minimum R-R interval in the cycles. Vertical line indicates timing of all sinus arrhythmia troughs (mean ± SE). Horizontal wave with vertical SE points is the mean heart period for all subjects averaged at the same point in each respiratory cycle. This waveform underestimates the size of sinus arrhythmia when the timing of the maximum and minimum heart period in the cycle varies greatly between subjects (A) but estimates the size better the more their timing coincides (B). ns, \( P > 0.05 \) vs. eupnea. NS, \( P > 0.05 \); xxxx \( P < 0.005 \); xxxxx \( P < 0.001 \) vs. positive-pressure mechanical hyperventilation in normocapnia (ANOVA with Student’s paired \( t \)-test).

Fig. 5. Respiratory rhythm and sinus arrhythmia in subject 1 during hyperventilation. A: instantaneous heart period, inspiratory muscle EMG activity, and tidal volume during voluntary hyperventilation. Inflation is indicated by positive (upward) volume. Inspiratory EMG activity coincides with voluntary inspiration. \( (\text{Because EMG is recorded from surface electrodes, there is a large ECG artifact that has not been removed from the EMG signal.}) \) B and C: instantaneous heart period, inspiratory muscle EMG activity, and airway pressure during mechanical hyperventilation \( (\text{synchronized intermittent mandatory hyperventilation mode}) \) in normocapnia \( (B) \) and hypocapnia \( (C) \). Inflation is indicated by positive (upward) pressure. Respiratory rhythm is shown in \( B \) \( (\text{normocapnia}) \) as irregularities in the rising positive pressure wave and as simultaneous inspiratory muscle EMG activity. Respiratory rhythm is absent in \( C \) \( (\text{hypocapnia}) \), where the rising positive pressure wave has no irregularities and no inspiratory muscle EMG activity is visible above background noise.
DISCUSSION

We believe these results show the importance of the contribution of the respiratory rhythm to sinus arrhythmia in normal unanesthetized subjects.

Persistence of the respiratory rhythm during mechanical ventilation with positive pressure in normocapnia. The respiratory rhythm remained detectable from airway pressure and EMG activity (20, 39, 40, 43, 65) in all subjects during our regimen of positive-pressure mechanical ventilation in normocapnia. We know this pressure and EMG activity is inspiratory, because the corresponding measurements always indicated inspiration in eupnea and when eupneic breathing triggered access to ventilator gases. These are the most sensitive and noninvasive measures of the respiratory rhythm available for humans.

In many (25, 35, 40, 43, 46, 62–65), but not all (3, 34, 54), studies on normal awake subjects, the respiratory rhythm was inhibited during positive-pressure mechanical ventilation in normocapnia. This “neuromechanical inhibition” may occur for a number of reasons.

First, the presence of such neuromechanical inhibition in normocapnia depends on the frequency, inspiratory time, and inflation volumes used in positive-pressure mechanical ventilation, as well as on the relation of these to the eupneic pattern of the subject and on the precise mode of mechanical ventilation. Our mechanical ventilation settings are slightly different from those used previously, and we also used a different model of ventilator.

Second, there is less neuromechanical inhibition during mechanical ventilation in normocapnia during sleep (20, 30, 48, 62). Our subjects would, however, appear to have been awake or, at most, in only stage I sleep because of the short duration of apnea (11 ± 3 s) when they were switched in hypocapnia (24 ± 1 mmHg) to permit spontaneous breathing. Thus Datta et al. (20) found that disconnecting the ventilator in hypocapnia (mean 27–35 mmHg) produced a mean apnea duration of only 4 s during wakefulness and 14 s during stage I sleep, whereas the durations were much longer in deeper sleep (23 s in stage II, 79 s in stage III, 52 in stage IV, and 27 s in rapid eye movement sleep). Similar results have been found with disconnection in hypocapnia during sleep (30), and a large number of similar studies confirm that hypocapnic apneas during wakefulness are short (3, 9, 16, 20, 34, 41, 45, 54). Strictly, however, we cannot exclude the possibility that our subjects may have been drowsy and have sometimes drifted into and out of a very light sleep during normocapnic mechanical ventilation.

Third, the absence of neuromechanical inhibition may be due to differences in experimental design. For example, our subjects always started with their eupneic breathing triggering access to ventilator gases; i.e., when they were first connected, their respiratory rhythm had to continue for them to obtain O2. In addition, some other studies first lowered Pco2 and then raised it (40, 43) and may therefore have discovered a hysteresis mechanism.

Fourth, normal unanesthetized and awake subjects are aware of mechanical ventilation and respond to it. Our subjects always inspired during mechanical inflation in normocapnia. Such breathing with the ventilator has been described previously in awake subjects when Pco2 levels are raised to reduce neuromechanical inhibition (64). Breathing with the ventilator is obviously the most comfortable strategy for an unanesthetized subject, although this behavior is the opposite of that found in mechanically ventilating anesthetized animals with intact vagus nerves (which tend to breathe against the ventilator) (11). We would easily have detected whether subjects had breathed against the ventilator, and they never did.

Finally, subjects respond to the continuous unloading of their inspiratory musculature in normocapnia with some degree of “allowing the ventilator to take over” or “being passive” (35); i.e., their respiratory rhythm continues in normocapnia, but it produces less respiratory motor output, and, hence, their O2 consumption and CO2 production fall (71).

Although there are good reasons why the respiratory rhythm is not detectable in other studies, it does continue during our regimen of positive-pressure mechanical ventilation. Unfortunately, our measures of it do not have sufficient sensitivity [unlike those of Wilson et al. (72) in sleeping or sedated subjects] to quantify whether its amplitude is exactly the same during mechanical hyperventilation and during eupnea or to quantify by how much it might be reduced. Other than by recording surface EMG activity and airway pressure, there is no more sensitive but noninvasive means to measure the respiratory rhythm to establish this in humans. Nevertheless, because the respiratory rhythm is at least present, we can investigate the effects of its CO2 sensitivity on sinus arrhythmia during mechanical hyperventilation.

Sinus arrhythmia during positive-pressure mechanical ventilation in normocapnia. We show that the amplitude of sinus arrhythmia in normocapnia during positive-pressure mechanical ventilation or hyperventilation when the respiratory rhythm is detectable is not significantly different from that during eupnea at the same PeTco2. Even if our mechanical ventilation regimen slightly reduces the respiratory rhythm in normocapnia, any reduction in sinus arrhythmia is too small to be statistically significant with the measurement techniques and protocols we used. Similarly, there was no detectable difference in sinus arrhythmia between breathing air and O2. The size of our sinus arrhythmia is also similar to that found in eupnea by others (38, 65), although it is slightly greater than that found by Sasano et al. (57) and is greater than that found when the respiratory rhythm is neuromechanically inhibited during positive-pressure mechanical hyperventilation (65).

The fact that subjects continue to inspire during positive-pressure inflation raises the question of whether the lack of effect on sinus arrhythmia is because the negative pressure of inspiration (from the continuing respiratory rhythm) reverses the positive pressure of mechanical inflation. Our pressure measurements in the face mask (Figs. 3C and 5B) show that this is not the case: inspiration produced only minor disturbances in the shape of the positive-pressure waveform and never reversed it.

Although positive-pressure ventilation does alter venous return and left ventricular stroke volume (1, 35, 53, 70), our results indicate that their alteration during our ventilation regimen in normocapnia has no significant effect on the size or timing of the fluctuations in systolic pressure during the inflation cycle or, remarkably, on those of sinus arrhythmia. More detailed analysis (13) of the relation between these systolic pressure fluctuations and sinus arrhythmia within the inflation
cycle during positive-pressure mechanical hyperventilation is, however, beyond the scope of this study.

The only obvious difference in sinus arrhythmia with mechanical hyperventilation is that its timing within the inflation cycle is more regular. This difference is not caused by the application of positive pressure but is caused by the fact that mechanical ventilation keeps all subjects at a constant frequency and inflation volume. During mechanical ventilation, we also did not detect any reversal of sinus arrhythmia (73), indicating that such reversal may be related more to the additional effects of anesthesia and paralysis.

In hypocapnia, the respiratory rhythm is undetectable and sinus arrhythmia is reduced. We show that in hypocapnia (24 mmHg) at constant mechanical ventilation the respiratory rhythm was undetectable. Thus there were no fluctuations in airway pressure, rhythmic EMG activity was absent [as found previously (3, 34, 54, 64)], and, when the ventilator was switched off, hypocapnia increased the delay to the first breath and the longest expiratory period in the first minute. No voluntary drive to breathe was therefore detectable during mechanical hyperventilation in hypocapnia (although clearly it reappeared in hypocapnia when the ventilator was switched to permit spontaneous breathing). There was, however, nothing to prevent subjects’ voluntarily continuing to breathe with the ventilator during hypocapnia if they wished, although clearly they did not.

This hypocapnia caused a substantial reduction (by 74%) in sinus arrhythmia (reducing the mean from 138 to 40 ms). This reduction is due to the reduction in the respiratory rhythm and cannot be due to any change in chest inflation, mean blood pressure, or mean heart period, because none of these were changed by hypocapnia. The hypocapnia reduced but did not abolish sinus arrhythmia. A number of factors may contribute to this residual sinus arrhythmia in hypocapnia during mechanical hyperventilation. The residual sinus arrhythmia may indicate the underlying influence of rhythmic chest inflation, acting via pulmonary (6, 7, 19, 65) and/or atrial stretch mechanisms (8, 13, 68). It remains possible that the respiratory rhythm still makes a small contribution, even at 24 mmHg, because such hypocapnia does not necessarily abolish the respiratory rhythm (11). Hypocapnia at 24 mmHg is, however, almost as low as PCO₂ can safely be taken in normal subjects, because paresthesiae and tetany occur at ~20 mmHg (42).

Our results show also that hypocapnia produces a greater reduction in sinus arrhythmia when caused by mechanical hyperventilation than previously found with voluntary hyperventilation. Whereas Sasano et al. (57) found that lowering mean PETCO₂ by 10 mmHg (from 40 to 30 mmHg) with voluntary hyperventilation reduced mean sinus arrhythmia by 30 ms (from 90 to 60 ms), we show that lowering mean PETCO₂ by 16 mmHg (from 40 to 24 mmHg) with mechanical hyperventilation reduces mean sinus arrhythmia by 98 ms (from 138 to 40 ms); i.e., mechanical hyperventilation doubles the reduction (6 vs. 3 ms/mmHg). This suggests that the voluntary drive to breathe does make a small contribution to sinus arrhythmia, even in hypocapnia. It might be argued that because at low voluntary hyperventilation frequencies the sinus arrhythmia trough leads airflow (5, 21, 26, 31, 38, 58, 60, 65, 68), sinus arrhythmia must be caused predominantly by the respiratory rhythm, rather than by inflation and its mechanical sequelae. Our evidence that the voluntary drive to breathe does make a small contribution to sinus arrhythmia would, however, complicate and weaken such an argument, because the voluntary drive leads both. Precisely how the voluntary drive to breathe might influence sinus arrhythmia is, however, not clear. Although humans have virtually no voluntary control of heart period, corticospinal, corticobulbar, and bulbospinal pathways are involved in the voluntary and chemical control of breathing (61). The precise details of how and at what level these pathways might interact and how they might also influence sinus arrhythmia are unclear.

The fact that hypocapnia with mechanical hyperventilation produces such a large reduction in sinus arrhythmia is, we believe, the strongest evidence that the respiratory rhythm might be the principal contributor to sinus arrhythmia during eupnea in normal unanesthetized subjects. Our results, however, do not differentiate between the many different mechanisms through which the respiratory rhythm might affect sinus arrhythmia (19), being consistent, for instance, with direct effects on cardiac vagal preganglionic neurons (27) and with effects on the sensitivity of the baroreflex (13, 22–24, 46).

Other effects of hypocapnia. Hypocapnia is known to constrict some vascular beds, and short periods of hypocapnic voluntary hyperventilation produce varied and transient effects on heart rate and blood pressure (36, 37, 44, 55, 57, 69). We show that maintaining hypocapnia at 24 mmHg, even for 20 min, with positive-pressure mechanical hyperventilation has no significant effect on mean heart period or averaged mean blood pressure, and this extends previous studies (17). Thus such transient cardiovascular effects may be related more to the process of voluntary hyperventilation (69) than to hypocapnia itself.

Our results, moreover, do not indicate the presence in unanesthetized humans of a pronounced and tonic CO₂-dependent drive to cardiac vagal preganglionic neurons (59), in that withdrawal of such a pronounced and tonic drive during hypocapnia should produce a tachycardia that we did not detect.

Pulmonary stretch afferents might be expected to augment sinus arrhythmia in hypocapnia at constant mechanical hyperventilation, because hypocapnia augments their potency [in anesthetized animals and in unanesthetized humans (10, 14, 32, 47)], because they contribute to sinus arrhythmia (6, 19, 65, 67), and because intact pulmonary vagus nerves appear to be obligatory for increasing inspiratory effort to increase sinus arrhythmia (65). We show, however, that any such augmentation is not discernable when other CO₂-dependent mechanisms have more pronounced effects on sinus arrhythmia.

In conclusion, we demonstrate that respiratory rhythm can continue in normocapnia during positive-pressure mechanical ventilation in normal, unanesthetized humans. We show a substantial contribution to sinus arrhythmia from the respiratory rhythm, rather than from rhythmic chest inflation and its mechanical sequelae. We believe this is the strongest evidence so far that the respiratory rhythm might make the principal contribution to sinus arrhythmia in eupnea, although the mechanisms involved are unclear. The fact that the respiratory rhythm can be detected during positive-pressure mechanical hyperventilation and expressed in sinus arrhythmia may also be important for management of patients receiving ventilatory support.


