Direct effect of \( \text{PaCO}_2 \) on respiratory sinus arrhythmia in conscious humans

NOBUKO SASANO,1 ALEX E. VESELY,1 JUNICHIRO HAYANO,2 HIROSHI SASANO,1 RON SOMOGYI,1 DAVID PREISS,1 KIYOYUKI MIYASAKA,1 HIROTADA KATSUYA,3 STEVE ISCOE,4 AND JOSEPH A. FISHER1

1Department of Anesthesia, University Health Network, University of Toronto, Toronto, Ontario, Canada M5G 2C4; 2Department of Internal Medicine; 3Department of Anesthesiology and Resuscitology, Nagoya City University Medical School, Nagoya, Japan 467-8601; and 4Department of Physiology, Queen’s University, Kingston, Ontario, Canada K7L 3N6

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Sasano, Nobuko, Alex E. Vesely, Junichiro Hayano, Hiroshi Sasano, Ron Somogyi, David Preiss, Kiyoyuki Miyasaka, Hirotada Katsuya, Steve Iscoe, and Joseph A. Fisher. Direct effect of \( \text{PaCO}_2 \) on respiratory sinus arrhythmia in conscious humans. Am J Physiol Heart Circ Physiol 282: H973–H976, 2002. First published November 15, 2001; 10.1152/ajpheart.00554.2001.—Respiratory sinus arrhythmia (RSA) may improve the efficiency of pulmonary gas exchange by matching the pulmonary blood flow to lung volume during each respiratory cycle. If so, an increased demand for pulmonary gas exchange may enhance RSA magnitude. We therefore tested the hypothesis that \( \text{CO}_2 \) directly affects RSA in conscious humans even when changes in tidal volume (\( V_T \)) and breathing frequency (\( F_B \)), which indirectly affect RSA, are prevented. In seven healthy subjects, we adjusted end-tidal \( \text{PCO}_2 \) (\( \text{PetCO}_2 \)) to 30, 40, or 50 mmHg in random order at constant \( V_T \) and \( F_B \). The mean amplitude of the high-frequency component of R-R interval variation was used as a quantitative assessment of RSA magnitude. RSA magnitude increased progressively with \( \text{PetCO}_2 \) (\( P < 0.001 \)). Mean R-R interval did not differ at \( \text{PetCO}_2 \) of 40 and 50 mmHg but was less at 30 mmHg (\( P < 0.05 \)). Because \( V_T \) and \( F_B \) were constant, these results support our hypothesis that increased \( \text{CO}_2 \) directly increases RSA magnitude, probably via a direct effect on medullary mechanisms generating RSA.

METHODS

Subjects. Seven healthy nonsmoking volunteers (6 male and 1 female), aged 20–23 yr, were studied following approval by our institutional ethics committee. None had a history of cardiopulmonary disease or was taking any medication. All subjects gave written informed consent.

Measurements. Experiments were performed at a room temperature of 23–25°C, and at least 3 h after the subjects ate their last meal and drank caffeine-containing beverages. Subjects were studied in the sitting position and breathed gas from a spirometer through a face mask with a nonre-breathing valve (Fig. 1). The electrocardiogram (ECG; model 78342A, Hewlett-Packard; Wilmington, DE), airway \( \text{P}_{\text{CO}}_2 \) (Capnomac, Datex; Helsinki, Finland), and expiratory flow (model SC 520, Vacumed; Ventura, CA) were measured and digitized at 1 kHz on a personal computer by an analog-to-digital converter (model DI200, Datapac Instruments; Akron, OH). Breath-by-breath \( V_T \) and \( F_B \) were measured by integrating the expiratory flow signal. Oxygen saturation (\( \text{SpO}_2 \)) was measured using a pulse oximeter (model 251 Pulse Oximeter, Datex) with a probe on the left ear and recorded every 30 s.

Protocol. Data were obtained at three different levels of \( \text{PetCO}_2 \) (30, 40, and 50 mmHg), all at constant \( V_T \), \( F_B \), and fraction of inspired \( \text{O}_2 \) (\( F_{\text{IO}}_2 \)) (0.21). We prevented the ventilation of the subjects from varying according to the \( \text{P}_{\text{CO}}_2 \) as follows. Before the experiment, the spontaneous minute ventilation (\( V_e \)) at a \( \text{PetCO}_2 \) of 50 mmHg was determined in each subject at a fixed \( F_B \) of 0.25 Hz (15/min). During the rest of the experiment, \( V_e \) was maintained at this level and \( F_B \) was maintained at 0.25 Hz across all \( \text{P}_{\text{CO}}_2 \). \( F_B \) was kept constant by having subjects breathe in synchrony with a computer-generated signal at the same frequency (inspiratory-to-expiratory duration ratio = 1:1) heard over headphones. \( V_T \) was kept constant by having each subject maintain the end-inspiratory and end-expiratory positions of the spirometer bell constant while gas flow into the spirometer was set equal to the individual's \( V_e \) previously determined at a \( \text{PetCO}_2 \) of 50 mmHg.
mmHg. The three target levels of PETCO2 were obtained by manipulating FiCO2 between 0 and 0.06. All subjects practiced the maneuvers at various levels of PETCO2 before actual measurements until they were able to perform them without discomfort. The order of conditions (PETCO2) was randomized among subjects and data were collected for at least 3 min after stabilization at each condition.

Assessment of RSA magnitude. Fluctuations in heart period, including those caused by RSA, were assessed quantitatively by power spectrum analysis of the R-R interval of the ECG. All R wave peaks were detected automatically with a fast peak-detection algorithm. The ECG waveforms, with markers indicating the positions of detected R wave peaks, were visually inspected for ectopic beats and artifacts. Any errors in R wave detection were edited manually, although no subject showed ectopic beats or artifacts. R-R interval sequences thus obtained were interpolated with a cubic spline function and resampled at 1 Hz to obtain time series with equidistantly spaced data points. Using a previously reported method (11, 12), we measured powers of autoregressive spectral components over the frequency range of 0.04–0.15 Hz and at 0.24–0.26 Hz, referred to as the low-frequency (LF) and high-frequency (HF) components, respectively (3). In the present study, a peak in the power spectrum was observed in the HF range in all subjects. The mean amplitude (square root of doubled power) of the HF component was used as a quantitative assessment of RSA (RSA magnitude). Mean R-R interval and the LF-to-HF power ratio (LF/HF) were used as indexes of cardiac autonomic tone and autonomic function, respectively.

Statistical analysis. One-way repeated-measures analysis of variance (ANOVA), followed by multiple comparisons with the Bonferroni correction, was used to evaluate the effects of PETCO2 on variables. Data are presented as means ± SE. A P value < 0.05 was considered to indicate significance.

RESULTS

All subjects were able to maintain FB and VT constant during measurements at the three different levels of PETCO2 (Table 1). Figure 2 shows the results obtained from a representative subject. Despite increasing levels of PETCO2 (the top border of the airway PCO2 trace), FB and VT did not change. At all three levels of PETCO2, the power spectra of R-R interval showed a narrow peak at 0.25 Hz of the HF component. The area under the peak corresponding to the magnitude of RSA increased with the level of PETCO2, as expected from the increasing magnitude of R-R interval fluctuation.

Despite constant Fl and VT, the magnitude of RSA increased with PETCO2. In six of seven subjects and repeated-measures ANOVA detected significant differences at the three levels of PETCO2 (P < 0.001 for all, Fig. 3). Mean R-R interval also increased with PETCO2, but a significant difference was detected only between PETCO2 of 30 and 40 mmHg (P < 0.05). No difference was observed in LF amplitude (42 ± 3, 42 ± 5, and 54 ± 11 ms for PETCO2s of 30, 40, and 50 mmHg) and the changes in LF/HF did not reach significance.

DISCUSSION

Increases in PETCO2 from 30 to 50 mmHg directly increased RSA magnitude when VT and FB were fixed. On the other hand, Spo2 and mean R-R interval between a PETCO2 of 40 and 50 mmHg did not change. Given the potential role of RSA in enhancing pulmonary gas exchange (13, 22), our results are consistent with the idea that increased demand for pulmonary CO2 elimination directly enhances gas exchange via increases in RSA magnitude.

Despite many studies of RSA, there are few studies of the effects of CO2 alone, particularly in conscious animals or humans. In unanesthetized trained dogs, Yasuma and Hayano (22) reported that hypercapnia (PETCO2 up to 54 mmHg) increases RSA magnitude by 62% with no concomitant changes in mean R-R interval. Our present observations are consistent with their findings. However, because their dogs increased VT and FB 3.7- and 1.2-fold, respectively, in response to hypercapnia, and because RSA magnitude increases with increasing VT and decreasing FB (8, 11, 14), their results provide no evidence for a direct effect of CO2 on RSA magnitude. Yen et al. (23) reported that vagal

### Table 1. Spo2, VT, and FB at three levels of PETCO2

<table>
<thead>
<tr>
<th>PETCO2, mmHg</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Spo2</td>
<td>98.8 ± 0.0</td>
<td>98.8 ± 0.1</td>
<td>98.7 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>VT, l</td>
<td>1.59 ± 0.06</td>
<td>1.57 ± 0.06</td>
<td>1.57 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>FB, min⁻¹</td>
<td>15.0 ± 0.1</td>
<td>15.0 ± 0.1</td>
<td>15.0 ± 0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 subjects. VT, tidal volume; FB, breathing frequency; Spo2, O2 saturation from pulse oximetry; PETCO2, end-tidal PCO2; NS, not significant.
outflow to the heart increases with hypercapnia and decreases with hypocapnia in unanesthetized decerebrate cats, although they did not report changes in RSA. Our present observations seem consistent with theirs, provided RSA magnitude reflects the tonic level of vagal activity (12, 16). In fact, the significant decreases in both RSA magnitude and mean R-R interval we observed at a PETCO2 of 30 mmHg are consistent with withdrawal of vagal tone. Moreover, even the increased RSA magnitude in the absence of changes in mean R-R interval at a PETCO2 of 50 mmHg may be compatible with increased vagal tone if the vagal effect on R-R interval was offset by a concomitant increase in sympathetic activity in response to the hypercapnia.

The most likely mechanism responsible for increased RSA magnitude in our study is chemostimulation that enhances respiratory modulation of vagal outflow. RSA arises primarily as a result of respiratory modulations of vagal outflow to the heart, mainly due to inhibition of vagal cardiac preganglionic neurons by medullary inspiratory neurons (10, 20) and phasic inspiratory gating by pulmonary stretch receptor input of excitatory inputs to these neurons (1, 5). Stimulation of carotid chemoreceptors by increased PaCO2 has primarily an excitatory effect on vagal preganglionic neurons to the heart, the so-called primary response, in the expiratory phase (6, 18). Hence, in the present study, CO2 likely increased vagal outflow during expiration. In contrast, during the inspiratory phase, vagal outflow would have been low because of the CO2-induced increase in respiratory drive, that is, greater inhibition by inspiratory neurons. In addition, gating of vagoexcitatory inputs by slowly adapting pulmonary stretch receptors may be greater at increased PaCO2 (9). The net effect will depend on the degree to which the primary excitatory effect is offset by the secondary inhibitory effect from both medullary inspiratory neurons and pulmonary stretch receptors (18). Taken together, increased CO2 enhances respiratory modulation of vagal outflow, even in the absence of changes in the overt ventilatory (VT and Fb) responses to increases in CO2. These putative mechanisms responsible for the CO2-induced increase in RSA magnitude may also explain why changes in RSA magnitude do not always parallel changes in vagal tone, that is, the net effect of excitatory and inhibitory inputs.

Regulation of RSA magnitude by PaCO2 could complement CO2-modulated changes in airway smooth muscle tone in controlling dead space. Hypercapnia both decreases tracheal diameter in anesthetized dogs (7) and causes bronchoconstriction in decerebrate cats (15). In anesthetized dogs, Hayano et al. (13) also showed that RSA reduces physiological dead space, that is, the alveolar dead space, by matching perfusion to ventilation during each respiratory cycle. Our results, in combination with these earlier findings, suggest that changes in PaCO2 are accommodated by dy-

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**Fig. 2.** Changes in R-R interval, airway PCO2, VT, and autoregressive power spectra of R-R interval at PETCO2 of 30, 40, and 50 mmHg in a representative subject. The magnitudes of respiratory sinus arrhythmia (RSA) measured as the mean amplitude of the high-frequency (HF) component (0.24–0.26 Hz) were 65, 104, and 123 ms, respectively. The mean amplitudes of the low-frequency (LF) component (0.04–0.15 Hz) were 57, 58, and 122 ms, respectively. PSD, power spectral density.

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**Fig. 3.** Effect of PETCO2 on the magnitude of RSA, mean R-R interval, and LF-to-HF ratio (LF/HF) of R-R interval power spectral components. Left, values in individual subjects; right, average values ± SE for the 7 subjects. Overall significances of the effect of PETCO2, according to repeated-measures ANOVA, are P < 0.001, P < 0.001, and not significant for the magnitude of RSA, mean R-R interval, and LF/HF, respectively. *P < 0.05 vs. 40 mmHg (multiple comparisons by the Bonferroni method).
nationally altering both anatomical and alveolar dead space to modify the efficiency of CO₂ elimination.

In conclusion, increases in RSA magnitude with hypercapnia were not, in conscious humans, associated with changes in SpO₂ or, at PETCO₂ between 40 and 50 mmHg, mean R-R interval. Changes in RSA magnitude did, however, parallel increases in PETCO₂ in the absence of changes in VT and F₂. Our results are consistent with our hypothesis that increased CO₂ directly increases RSA magnitude even when changes in VT and F₂ are prevented. Given the potential role of RSA in improving pulmonary gas exchange, our results suggest that increased PCO₂ directly enhances RSA to facilitate CO₂ elimination.

REFERENCES