Augmentation of respiratory sinus arrhythmia in response to progressive hypercapnia in conscious dogs

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Yasuma, Fumihiko, and Jun-Ichiro Hayano. Augmentation of respiratory sinus arrhythmia in response to progressive hypercapnia in conscious dogs. Am J Physiol Heart Circ Physiol 280: H2336–H2341, 2001.—Respiratory sinus arrhythmia (RSA) may serve to enhance pulmonary gas exchange efficiency by matching pulmonary blood flow with lung volume within each respiratory cycle. We examined the hypothesis that RSA is augmented as an active physiological response to hypercapnia. We measured electrocardiograms and arterial blood pressure during progressive hypercapnia in conscious dogs that were prepared with a permanent tracheostomy and an implanted blood pressure telemetry unit. The intensity of RSA was assessed continuously as the amplitude of respiratory fluctuation of heart rate using complex demodulation. In a total of 39 runs of hypercapnia in 3 dogs, RSA increased by 38 and 43% of the control level when minute ventilation reached 10 and 15 l/min, respectively (P < 0.0001 for both), and heart rate and mean arterial pressure showed no significant change. The increases in RSA were significant even after adjustment for the effects of increased tidal volume, respiratory rate, and respiratory fluctuation of arterial blood pressure (P < 0.001). These observations indicate that increased RSA during hypercapnia is not the consequence of altered autonomic balance or respiratory patterns and support the hypothesis that RSA is augmented as an active physiological response to hypercapnia.

METHOdS

Study animals and preparations. This study was performed in compliance with the “Animal Experimentation Guide of the Nagoya University School of Medicine” (1989) and the National Institutes of Health Guiding Principles in the Care and Use of Animals (Revised 1996). The subjects were three adult mongrel dogs (weighing 22–28 kg) that were trained to lie down quietly in their assigned place in the laboratory (28). Months before the studies, under general anesthesia and aseptic conditions, each dog was prepared surgically with a permanent side-hole tracheostomy. Anesthesia was induced with a short-acting barbiturate (thiamyl sodium, 10–20 mg/kg iv) and maintained with a long-acting barbiturate (pentobarbital sodium, 30–50 mg/kg iv). Antibiotics (penicillin G-potassium, 0.02–0.03 mg/kg im) were administered postoperatively. Weeks before the studies, while under general anesthesia, the dogs were also implanted with a one-channel telemetry unit for blood pressure (TA11PA-D70, Data Sciences; St. Paul, MN). The methods of anesthesia and postoperative administration of antibiotics were the same as described above. The femoral artery was exposed and an arterial catheter, which was connected to the pressure sensor in the transmitter, was inserted and advanced to the external iliac artery. Silk ligatures secured the catheter in the proximal artery and were used to tie off the distal deep femoral artery.

Study protocol. All experiments were performed before the daily feeding and without anesthetic or analgesic agents. During the experiment, the dogs breathed through a cuffed tracheal tube inserted through the tracheostomy. Progressive hyperoxic hypercapnia was applied using a modification of the method of Read (21). The dog rebreathed from a bag containing a mixture of 7% CO2-93% O2. Each rebreathing
run was undertaken with the dog lying on its left side and was terminated when the dog moved. The dog was kept awake during the measurements as revealed by the electroencephalograms and behavioral criteria (20, 28). Each rebreathing run lasted for 3–4 min. Between the runs at least 10 min of room air breathing was allowed for all variables to recover; during this time the dog was also lying down quietly.

Measurements. Respiratory airflow was measured with a hot-wire pneumotachograph (Mini Sensor, Minato; Osaka, Japan) attached to the tube. Airway CO2 and O2 concentrations were measured continuously with a medical gas analyzer (model MG-360, Minato). The signals were directed to a respirometabolic monitor (model RM-300, Minato), and breath-by-breath respiratory parameters [tidal volume, respiratory rate, arterial O2 saturation (SaO2), and end-tidal Pco2 and minute ventilation volume (Vi)] were determined. Arterial blood pressure was measured with a telemetry system (3). The radio-frequency signal emitted from the implanted device was received with a water-resistant receiver (model RLA2000, Data Sciences) that was positioned close to the dog. Because the pressure implant sensed the absolute pressure (i.e., relative to a vacuum), an electric barometer (model C11PR, Data Sciences) was incorporated into the system by which the influence of the changes in the barometric pressure was adjusted. Electroencephalograms and electrocardiograms were measured using subcutaneous needle electrodes (model 45126, NEC-Sanei; Tokyo, Japan). All respiratory and cardiovascular parameters were monitored with a polygraph (NEC-Sanei) and recorded with a thermal chart recorder (Omnia, NEC-Sanei) and an FM-tape data recorder (model MR-30, TEAC; Tokyo, Japan) for off-line analyses.

Data processing. Electrocardiogram and blood pressure signals were played back from the FM tape and digitized on a personal computer (model P5–150, Gateway 2000, Sioux City, SD) with a 12-bit analog-to-digital converter (model DI-200, DATAQ Instruments; Akron, OH) at a sampling frequency of 1 kHz. All R wave peaks were detected automatically with a fast peak-detection algorithm. The electrocardiogram with markers indicating the positions of detected R wave peaks was visually inspected for ectopic beats and artifacts, and any errors in R wave detection were edited manually. Mean arterial pressure (MAP) for each cardiac cycle was calculated as the area under the blood pressure waveform divided by the RRI. When R waves were ectopic or in some other way abnormal, the RRI and corresponding MAP were deleted from the series of data. Each normal-to-normal RRI and MAP time series was interpolated with a cubic spline function and resampled at 2 Hz to obtain a time series of equidistantly spaced data points.

Dynamic changes in the amplitude of oscillatory components of RRI and MAP were assessed continuously with complex demodulation (8, 9). Complex demodulation is a nonlinear time-domain method of time-series analysis developed for assessing oscillatory components in nonstationary data. In contrast to spectral analysis, which provides the time-averaged properties (power and frequency) of oscillatory components over a stationary segment of data, the complex demodulation provides time-dependent changes in the instantaneous amplitude of an oscillatory component within a given frequency range. Computations for the analysis were performed on the personal computer using a subroutine called Complex Demodulation, which we deposited with the National Auxiliary Publications Service (8).

Oscillations of RRI and MAP in low-frequency (0.04–0.15 Hz) and high-frequency (0.15–0.80 Hz) bands were assessed. These frequency ranges were selected in accordance with earlier reports (1, 2, 9, 17) and the maximum instantaneous respiratory rate observed in the present study (47 breaths/min). The amplitude of RRI oscillation in the high-frequency band (RRIHF) was used to denote the intensity of RSA. The low frequency-to-high frequency ratio in squared amplitude of RRI oscillation (LF/HF), the amplitude of MAP oscillation in the high-frequency band (MAPHF), and that in the low-frequency band (MAPLF) were also evaluated.

For each run, we measured all respiratory and cardiovascular variables at the control condition just before the loading of hypercapnia and during hypercapnia, when Vt first reached 10 and 15 l/min; this allowed us to compare the cardiovascular variables between the intensities of hypercapnia standardized by the degree of ventilatory response. For each of these conditions, the respiratory variables were measured as the averages over 3 continuous breaths, and the cardiovascular variables were also averaged over the corresponding periods.

Statistical analysis. A Statistical Analysis System program package (SAS Institute; Cary, NC) was used for statistical analysis. All variables were examined for the distribution; when a variable did not distribute normally, an appropriate transformation was performed to obtain a normal distribution. The effects of hypercapnia on the variables were evaluated by a two-way repeated-measures ANOVA (level of hypercapnia × dog) with contrast transformations (the values at Vt of 10 and 15 l/min were contrasted to the value for control). Considering the effects of respiratory variables on the intensity of RSA (10, 16, 22), the effect of hypercapnia on RRIHF was also evaluated with an analysis of covariance by which the comparison between conditions (control and at Vt of 10 and 15 l/min) was adjusted for the effects of tidal volume, respiratory rate, and MAPHF. The Bonferroni method was used for multiple comparisons to guard against an increase in type I error level. Data were presented as means ± SE in Fig. 2 and Table 1 except for RRIHF adjusted for the effects of tidal volume, respiratory rate, and MAPHF, which are presented as the least squares means ± SE. P < 0.05 was considered as significant in all of the statistical analyses.

RESULTS

For the 3 dogs, 13, 18, and 8 runs of progressive hypercapnia were obtained (Fig. 1 shows representative tracings of a run for dog 2). The electrocardiograms showed that all measurements were obtained during sinus rhythm without ectopic beat or heart block. In control conditions and at Vt of 10 and 15 l/min, all variables showed a normal distribution except for LF/HF, which was transformed into a natural logarithmic value [ln(LF/HF)] to obtain a normal distribution in all conditions. The two-way repeated measures ANOVA showed no significant effect of the interaction between the level of hypercapnia and the dog for any variables, which indicates that the patterns of the changes with hypercapnia did not differ between the three dogs.

On average over all the runs in the three dogs, RRIHF (the intensity of RSA) increased by 38 and 43% of the control level when Vt reached 10 and 15 l/min, respectively, during progressive hypercapnia (P < 0.0001 for both); however, no significant change was observed in heart rate, MAP, ln(LF/HF), or MAPLF throughout the hypercapnia (Fig. 2). MAPHF also in-
increased when $\dot{V}l$ reached 10 and 15 l/min ($P < 0.0001$ for both). Both tidal volume and respiratory rate (except the value at a $\dot{V}l$ of 10 l/min) increased significantly with progressive hypercapnia; however, the progressive increase in RRIHF was still significant even after adjustment for the effects of tidal volume, respiratory rate, and MAPHF (Table 1).

DISCUSSION

Our results support the hypothesis that RSA is augmented as an active physiological response to hypercapnia. We observed in conscious dogs that progressive hypercapnia increased the intensity of RSA without changing heart rate, MAP, MAPLF, or $\ln$(LF/HF). Furthermore, the increase in RSA intensity was not attributable to the effects of concomitant changes in tidal volume, respiratory rate, or MAPHF. These observations indicate that the increased RSA during hypercapnia is not the consequence of the changes in autonomic balance or respiratory patterns but a direct response of central chemostimulation.

Compared with earlier work our study is unique in the following two aspects. First, we used trained dogs that were able to lie down calmly during the loading of hypercapnia. This allowed us to collect data with the dogs awake and in well-standardized conditions, thereby avoiding the influences of anesthetics, consciousness levels, and physical activities on the auto-

**Fig. 1.** Representative tracings during progressive hypercapnia in dog 2: R-R interval (RRI), mean arterial pressure (MAP), amplitudes of RRI oscillation in high-frequency band (RRIHF) and in low-frequency band (RRILF), amplitudes of MAP oscillation in high-frequency band (MAPHF) and in low-frequency band (MAPLF), minute volume of inspiration ($\dot{V}l$), tidal volume (TV, thick line), respiratory frequency (RR, thin line), saturation of arterial blood ($Sao_2$, thick line), and partial pressure of end-tidal CO$_2$ (PETCO$_2$, thin line). Note that respiratory sinus arrhythmia (the magnitude of respiratory oscillation of RRI and its quantitative reflection in RRIHF) increases with the progression of hypercapnia.
nomic nervous and respiratory functions (6). Also, the dog seems to be an ideal animal for studying the physiology of RSA because it shows the most distinct RSA (7, 23), and the properties of autonomic modulation of heart rate have been well characterized in this animal (2, 17). The second aspect of the uniqueness of our study is that we used complex demodulation to analyze the time series of cardiovascular parameters. This method is advantageous over spectral analysis in that it provides the changes in amplitude of an oscillatory component of nonstationary data as a function of time (8, 9). Using the complex demodulation, we were able to delineate the dynamic response of RSA intensity to progressive hypercapnia.

The results of the present study are apparently inconsistent with the conventional concept that the intensity of RSA measured as RRIHF reflects cardiac vagal activity (5, 10, 12, 16, 17, 22). From this conventional concept, an increase in RRIHF would be accompanied by a decrease in heart rate due to cardiac vagal

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<th>Table 1. Respiratory parameters and adjusted amplitude of R-R interval oscillation in high-frequency band during hypercapnia</th>
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Values are means ± SE except for the adjusted amplitude of R-R interval oscillation in high-frequency band (RRIHF), which is expressed as least squares mean ± SE adjusted for the effects of tidal volume, respiratory rate, and amplitude of mean arterial pressure oscillation in high-frequency band. 

Fig. 2. Cardiovascular parameters and the oscillatory components at control condition and during hypercapnia when V1 first reached 10 and 15 L/min: heart rate (HR) and the natural logarithm of low frequency-to-high frequency ratio in squared amplitude of RRI oscillation [ln(LF/HF)]. Note RRIHF increases with hypercapnia despite no change in HR, MAP, ln(LF/HF), and MAPLF. *P < 0.05 vs. control. bpm, Beats/min.

Fig. 3. Conceptual schema explaining the differential effects of vagal activation and hypercapnia on the respiratory modulation of the vagal outflow. Vagal outflow is modulated directly by the respiratory center (stimulated during expiration and suppressed during inspiration) and indirectly by the inspiratory gating mechanism driven by the afferent input from the pulmonary stretch receptors (by which vagal outflow is attenuated during inspiration). A: vagal activation is accompanied by an increase in the rate of respiratory modulation of vagal outflow that is proportional to the mean level of vagal outflow. B: in contrast, hypercapnia increases the range of respiratory modulation through enhancing the strength of the modulation without changing mean vagal tone. *Inspiration; dashed lines, mean level of vagal outflow.
activation. We observed, however, no significant decrease in heart rate during hypercapnia despite the presence of a progressive increase in RRiHF. Our findings suggest that hypercapnia could lead to dissociation between the intensity of RSA and the cardiac vagal outflow.

Two possible mechanisms may be considered for this dissociation. First, the concomitant sympathetic activation may have counterbalanced the effect of increased cardiac vagal outflow. This seems unlikely from our results, because we observed no significant change in MAP, ln(LF/HF), or MAPLF, which are assumed to be potential markers of sympathetic activity within individual animals or humans (4, 13, 16–19, 26, 27). In contrast to our observations, Somers and co-workers (25) reported that an acute increase in PCO2 augments the sympathetic nerve activity recorded from the peroneal nerve in conscious humans; however, they did observe concomitant increases in heart rate and MAP.

The second possible mechanism for the dissociation between the intensity of RSA and the cardiac vagal outflow is that hypercapnia may directly affect the phasic respiratory modulation of cardiac vagal outflow. In the conventional concept (Fig. 3A), the range of phasic respiratory vagal modulation, which determines the intensity of RSA, correlates with the level of mean vagal tone (5, 10, 12, 16, 17, 22). Our observations, however, suggest that hypercapnia may increase the range of phasic modulation without changing the level of mean vagal tone (Fig. 3B). The mechanisms generating the phasic respiratory vagal modulation are known to include a direct modulation by the respiratory center (the activity of the vagal motoneurons is stimulated during expiration and suppressed during inspiration) and an inspiratory gating mechanism driven by the afferent input from the pulmonary stretch receptors (the central and baroreflex stimuliations to the vagal outflow only appear during expiration but almost disappear during inspiration) (14). These mechanisms might be reinforced by central chemostimulation with hypercapnia. This hypothesis is consistent with the report of a canine study by Shykoff and co-workers (24), who reported that RSA is centrally mediated and directly related to the respiratory drive.

The present study and a previous study of ours (11) suggest that augmentation of RSA with hypercapnia may be a coping response aimed at expelling CO2 effectively from the pulmonary circulation. In the previous study (11), we investigated the effects of RSA on pulmonary gas exchange using an artificial RSA, which was simulated by the phasic vagal stimulation in synchrony with pulmonary ventilation. We observed that the presence of RSA reduced the physiological dead space (Vd/Vt, where Vd is physiological dead space and Vt is tidal volume) and the intrapulmonary shunt fraction (Qs/Qt, where Qs is physiological shunt and Qt is cardiac output or blood flow through the lungs) and consequently enhanced oxygen transport compared with the constant distribution of the equal number of heartbeats per minute. In contrast, an inversion of the phase relationship between the temporal density of heartbeats and lung volume termed the “reverse RSA” increased Vd/Vt and Qs/Qt significantly. This suggests that RSA enhances the pulmonary gas exchange efficiency by matching perfusion to ventilation within each respiratory cycle. To determine the extent to which RSA improves the removal of CO2 and accelerates the recovery from hypercapnia, further studies are required.

In conclusion, we demonstrated that hypercapnia in conscious dogs augments RSA independently of the effects on autonomic balance or respiratory patterns. Our findings suggest that the central chemostimulation by CO2 directly intensifies the phasic respiratory modulation of vagal outflow and supports the hypothesis that RSA is augmented as an active physiological response to hypercapnia.

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REFERENCES


