Impact of acute hypoxia on heart rate and blood pressure variability in conscious dogs

FUMIHIKO YASUMA1 AND JUN-ICHIRO HAYANO2
1First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya 466-8550; and 2Third Department of Internal Medicine, Nagoya City University Medical School, Nagoya 467-8601, Japan

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Yasuma, Fumihiko, and Jun-Ichiro Hayano. Impact of acute hypoxia on heart rate and blood pressure variability in conscious dogs. Am J Physiol Heart Circ Physiol 279: H2344–H2349, 2000.—To examine whether the impacts of hypoxia on autonomic regulations involve the phasic modulations as well as tonic controls of cardiovascular variables, heart rate, blood pressure, and their variability during isocapnic progressive hypoxia were analyzed in trained conscious dogs prepared with a permanent tracheostomy and an implanted blood pressure telemetry unit. Data were obtained at baseline and when minute ventilation (Vt) first reached 10 (Vt10), 15 (Vt15), and 20 (Vt20) l/min during hypoxia. Time-dependent changes in the amplitudes of the high-frequency component of the R-R interval (RRIHF) and the low-frequency component of mean arterial pressure (MAPLF) were analyzed by complex demodulation. In a total of 47 progressive hypoxic runs in three dogs, RRIHF decreased at Vt15, whereas heart rate and arterial pressure increased progressively with advancing hypoxia. We conclude that the autonomic responses to isocapnic progressive hypoxia involve tonic controls and phasic modulations of cardiovascular variables; the latter may be characterized by a progressive reduction in respiratory vagal modulation of heart rate and a transient augmentation in low-frequency sympathetic modulation of blood pressure.

METHODS

Animal preparations. This study was performed in compliance with the Animal Experimentation Guide of the Nagoya University School of Medicine (1989) and the Guiding Principles in the Care and Use of Animals (Revised 1996). Three adult mongrel dogs (22–28 kg body wt) were trained to lie quietly in their assigned place in the laboratory. Months before the experiments, the dogs were prepared surgically.

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with a permanent side-hole tracheostomy under general anesthesia. Anesthesia was induced with short-acting barbiturate (thiamyl sodium, 10–20 mg/kg iv) and maintained with long-acting barbiturate (pentobarbital sodium, 30–50 mg/kg iv). Intramuscular antibiotics (penicillin G potassium, 0.02–0.03 mg/kg) were administered postoperatively. Before the experiments, the dogs were implanted with a blood pressure telemetry device (model TA11PA-D70, Data Sciences, St. Paul, MN) also under general anesthesia. The methods of anesthesia and postoperative administration of antibiotics were as described above. The femoral artery was exposed, and an arterial catheter, connected to the pressure sensor in the transmitter, was inserted and advanced into the external iliac artery. Silk ligatures secured the catheter in the proximal artery and were used to tie off the distal deep femoral artery.

**Respiratory and hemodynamic measurements.** All experiments were performed before the daily feeding without anesthetic or analgesic agents. During the experiments, the dogs breathed through a cuffed endotracheal tube inserted through the tracheostomy. Respiratory airflow was measured with a hot-wire pneumotachograph (Mini Sensor, Minato, Osaka, Japan) attached to the tube. Airway CO$_2$ and O$_2$ concentrations were measured continuously with a medical gas analyzer (model MG-360, Minato). The airflow, gas, and calculated metabolic parameters were directed to a respirometabolic monitor (model RM-300, Minato), and the following breath-by-breath parameters were stored in a personal computer (model PC-9801-F, NEC, Tokyo, Japan): tidal volume, instantaneous respiratory rate, inspiratory and expiratory durations, inspiratory and expiratory volumes, inspired Pco$_2$ and Po$_2$, and end-tidal Pco$_2$ and Po$_2$. The minute volumes of inspiration and expiration were also calculated. Buccal arterial O$_2$ saturation (Sa O$_2$) was monitored with a pulse oximeter (Biox-3700, Ohmeda, Boulder, CO). To know when the dog was awake, an electroencephalogram was recorded from the scalp with subcutaneous needle electrodes (model 45126, NEC-Sanei, Tokyo, Japan). A bipolar electrocardiogram was also recorded from the trunk with the subcutaneous needle electrodes.

Arterial blood pressure was measured with a telemetry system (4). The radio-frequency signal emitted from the implanted device was received with a water-resistant receiver unit (model RLA2000, Data Sciences) placed close to the dog. Because the pressure implant device measured absolute pressure (i.e., relative to vacuum), an electric barometer (model C11PR, Data Sciences) was incorporated into the system, by which the influence of the change in barometric pressure was adjusted. All signals were recorded with a thermal chart recorder (Omnimec, NEC-Sanei) and on FM tapes with a data recorder (model MR-30, TEAC, Tokyo, Japan) for off-line analysis.

**Impostion of hypoxia.** Progressive isocapnic hypoxia was induced using a modification of the method of Rebuck and Campbell (34). The dogs rebreathed from a bag containing a mixture of 15% O$_2$–85% N$_2$. End-tidal CO$_2$ concentration, an index of alveolar CO$_2$ was maintained within ±0.03% of the control level by means of an automated control system and CO$_2$ absorber circuit (40). Each run was undertaken with the dog lying on its left side and terminated when the dog moved. The dog was kept awake during the measurements, as revealed by the electroencephalogram and behavioral criteria (32, 43). Each rebreathing run lasted ~5 min. Between the runs, ≥10 min of room air breathing were allowed for all variables to return to the control level.

**Data processing.** Electrocardiogram and blood pressure signals were played back from the FM tapes 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. The temporal positions of all R-wave peaks were determined automatically with a fast peak detection algorithm. The electrocardiogram waveform with markers indicating the position 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) of each heartbeat was measured as the area under the blood pressure waveform divided by the R-R interval. When an R-R interval was ectopic or in some other way abnormal, the interval and corresponding MAP were deleted from the series of data. Each normal-to-normal R-R interval and MAP time series was interpolated with cubic spline function and resampled at 2 Hz to obtain a time series of equidistantly spaced data points.

**Complex demodulation.** Dynamic responses of the amplitudes of RSA and Mayer waves to hypoxia were assessed with complex demodulation (15, 16). Complex demodulation is a nonparametric time-domain method of time series analysis developed for assessing oscillatory components in nonstationary data. In contrast to spectral analysis, which provides time-averaged properties (power and frequency) of oscillatory components over a period (usually >1 min) with the assumption that the data are stationary during the period, complex demodulation provides time-dependent changes in the instantaneous amplitude of the oscillatory component within a given frequency range. To assess the magnitude of RSA and of Mayer waves, we demodulated the amplitude of the R-R interval oscillation in the high-frequency band (0.15–0.80 Hz; RRIHF) and that of MAP oscillation in the low-frequency band (0.04–0.15 Hz; MAPLF), respectively. These frequency ranges were selected according to earlier reports (1, 3, 5) and to the maximum instantaneous respiratory rate observed in the present study (47 breaths/min).

The analysis was performed on a personal computer (model P5–150, Gateway 2000) with a subroutine complex demodulation written in FORTRAN that we deposited with the National Auxiliary Publications Service (15). For analysis of RRIHF and MAPLF, the reference frequencies were set at 0.475 and 0.095 Hz, respectively. The low-pass filtering was performed with a zero-phase-shift least-squares filter with convergence factors. The length of the filter was set at 61 terms, resulting in a transitional bandwidth of 0.033 Hz. The low-pass corner frequencies were set at 0.325 and 0.055 Hz for RRIHF and MAPLF, respectively, so that the frequency bands for demodulating these components were 0.15–0.80 and 0.04–0.15 Hz, respectively. The amplitude of the R-R interval oscillation in the low-frequency band (0.04–0.15 Hz) was also demodulated, and its ratio to RRIHF was calculated as LF/HP (the ratio was squared to be expressed as power ratio).

**Statistical analysis.** For each run, we measured all variables at the control condition (CC) just before the start of rebreathing and during progressive hypoxia when minute ventilation (Vt) first reached 10 l/min (Vt10), 15 l/min (Vt15), and 20 l/min (Vt20); this allowed us to compare the cardiovascular variables among the intensities of hypoxia standardized by the degree of ventilatory response. For each of CC, Vt10, Vt15, and Vt20, the respiratory variables were measured as the average over three continuous breaths and the cardiovascular variables were also averaged over the same period.

A program package of the Statistical Analysis System (SAS, Cary, NC) was used for the statistical analysis. The effects of hypoxia on the variables were evaluated by a
two-way repeated-measure ANOVA with contrast transformations of the values for $\dot{V}I_{10}$, $\dot{V}I_{15}$, and $\dot{V}I_{20}$ against the value for CC. Considering the effects of respiratory variables on the magnitude of RSA (13, 18, 27), the impact of hypoxia on RRIHF was also evaluated with an analysis of covariance, by which the comparison between conditions (CC, $\dot{V}I_{10}$, $\dot{V}I_{15}$, and $\dot{V}I_{20}$) was adjusted for the effects of concomitant changes in respiratory rate and tidal volume. The Bonferroni method was used for multiple comparisons to guard against an increase in type I error level. Values are means $\pm$ SE, except as otherwise noted (i.e., adjusted RRIHF in Table 1, which is presented as least-square mean $\pm$ SE). An $\alpha = 0.05$ was considered significant in all statistical analyses.

RESULTS

In dogs 1, 2, and 3, we obtained 20, 18, and 9 runs of isocapnic rebreathing hypoxia after each control measurement, respectively. A representative trace is shown in Fig. 1. The averaged responses of heart rate, MAP, RRIHF, LF/HF, and MAPLF at CC, $\dot{V}I_{10}$, $\dot{V}I_{15}$, and $\dot{V}I_{20}$ are displayed in Fig. 2. $\text{SaO}_2$, end-tidal $\text{PCO}_2$, respiratory rate, tidal volume, and adjusted RRIHF at CC, $\dot{V}I_{10}$, $\dot{V}I_{15}$, and $\dot{V}I_{20}$ are summarized in Table 1.

Heart rate, MAP, respiratory rate, and tidal volume increased with progressive hypoxia. RRIHF decreased with increasing intensity of hypoxia, and this change was also significant even after adjustment for the effects of respiratory rate and tidal volume. In contrast, MAPLF was increased at $\dot{V}I_{10}$ and $\dot{V}I_{15}$, but not at $\dot{V}I_{20}$. Additionally, LF/HF was increased only at $\dot{V}I_{15}$.

DISCUSSION

This is the first study to investigate the direct effects of isocapnic hypoxia on heart rate and blood pressure variability in conscious dogs. We observed that RRIHF reduced progressively during moderate-to-severe hypoxia and that MAPLF increased with mild-to-moderate hypoxia but returned toward the control level during severe hypoxia. Heart rate and MAP, on the other hand, showed a consistent increase with advancing hypoxia. These results indicate that the autonomic responses to isocapnic hypoxia involve the tonic control and phasic modulation of cardiovascular variables, but their responses to increasing intensity of hypoxia may differ.

The strength of the present study seems twofold. First, we investigated the cardiovascular responses to hypoxia in dogs under unanesthetized and standardized conditions. Cardiovascular and respiratory vari-
awake and lying quietly on the left side. Second, we employed complex demodulation to analyze the time series data. Cardiovascular variables and their oscillations during acute and progressive hypoxia could show rapid and dynamic changes, and, hence, the data could be highly nonstationary. Complex demodulation allowed us to delineate the time-dependent changes in the oscillatory components in R-R interval and MAP during advancing hypoxia.

In the present study, we observed that RRIHF was decreased by 20 and 32% during hypoxia at V˙I15 and V˙I20, respectively, compared with CC. RRIHF is a quantitative reflection of RSA, which is mediated purely by the vagus, and, hence, its amplitude is thought to reflect the respiratory modulation of cardiac vagal outflow (9, 21). However, the vagal modulation of heart period could be affected by respiratory parameters (3, 38). The magnitude of RSA has been known to decrease with increased respiratory rate and decreased tidal volume (18, 27), and these changes could occur without changes in tonic/mean level of cardiac vagal activity (13). In light of our data, although respiratory rate and tidal volume increased with progressive hypoxia, we found that the simultaneous decrease in RRIHF was not attributable to these changes in respiratory rate and tidal volume. Thus our observations lead to the assumption that isocapnic progressive hypoxia may cause a progressive reduction in the respiratory modulation of cardiac vagal outflow.

The RRIHF has often been interpreted as reflecting the tonic/mean level of cardiac vagal outflow on the basis of the assumption that the magnitude of respiratory oscillation in cardiac vagal outflow is closely correlated with its tonic/mean level (9). Such a correlation may be lost in certain conditions such as pharmacological baroreflex stimulation (12). In most physiological conditions, on the other hand, there is supportive evidence for this assumption (14, 21). Together with the concomitant reciprocal increase in heart rate (by 17 and 19% at V˙I15 and V˙I20, respectively, compared with CC), the changes in RRIHF with advancing hypoxia seem consistent with the progressive reduction in cardiac vagal tone.

During advancing hypoxia, MAP increased progressively, whereas MAPLF showed a nonlinear response; it showed the maximum increase by 121% at V˙I10 and a lesser increase by 51% at V˙I15 and returned toward the control level at V˙I20. The changes in MAP seem consistent with a progressive increase in sympathetic nerve activities, which have been demonstrated by many studies with direct measurement of sympathetic nerve activity in humans (36, 37), anesthetized dogs (19), and anesthetized (10) or unanesthetized rats (26) and by measurements of plasma norepinephrine turnover in conscious dogs (35) and rats (23). On the other hand, MAPLF reflects the magnitude of low-frequency arterial pressure oscillation known as Mayer waves. Although the amplitude of Mayer waves has been proposed as an index of tonic/mean level of sympathetic activity (20, 28), several studies have been contradictory, showing the discrepancies between the Mayer wave amplitude and direct measure of sympathetic nerve activity (39, 41). In this context, our observation of the nonlinear response of MAPLF to progressive hypoxia may be additional evidence against the thesis that Mayer waves provide an index of sympathetic activity.

Concerning the mechanisms of nonlinear MAPLF response, there may be a potential involvement of several factors that we should consider. There is controversy over the origin of Mayer waves, and at least two possible mechanisms have been suggested: the presence of a central oscillator (6) and the resonance within the baroreflex control system (24). In either case, however, low-frequency oscillation in the vasomotor sympathetic nerve activity is thought to mediate Mayer waves (5, 8, 24). In fact, a close coherence has been reported between arterial pressure and sympathetic discharge in the frequency band of Mayer waves recorded from the muscle sympathetic nerves (29, 31). Nevertheless, the magnitude of Mayer waves may be affected not only by the magnitude of low-frequency oscillations in sympathetic neural activity but also by the responsiveness of blood pressure to neural stimuli.

Fig. 2. Cardiovascular variables and their oscillatory components at control condition (CC) and during hypoxia when V˙I first reached 10 l/min (V˙I10), 15 l/min (V˙I15), and 20 l/min (V˙I20). HR, heart rate; LF/HF, high frequency-to-low frequency ratio in amplitude of R-R interval oscillation (LF/HF). *P < 0.05 vs. CC.
Concerning the determinants of the magnitude of Mayer waves, we can obtain important insights from clinical observations in patients with heart failure, which is another situation with systemic hypoxia. In patients with severe congestive heart failure, the low-frequency oscillation in muscle sympathetic nerve activity as well as that in blood pressure is reduced (2, 42). However, Ando et al. (2) observed that the coherence and gain of the transfer function from the neural activity to blood pressure were also reduced in the frequency band of Mayer waves in these patients. These observations suggest that the nonlinear MAPLF response to progressive hypoxia could result from the interactions between the hypoxic effects on the low-frequency oscillation in sympathetic nerve activity and the responsiveness of blood pressure to the neural stimuli.

The responses in cardiovascular variables and their oscillation might reflect the functional consequences of the systematic defensive responses to the threat of hypoxia of vital organs. The observed tonic cardiovascular responses to hypoxia are consistent with the suppressed cardiac vagal activity and the augmented vasomotor sympathetic activity. During advancing hypoxia, the maintenance of systemic oxygenation is critical for the organism; consequently, a redistribution of blood flow to the vital organs is necessary for survival. For this purpose, such circulatory adjustments as sinus tachycardia to increase cardiac output and an elevation in blood pressure, presumably suitable for the redistribution of blood flow, need to occur. Although the physiological role of Mayer waves is not clear, these waves are known to increase in situations that require redistribution of blood flow, such as hemorrhage (25) or upright posture (16, 33).

In a previous study of dogs, we experimentally demonstrated that RSA itself has an active physiological role, which improves the pulmonary gas exchange efficiency by temporally matching pulmonary blood flow and lung volume in each respiratory cycle and, hence, the energy efficiency of pulmonary circulation by “saving unnecessary heartbeats” during expiration (17). In the present study, we observed that RRIHF, i.e., the magnitude of RSA, decreased progressively with hypoxia. Therefore, it is assumed that the beneficial role of RSA on the pulmonary circulation might be traded off for the purpose of coping with the threat of hypoxia in the systemic circulation.

In conclusion, the autonomic responses to isocapnic progressive hypoxia involve tonic controls and phasic modulations of cardiovascular variables. The former may be characterized by the suppressed cardiac vagal activity and the probable augmented sympathetic activity, which lead to sinus tachycardia and elevated blood pressure. The latter may be characterized by the progressive reduction in respiratory vagal modulation of heart rate and the transient augmentation in low-frequency sympathetic modulation of blood pressure.

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