Effects of respiratory interval on vagal modulation of heart rate

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Hayano, Junichiro, Seiji Mukai, Masahito Sakakibara, Akiyoshi Okada, Kazuyuki Takata, and Takao Fujinami. Effects of respiratory interval on vagal modulation of heart rate. *Am. J. Physiol.* 267 (*Heart Circ. Physiol.* 36): H33–H40, 1994.—To determine whether paced breathing (PB) and respiratory interval of PB modify the relationship between spectral components of heart rate variability (HRV) and cardiac vagal tone, we studied seven healthy young males under the condition of β-adrenergic blockade by intravenous propranolol (0.2 mg/kg). Compared with spontaneous breathing, PB at the same respiratory interval as that of individual spontaneous breathing showed no significant effect on the amplitude of the high-frequency (HF) component or the mean R-R interval in either the supine or tilt position, whereas the PB decreased the amplitude of the low-frequency (LF; 0.04–0.15 Hz) component in both positions ($P = 0.004$ and 0.042, respectively). When the respiratory interval was increased from 3 to 6 s, the HF amplitude showed a progressive increase in both positions ($P = 0.001$ and 0.035, respectively), while the LF amplitude and mean R-R interval remained unchanged. These results indicate that the effects of PB and respiratory interval on the spectral components of HRV are not mediated by the changes in mean cardiac vagal tone and support the hypothesis that increased respiratory interval amplifies the respiratory-related vagal modulation of heart rate.

heart rate variability; respiratory sinus arrhythmia; power spectrum analysis; respiration; head-up tilt; humans

ANALYSIS OF HEART RATE VARIABILITY (HRV) has been widely used as an assessment of autonomic activity, particularly of cardiac vagal tone (3, 5, 29), but limitations of this method in humans have also been reported (6, 11, 19). Most of the criticism seems to focus on the influences of the respiratory parameters on the high-frequency (HF, >0.15 Hz) component of HRV, which corresponds to respiratory sinus arrhythmia (RSA). The HF component, or RSA, has been known to result from respiratory-related vagal modulation of heart rate (4, 5, 7, 24–26), and the amplitude has been demonstrated to correlate with cardiac vagal tone (5, 8, 10, 15, 18). However, it has also been well recognized that the amplitude decreases with respiratory frequency and increases with tidal volume (2, 6, 8, 11, 13, 17, 22, 26, 27). Furthermore, controversy remains as to whether these changes in the amplitude along with the respiratory parameters are mediated by the changes in the mean level of cardiac vagal tone or by the changes in the magnitude of respiratory-related vagal modulation of heart rate (11, 19, 20).

This study aimed to examine whether or not the respiratory interval modifies the relationship between the HF amplitude of HRV and cardiac vagal tone in humans. The HF amplitude was assessed by autoregressive power spectral analysis, with the mean R-R interval of electrocardiogram (ECG) under complete β-adrenergic blockade being taken as the measure of mean cardiac vagal tone. Although the respiratory interval was controlled by the paced-breathing technique, this technique has been reported to enhance cardiac vagal tone, based on the observation that paced breathing increases the relative power of the HF component to the total power of HRV (20, 23). Thus we also evaluated the effects of paced breathing at the same respiratory interval as that of individual spontaneous breathing on mean cardiac vagal tone.

METHODS

Subjects. We studied nine nonsmoking male medical students. All subjects were screened by careful medical history taking, physical examination, routine laboratory tests, chest X-ray, and resting and exercise ECG to ensure that they were in good health and without cardiovascular disease, pulmonary disease, diabetes mellitus, or uremia. No subject took any medications during the 3-wk period before the study. Two of the subjects were subsequently excluded from the study because of development of a sign or symptom of presyncope during a tilt test in this study. The mean age of the remaining seven subjects was 23 ± 2 (SD) yr. All subjects gave their written informed consent, and the procedure was performed according to Nagoya City University Medical School's ethical guidelines.

Study design. This study was designed to examine the effects of paced breathing and respiratory interval under four different conditions, i.e., combinations among two postures (supine and 70° head-up tilt) and two drug treatments (control and propranolol). The effects of the paced breathing were evaluated in the contrast between spontaneous breathing and paced breathing at the same respiratory interval as that of individual spontaneous breathing. The paced breathing was also performed at respiratory intervals of 3, 4, 5, and 6 s. The order of the drug treatments was randomized among subjects, although data were collected first in the supine position in all subjects. The spontaneous respiratory interval was determined individually in each of the four different conditions, and therefore, data were always collected first under spontaneous breathing followed by the paced breathing. The order of different respiratory intervals of the paced breathing was randomized among subjects but was fixed within each subject.

Protocol. Experiments under the two drug conditions were performed on different days at an interval of 1 wk. For both days, subjects were instructed not to consume food or beverages containing caffeine or alcohol after 2100 h the previous night. Subjects were studied in an air-conditioned laboratory (24°C) between 1000 and 1500 h more than 3 h after a light meal.

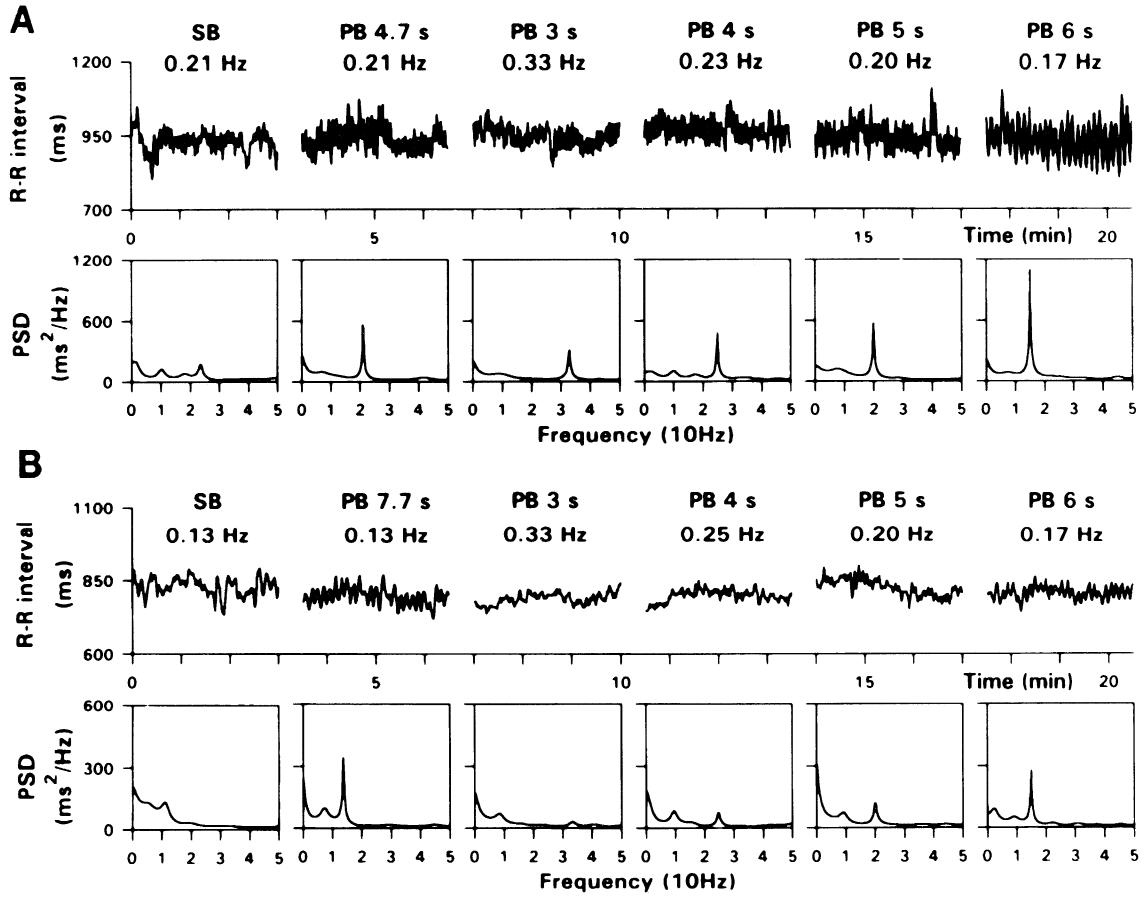

Fig. 1. Changes in R-R interval and autoregressive power spectra of R-R interval variability during spontaneous breathing (SB) and paced breathing (PB) in a representative subject under β-adrenergic blockade caused by propranolol (0.2 mg/kg). Paced breathing was performed at different respiratory intervals between 3 and 6 s, including same interval as that of spontaneous breathing in supine (*A*) and 70° head-up tilt (*B*) positions. PSD, power spectral density.

Before the experiment, subjects practiced paced breathing. We developed a computer program that generates an audio signal consisting of high (880 Hz)- and low (440 Hz)-pitched sounds, which appeared alternatively at a programmable interval. Subjects were instructed to breathe in when hearing the high-pitched sound (880 Hz) and to breathe out during the low-pitched sound (440 Hz). The durations of inspiratory and expiratory phases were set at an equal length (50:50 inspiration-to-expiration ratio). Subjects were instructed to adjust their tidal volume to a comfortable level according to the changes in respiratory interval from 3 to 6 s. They continued the training until they were familiar with this maneuver, and all subjects became able to breathe comfortably in synchrony with the audio signal at all of these respiratory intervals.

Subjects were placed on a tilt table and supported by a foot rest. ECG (CM_5 lead) and uncalibrated impedance spirogram (AI-601G; Nihon Koden, Tokyo, Japan) were recorded continuously on a frequency-modulated tape recorder (MR-30; Teac, Tokyo, Japan). Transcutaneous P_{O_2} and P_{CO_2} (OKV-7101; Nihon Koden) were monitored in four subjects under both the control and β-adrenergic blockade conditions. For on-line measurement of spontaneous respiratory interval, the respiratory waveform was also supplied directly to a microcomputer (PC9801EX; NEC, Tokyo, Japan), on which the data were digitized at 2 Hz using an analog-to-digital converter (ADX-98E; Canopus Electronics, Kobe, Japan) and stored in memory. For the purpose of other studies, noninvasive continuous blood pressure by means of arterial tonometry (CBM-7000; Colin Electronics, Komaki, Japan) was recorded from the radial artery of the left arm that was supported by a side board attached to the table.

After 30 min of supine rest, propranolol (0.2 mg/kg) was injected intravenously over 5 min in the β-adrenergic blockade condition, while the same volume of saline was injected in the control condition. After a 5-min equilibrium period, data were collected for 3 min under spontaneous breathing in the supine position while subjects were resting quietly. The power spectrum of the respiratory waveform during this period was then immediately obtained, and the spontaneous respiratory interval was determined from the mean respiratory frequency. The spectral analysis of respiratory waveform was performed by the same method as that used for spectral analysis of R-R interval described below, except that the order of autoregressive model was determined simply by Akaike's final prediction error criteria (1).

Paced breathing sessions (4 min each) were performed at five different respiratory intervals, the spontaneous respiratory interval and 3, 4, 5, and 6 s, in a randomized order. Each session was followed by a 1-min rest period, during which subjects breathed spontaneously.

Subjects were then tilted to the 70° head-up position. After 2 min, data were collected for spontaneous breathing session in the head-up tilt position. The spontaneous respiratory interval was determined, and paced breathing was performed in the same procedure as in the supine position.

Data analysis. The ECG data were played back from the frequency-modulated tape and digitized at 1 kHz by an analog-to-digital converter (ADX-98E; Canopus Electronics) and analyzed on a microcomputer (PC-9801DA; NEC). The temporal positions of all R-wave peaks were determined by a fast peak detection algorithm. The original ECG waveform with markers indicating the positions of detected R-wave peaks was completely scanned on display, all ectopic beats were checked, and any errors in R-wave detection were edited manually. No subject presented any ectopic beats during the experiment. All R-R intervals were measured, and the mean R-R interval during each experimental session was calculated. The beat-to-beat R-R intervals were then interpolated by cubic spline function and resampled at 1 Hz, yielding an equidistantly sampled R-R interval function.

Autoregressive power spectrum analysis was performed on the R-R interval function during the last 3 min in each 4-min session. The magnitude and center frequency of the low-frequency (LF) and HF components of the R-R interval variability were assessed. The detail of this method has been reported elsewhere (14–16). Briefly, the autoregressive model order was determined by Akaike's final prediction error criteria (1), but when the criteria provided an order of < 14, 14 was used to obtain well-defined peaks for the LF and HF components (9). Autoregressive coefficients were computed using the Marple algorithm (21). Spectral decomposition was accomplished by means of an algorithm based on residual integration (30), and the power and center frequency of individual spectrum components were determined. We defined the HF components as the center frequency of the spectral component being equal to the respiratory frequency corresponding to respiratory interval in each experimental session. The LF component was defined as the center frequency of the component being between 0.04 and 0.15 Hz when the respiratory frequency was > 0.15 Hz, and between 0.04 Hz and the respiratory frequency when the respiratory frequency was ≤ 0.15 Hz.

In this study, the magnitudes of the LF and HF components were expressed as the mean amplitude that was calculated by the following formula

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

We also evaluated the ratio of the mean amplitudes of the LF to HF components (LF/HF).

Statistical analysis. SAS programs (SAS Institute, Cary, NC) were used for statistical analysis. The effects of paced breathing and respiratory interval on the variables (mean R-R interval, the amplitude of the LF and HF components, and LF/HF) were analyzed separately for the four different conditions (2 postures × 2 drug treatments). The within-subject effects of paced breathing and respiratory interval were evaluated by paired t test and repeated-measure analysis of variance, respectively. Data are presented as the means ± SE unless otherwise noted. A P value < 0.05 was considered significant in all statistical analyses.

RESULTS

β-Adrenergic blockade condition. Figure 1 shows representative results obtained from a subject under β-adrenergic blockade. In both the supine and head-up tilt positions, the mean level of the R-R interval appeared unchanged, while its variability is clearly changed with the paced breathing and with the respiratory interval of the paced breathing. These changes in the R-R interval variability were reflected by the changes in autoregressive power spectra. In both the supine and tilt positions, all spectra demonstrated an HF component peak at the respiratory frequency of the paced breathing in individual sessions and an LF component peak at a frequency of ~0.1 Hz. The HF component appeared as wide-based and multiple peaks during the spontaneous breathing, while it appeared as a single sharp peak during the paced breathing, and the longer the respiratory interval, the larger the size of the HF peak in both positions.

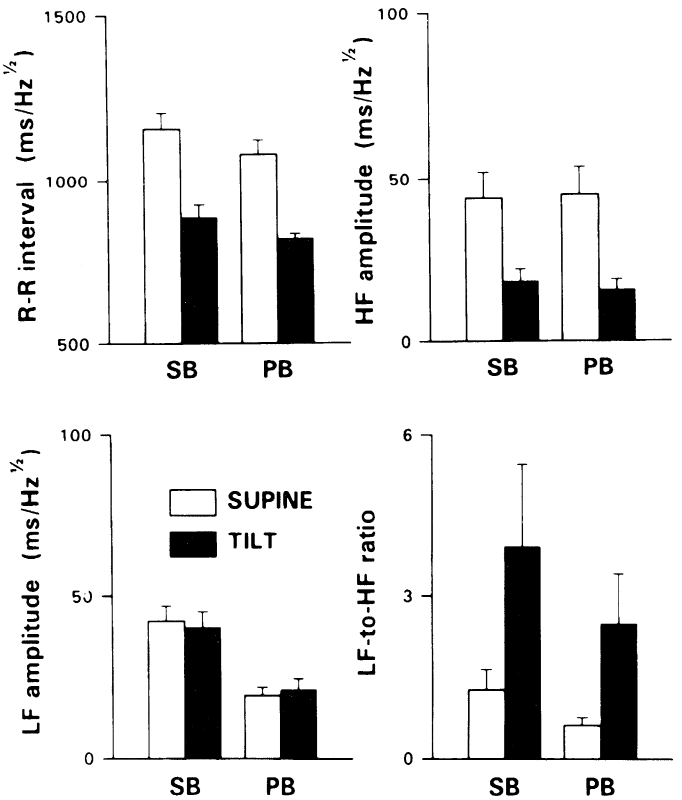

Fig. 2. Effects of paced breathing (PB) on mean R-R interval, mean amplitude of high-frequency (HF) and low-frequency (LF) components, and LF/HF of heart rate variability in 7 young healthy male subjects under β-adrenergic blockade. Paced breathing was performed at same respiratory interval as that of individual spontaneous breathing (SB) in each of supine and head-up tilt positions.

To evaluate the effects of the paced breathing, we compared spontaneous breathing and paced breathing

Table 1. *Effects of paced breathing on center frequency of low-frequency component of heart rate variability and transcutaneous P_{O_2} and P_{CO_2} in healthy young male subjects under β-adrenergic blockade condition*

	Spontaneous Breathing	Paced Breathing	P Value
Low-frequency component, Hz			
Supine	0.095 ± 0.004	0.082 ± 0.007	NS
Tilt	0.085 ± 0.005	0.077 ± 0.007	NS
Transcutaneous P_{O_2}, mmHg			
Supine	86 ± 3	86 ± 2	NS
Tilt	81 ± 5	82 ± 5	NS
Transcutaneous P_{CO_2}, mmHg			
Supine	39 ± 2	36 ± 4	NS
Tilt	37 ± 4	35 ± 4	NS

Values are means ± SE of 7 subjects. NS, not significant.

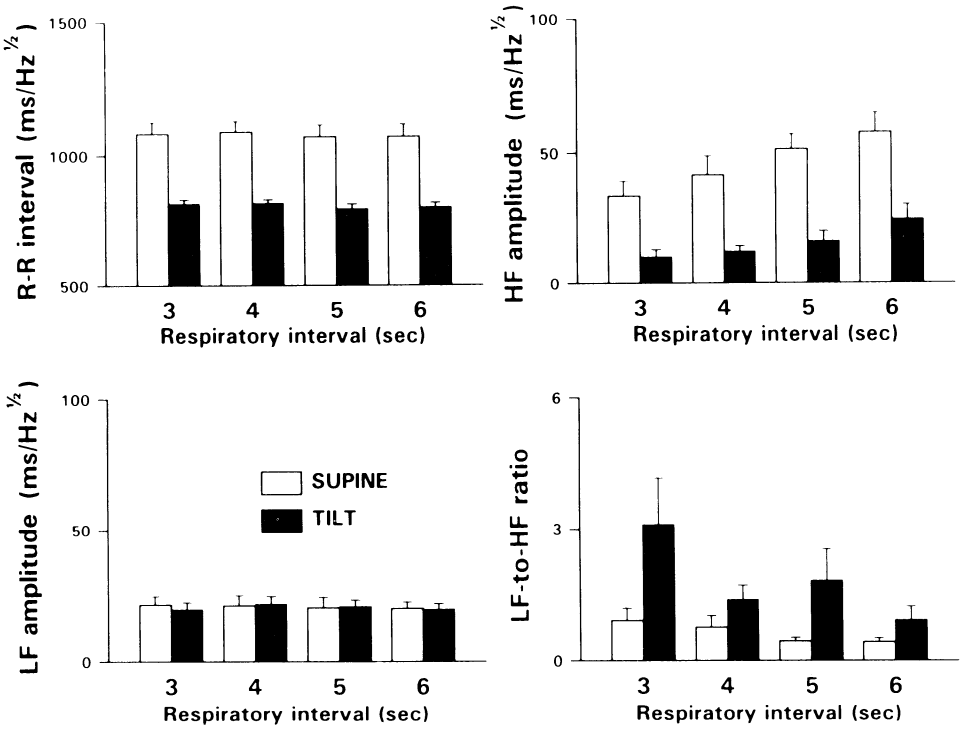

Fig. 3. Effects of respiratory interval on mean R-R interval, mean amplitude of high-frequency (HF) and low-frequency (LF) components, and LF/HF of heart rate variability in 7 young healthy male subjects under β-adrenergic blockade in supine and head-up tilt positions.

at the same respiratory interval as that of the spontaneous breathing observed under the conditions of individual postures and drug treatment in each subject. The mean R-R interval under the β-adrenergic condition decreased with the paced breathing in both the supine and tilt positions, but these changes did not reach the significant level (Fig. 2). The HF amplitude was not affected by the paced breathing in either position. Although the center frequency of the LF component did not change significantly in either position (Table 1), the LF amplitude showed a significant decrease with the paced breathing in both the supine and tilt positions (P = 0.004 and 0.042, respectively). The LF/HF decreased with the paced breathing, although the change was significant only for the supine position (P = 0.020). No significant change was observed in transcutaneous P_{O_2} or P_{CO_2} in either position (Table 1).

Figure 3 shows the effects of the respiratory interval of the paced breathing under the β-adrenergic blockade condition. While the mean R-R interval was unchanged in either position, the HF amplitude in the supine and tilt positions increased on average 1.7 and 2.3 times, respectively, when the respiratory interval was increased from 3 to 6 s (P = 0.001 and 0.035, respectively). Neither the amplitude nor the frequency of the LF component changed in either position (Table 2), but the LF/HF was influenced significantly by the respiratory interval (P = 0.049 and 0.046, respectively). No significant change was observed in transcutaneous P_{O_2} or P_{CO_2} in either position (Table 2).

Control condition. The effects of paced breathing and respiratory interval observed under the β-adrenergic blockade condition may be attributable to the effect of propranolol. To examine this possibility, we performed the same experiments without propranolol (control condition).

As shown in Table 3, the mean R-R interval under the control condition decreased with the paced breathing in

Table 2. *Effects of respiratory interval of paced breathing on center frequency of low-frequency component of heart rate variability and transcutaneous P_{O_2} and P_{CO_2} in healthy young male subjects under β-adrenergic blockade condition*

	Respiratory Interval, s				*P* Value
	3	4	5	6	
Low-frequency component, Hz					
Supine	0.080 ± 0.008	0.095 ± 0.006	0.089 ± 0.008	0.077 ± 0.004	NS
Tilt	0.086 ± 0.003	0.088 ± 0.010	0.087 ± 0.008	0.087 ± 0.005	NS
Transcutaneous P_{O_2}, mmHg					
Supine	87 ± 3	88 ± 3	88 ± 2	87 ± 3	NS
Tilt	81 ± 3	82 ± 3	83 ± 3	80 ± 3	NS
Transcutaneous P_{CO_2}, mmHg					
Supine	35 ± 2	34 ± 3	37 ± 4	36 ± 4	NS
Tilt	33 ± 2	35 ± 4	36 ± 4	34 ± 3	NS

Values are means ± SE of 7 subjects.

Table 3. *Effects of paced breathing on heart rate variability in healthy young male subjects under control (drug-free) condition*

	Spontaneous Breathing	Paced Breathing	*P* Value
R-R interval, ms			
Supine	1,006 ± 44	979 ± 50	NS
Tilt	710 ± 38	652 ± 26	0.046
HF amplitude, ms/Hz$^{1/2}$			
Supine	42.4 ± 8.2	39.1 ± 7.9	NS
Tilt	15.2 ± 4.2	22.4 ± 9.0	NS
LF amplitude, ms/Hz$^{1/2}$			
Supine	23.5 ± 3.0	19.5 ± 4.3	NS
Tilt	34.0 ± 5.1	27.1 ± 2.5	0.020
LF component, Hz			
Supine	0.098 ± 0.004	0.091 ± 0.010	NS
Tilt	0.083 ± 0.004	0.072 ± 0.007	NS
LF/HF			
Supine	1.0 ± 0.2	0.8 ± 0.1	0.019
Tilt	2.3 ± 0.6	2.6 ± 1.2	NS
Transcutaneous P_{O_2}, mmHg			
Supine	87 ± 4	85 ± 4	NS
Tilt	83 ± 2	83 ± 4	NS
Transcutaneous P_{CO_2}, mmHg			
Supine	36 ± 2	36 ± 3	NS
Tilt	36 ± 3	35 ± 3	NS

Values are means ± SE of 7 subjects. HF, high frequency; LF, low frequency; LF/HF, ratio of low-frequency to high-frequency component.

both the supine and tilt positions, but the change was significant only for the tilt position ($P = 0.046$). The HF amplitude was not affected by the paced breathing in either position, while the LF amplitude showed a significant decrease with the paced breathing in both the supine and tilt positions ($P = 0.018$ and 0.020, respectively). The center frequency of the LF component did not change significantly in either position. The LF/HF decreased with the paced breathing in both the supine and tilt positions ($P = 0.019$ and 0.040, respectively). No significant change was observed in transcutaneous P_{O_2} or P_{CO_2} in either position.

The effects of the respiratory interval under the control condition are shown in the Table 4. The results were basically the same as those obtained under the β-adrenergic blockade condition. While the mean R-R interval was unchanged in either position, the HF amplitude in both the supine and tilt positions increased with the respiratory interval ($P = 0.002$ and 0.001, respectively). Neither the amplitude nor the center frequency of the LF component changed in either position, but the LF/HF decreased with the respiratory interval ($P = 0.027$ and 0.046, for the supine and tilt positions, respectively). No significant change was observed in transcutaneous P_{O_2} or P_{CO_2} in either position.

DISCUSSION

In this study, we examined whether or not paced breathing and the respiratory interval modify the relationship between cardiac vagal tone and the spectral components of HRV. Contrasting between spontaneous breathing and paced breathing at the same respiratory interval as that of the individual spontaneous breathing, we found that the paced breathing did not increase the mean R-R interval under either the β-adrenergic blockade or control condition. We also found that the mean R-R interval was not changed by the voluntary changes in the respiratory interval from 3 to 6 s in either the supine or tilt position under either the β-adrenergic blockade or control condition, while the HF amplitude increased progressively with the increase in respiratory interval. These results indicate that the paced breathing, at least according to the method we used in this study, does not increase the mean cardiac vagal tone and that the increase in the respiratory interval increased

Table 4. *Effects of respiratory interval of paced breathing on heart rate variability in healthy young male subjects under control (drug-free) condition*

	Respiratory Interval, s				*P* Value
	3	4	5	6	
R-R interval, ms					
Supine	1,003 ± 50	1,002 ± 46	993 ± 50	979 ± 50	NS
Tilt	668 ± 30	651 ± 18	645 ± 17	654 ± 16	NS
HF amplitude, ms/Hz$^{1/2}$					
Supine	28.8 ± 5.4	50.8 ± 9.4	54.4 ± 11.8	79.5 ± 16.1	0.002
Tilt	9.7 ± 2.5	14.1 ± 3.7	19.5 ± 4.0	32.6 ± 5.4	0.001
LF amplitude, ms/Hz$^{1/2}$					
Supine	22.8 ± 6.1	21.1 ± 3.4	22.6 ± 3.9	19.8 ± 1.5	NS
Tilt	28.3 ± 6.1	28.5 ± 4.8	36.7 ± 8.3	27.5 ± 2.9	NS
LF component, Hz					
Supine	0.099 ± 0.006	0.100 ± 0.004	0.092 ± 0.009	0.084 ± 0.005	NS
Tilt	0.079 ± 0.007	0.085 ± 0.003	0.074 ± 0.006	0.073 ± 0.004	NS
LF/HF					
Supine	1.1 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.027
Tilt	3.9 ± 1.2	2.0 ± 0.5	1.7 ± 0.5	1.2 ± 0.2	0.046
Transcutaneous P_{O_2}, mmHg					
Supine	88 ± 3	90 ± 3	88 ± 3	87 ± 4	NS
Tilt	80 ± 4	83 ± 3	81 ± 4	82 ± 3	NS
Transcutaneous P_{CO_2}, mmHg					
Supine	36 ± 2	36 ± 4	37 ± 3	36 ± 4	NS
Tilt	33 ± 4	32 ± 4	34 ± 4	33 ± 4	NS

Values are means ± SE of 7 subjects.

the HF amplitude without changing the mean cardiac vagal tone.

Pagani et al. (23) reported that paced breathing decreased the LF component, increased the HF component, and consequently decreased the LF/HF in both the supine and tilt positions in healthy young subjects. Based on these observations, they concluded that during paced breathing there is enhanced vagal tone. Despite the fact that in their study the frequency of the paced breathing was set at 0.33 Hz regardless of the individual differences in the frequency of spontaneous breathing and that the magnitudes of the LF and HF components were expressed in normalized power (relative power to the total power of oscillating components), their findings seem consistent with ours concerning the changes in the spectral components of HRV. However, we observed that paced breathing did not increase the mean R-R interval under β-adrenergic blockade. Therefore, the decrease in the LF/HF with the paced breathing is not attributable to enhanced vagal tone.

Although the effects of respiratory parameters on the relationship between HRV and cardiac vagal tone have been investigated in earlier studies (11, 19), in such studies the HF/RSA amplitude was determined by a nonspectral method, i.e., it was measured as the difference between respiratory-related minimum and maximum R-R intervals (12). Kollai and Mizsei (19) examined the effects of the respiratory interval on RSA amplitude in healthy young subjects under β-adrenergic blockade and have reported that RSA amplitude invariably increased with slow breathing, but that the response of the mean R-R interval varied among subjects; it decreased, increased, or stayed at approximately the same level. They determined both RSA amplitude and mean R-R interval during paced breathing from a short segment of data (within several breath cycles, see Figs. 1 and 2 in Ref. 19). These measures could be confounded by nonrespiratory fluctuations of R-R interval; indeed, we observed that such fluctuations resulted in the changes in the mean R-R interval as well as in the RSA waveform during paced breathing (Fig. 1). However, both the observations of their study and ours commonly indicate that augmented RSA amplitude with slower respiration is not explained by an enhancement of cardiac vagal tone.

In this study, we did not control the tidal volume during paced breathing. Because no significant change was observed in transcutaneous P_{O_2} or P_{CO_2}, slower paced breathing was expected to be accompanied by a greater tidal volume, which could have also contributed to the changes in the HF amplitude. Although this study was not designed to examine separately the effects of these two factors, both increased respiratory interval and increased tidal volume have been reported to increase the HF/RSA amplitude (8, 11, 17). Our results, therefore, suggest that neither the effect of the respiratory interval nor that of the tidal volume on the HF amplitude is mediated by the changes in the mean vagal tone. This is supported by the findings of Grossman et al. (11) who have reported that a lengthening of the respiratory interval under a fixed tidal volume increases the RSA amplitude without changing the mean R-R interval under β-adrenergic blockade, although RSA and the mean R-R interval in their study were determined from a short segment of data by a nonspectral method (12).

Our observations seem consistent with those of Triedman et al. (28), who investigated the effect of hypovolemia induced by blood donation and postural change on the transfer magnitude of the modulation of heart rate by lung volume in normal subjects. They found that the transfer magnitude in the 0.12- to 0.5-Hz frequency band decreases progressively with increasing hypovolemia. However, their data also showed that at any level of hypovolemia, the transfer magnitude in this frequency band decreased uniformly with increasing frequency of lung volume fluctuation. Thus their observations also suggest that the effect of respiratory frequency on the HRV in the HF band (>0.12 Hz in their study) is independent of the changes in autonomic tone induced by hypovolemia.

Our observation that an increase in respiratory interval enhanced the HF amplitude without changing the mean cardiac vagal tone seems consistent with the currently accepted hypothesis concerning the mechanisms of RSA and that concerning vagal innervation of the sinus node. Two major physiological mechanisms have been thought to be involved in the genesis of RSA: *1*) the phasic excitatory/inhibitory modulation of vagal cardiomotor neurons by the central respiratory generator (5, 17, 25) and *2*) the phasic gating of vagoexcitatory inputs in the reflex networks receiving baroreceptor and chemoreceptor afferent barrages by pulmonary stretch receptors (5). These mechanisms could yield maximum vagal excitation during expiration and minimal or near-absent vagal activity during inspiration (18). The increase in tidal volume has been suggested to cause more effective gating of vagoexcitatory input by the lung inflation receptors, resulting in augmented RSA through increased vagal inhibition during inspiration (5, 7). On the other hand, the response of the sinus node to direct stimulation of the cardiac vagal nerve has been reported to have a low-pass filter property with a decay constant of ~ 1 s, although the sinus node response to stimulation of cardiac sympathetic nerve has a much longer decay constant, specifically, >15 s (4, 5). This property of vagal action on the sinus node seems mainly responsible for the effect of the respiratory interval on the HF amplitude.

Figure 4 represents a simulation of this effect. If the phasic respiratory variation of the vagal outflow can be simulated as a step function, the R-R interval during expiration phase would increase from an end-inspiratory level (I) toward the upper saturation level of R-R interval (E_s) that is determined by the expiratory level of vagal outflow. This process can be expressed as

$$RR(t) = E_s - (E_s - I)e^{-t/\tau} \qquad (2)$$

where $RR(t)$ is the R-R interval at time t, and τ is the decay constant for the effect of vagal activation on the R-R interval. Similarly, the R-R interval during inspiration phase decreases from an end-expiration level (E)

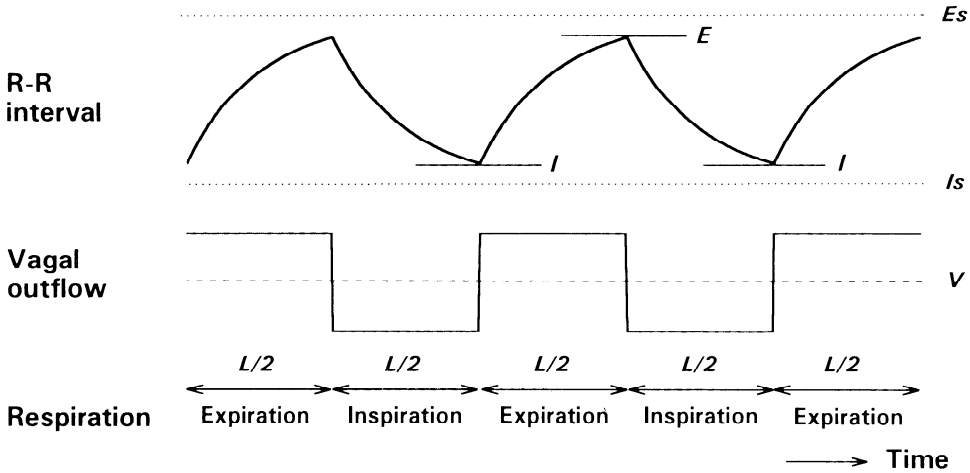

Fig. 1. Simulation model of respiratory sinus arrhythmia (RSA). Respiratory variation of vagal outflow is expressed as a step function with mean level of vagal outflow (V). R-R interval increases during expiration from an end-inspiratory level (I) toward upper saturation level (E_s) that is determined by vagal outflow during expiratory phase and decreases during inspiration from end-expiratory level (E) toward lower saturation level (I_s) that is determined by vagal outflow during inspiratory phase. These processes are expressed as $RR(t) = E_s - (E_s - I)e^{-t/\tau}$ and $RR(t) = I_s + (E - I_s)e^{-t/\tau'}$, respectively, where $RR(t)$ is R-R interval at time t and τ and τ' are decay constant for effects of vagal modulation of R-R interval. When respiratory interval (L) comprises an equal length of expiratory and inspiratory phases, amplitude of RSA is expressed as $E - I = (E_s - I_s)(1 - e^{-L/2\tau})/(1 + e^{-L/2\tau'})$.

toward the lower saturation level (I_s) that is determined by the inspiratory level of vagal outflow, and this process can be expressed as

$$RR(t) = I_s + (E - I_s)e^{-t/\tau'} \quad (3)$$

where τ' is the decay constant for the effect of vagal withdrawal on the R-R interval. When the respiratory interval is controlled to be L seconds, *Eqs. 2* and *3* can be written as

$$E = E_s - (E_s - I)e^{-L/2\tau} \quad (4)$$

and

$$I = I_s + (E - I_s)e^{-L/2\tau'} \quad (5)$$

If we assume that $\tau = \tau' = 1$ s as reported, *Eqs. 4* and *5* would yield

$$E - I = (E_s - I_s)(1 - e^{-L/2})/(1 + e^{-L/2}) \quad (6)$$

In *Eq. 6*, $E - I$ represents the HF amplitude and $E_s - I_s$ is thought to be determined by the difference in cardiac vagal outflow between the inspiration and expiration phases. Thus, when the vagal outflow during the inspiratory phase could be assumed to be approximately zero, the $E_s - I_s$ would be equal to two times mean cardiac vagal control, and thus, proportional to the mean vagal outflow. According to *Eq. 6*, the HF amplitudes at a respiratory interval (L) of 3, 4, 5, and 6 s are calculated as 64, 76, 85, and 91% of $E_s - I_s$, respectively. Therefore, the increase in the HF amplitude with the increase in the respiratory interval from 3 to 6 s is estimated as 1.43 times. This value appears to be smaller than those actually observed in this study (1.7 and 2.3 times in the supine and tilt positions, respectively), suggesting possible underestimation of the τ value, or the involvement of other factors in the increase in the HF amplitude, among which the effect of the increasing tidal volume on the pulmonary inflation reflex is most likely, which decreases I_s in *Eq. 6* through more effective gating of vagoexcitatory input during the inspiratory phase (5).

Our finding that respiratory interval modifies the relationship between cardiac vagal tone and the spectral components of HRV clearly indicates the importance of controlling and measuring respiration when assessing autonomic nervous activity by HRV. In both clinical and basic researches, the amplitude of the HF component and the LF/HF have been widely used as an index of cardiac vagal tone and of sympathovagal balance, respectively (3, 5, 29). In this study, however, we found that an increase in the respiratory interval augments the HF amplitude and decreases the LF/HF without changing the mean R-R interval under both the control and β-adrenergic blockade conditions. These observations indicate that the increase in the HF amplitude and the decrease in the LF/HF with the increasing respiratory interval do not reflect either an increase in cardiac vagal tone or a decrease in sympathovagal balance. Thus, in the assessment of autonomic activity by HRV, it is important to compare both between individuals and within individuals with similar respiratory frequency contents.

Conclusion. We investigated the effects of paced breathing and respiratory interval on the relationship between the spectral components of HRV and the mean cardiac vagal tone. Under both the control and β-adrenergic blockade conditions, we found that paced breathing decreased the LF/HF without an increase in the mean R-R interval and that an increase in the respiratory interval augmented the HF amplitude without changing the mean R-R interval. The results of this study suggest that neither the increase in the LF/HF with paced breathing nor the increase in the HF amplitude with the increasing respiratory interval is mediated

by an increase in the mean cardiac vagal tone. Our findings support the hypothesis that an increase in the respiratory interval amplifies the respiratory-related vagal modulation of heart rate.

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