J Anesth (1995) 9:229–234

Journal of
Anesthesia

Spontaneous respiration should be avoided in frequency domain analysis of heart rate variability

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Abstract: To determine whether spontaneous respiration is suitable for frequency domain analysis of heart rate (R–R interval) variability, we studied 15 volunteers (5 men and 10 women, aged 22–34 years) and evaluated the reproducibility of the power spectrum. Electrocardiograms were recorded for 5 min each with spontaneous and rate-controlled respiration (15 breaths·min^{-1}), repeating the same protocol 1 week later. Fast Fourier transformation was performed using the digitized data of the R–R intervals. Mean heart rate, arterial pressure, and plasma catecholamines remained constant during the measurements. In spontaneous respiration, however, the respiratory rate was significantly lower during the second measurement (9.4 ± 2.1 breaths·min^{-1}) than during the first measurement (10.9 ± 2.6 breaths·min^{-1}), and the low-frequency power increased from 2.61 ± 2.36 to 5.14 ± 5.06 sec^2·Hz^{-1}·10^{-3}. After deleting five data sets because the respiratory peak was inseparable from the low-frequency area, there was no correlation in power spectra in four out of ten subjects between the two measurements. Data were comparable for rate-controlled respiration. Since respiratory parameters strongly influenced the low- and the high-frequency R–R interval power spectra, spontaneous respiration should be avoided. A constant respiratory condition is required to interpret results of frequency domain analysis of R–R interval variability.

Key words: R–R interval, Power spectrum, Respiration

Introduction

Since its introduction in 1973 by Sayers [1], frequency domain analysis of the heart rate (R–R interval) has been useful in studying autonomic control of the heart. There are two major spectral components in power spectrum density analysis: a high-frequency (HF) component at about the frequency of respiration, and a low-frequency (LF) component at about 0.1 Hz. The HF component reflects only parasympathetic activitiy while the LF component reflects sympathetic as well as vagal cardiac outflow [2].

The variability of the R–R interval is influenced by changes in respiratory depth, interval, quantity, and periodicity. The relationship between respiratory phase and the R–R interval was investigated by Eckberg [3], who found a phase effect of vagal cardiac outflow on the control of heart rate.

Considering the relationship between respiration and R–R interval variability, it is important to control the respiration in studying the variability of the R–R interval regardless of method. Nevertheless, many investigators still employ spontaneous respiration, especially in the studies of frequency domain analysis [4]. Some authors [5,6] argue that rate-controlled respiration modifies the respiratory wave form and subsequently affects the low- as well as the high-frequency power spectrum. However, when using this method for monitoring autonomic cardiac function, especially during anesthesia, it is necessary to see if the power spectra measured under spontaneous respiration are interpretable and can be used for further analysis such as the integrated area under the curve and the LF/HF ratio. Our objective was to evaluate the consistency of the respiratory condition and the reproducibility of R–R interval power spectrum density analysis during spontaneous and rate-controlled respiration in the measurements which were repeated after 1 week.

Materials and methods

Subjects

A total of 15 healthy young adults, 5 men and 10 women, aged 23–34 (mean 24.5) years, were studied. This study was approved by the committee on human investigation of Kure National Hospital. Each subject

Address correspondence to: J. Koh
Received for publication on January 24, 1994; accepted on March 23, 1995

gave written informed consent before participating in the study.

Measurements

The subjects were studied in a quiet room while in the supine position. A catheter was placed in an antecubital vein to sample blood. Tidal volume and airway flow (taken as respiratory activity) were measured through a mouthpiece connected to a ventilator (Servo 900-C, Siemence-Elema AB, Solna, Sweden). The electrocardiogram (ECG) was recorded via chest leads. The beat-to-beat arterial pressure was estimated with a photoplethysmograph (Finapres Model 2300, Ohmeda, Englewood, CO, USA), that was placed on the middle digit of the middle finger. Arterial pressure was also measured every 2.5 min using an automated oscillometric device (Pulsemate BX-5, Colin Electronics, Komaki, Aichi, Japan). Physiologic signals were transcribed onto *magnetic tape* and electrostatic paper recorders.

Biochemical measurements

Blood was drawn from the antecubital vein into prechilled syringes during the last seconds of each measurement, and was immediately, transferred to glass tubes which contained ethylenediaminetetraacetic acid, and then placed on ice. The tubes were then centrifuged at 4°C at 3000 rpm for 10 min, transferred to silicone tubes, and frozen at −30°C. Plasma noradrenaline and adrenaline were measured by high-performance liquid chromatography by SRL (Special Reference Laboratory, Tokyo, Japan).

Experimental protocol

The experiment was begun after a 20-min rest period. We obtained two sets of 5-min recordings, one during spontaneous respiration and the other during rate-controlled respiration at 15 breaths/min (the first measurements). Each subject started an inspiration on hearing a sound from the metronome. The experiment was repeated after 1 week using the same protocol and the same room (the second measurements).

Data analysis

The ECG and data on tidal volume, airway flow, and beat-to-beat arterial pressure wave forms were digitized at 250 Hz using signal acquisition hardware and software (CODAS, Dataq Instruments, Akron, OH, USA) and stored in a personal computer. Peak and valley analysis were performed to determine the following: (1) respiratory interval, (2) tidal volume, (3) R–R interval, and (4) systolic and diastolic pressure. Subsequent analyses were performed with custom programs developed for DADiSP software (DSP Development, Cambridge, MA, USA).

R–R interval spectral power was derived by a periodogram method based on the Welch alogorithm [7]. The procedure involved analysis of 256 s of beat-to-beat R–R intervals. The time series was interpolated linearly at 8 Hz to obtain equidistant time intervals that were divided into seven equal overlapping segments. Each segment was detrended, Hanning window-filtered after putting 0 values until the segment's series became 256 long, and fast Fourier-transformed to its frequency representation squared. Modified periodograms were averaged to produce the spectrum estimate. The frequency resolution for this procedure was 0.0039 Hz [8]. To exclude the direct current component and to minimize the effect of LF noise component, the 0.05–0.5 Hz frequency band was used for statistical comparisons of correlation coefficients. The low- and high-frequency areas were defined as 0.05–0.15 and 0.15–0.5 Hz, respectively.

The use of power spectra computed with fast Fourier transformation requires that the signals be stationary. Although this stationary condition of defined according to strict mathematical guidelines, probably does not exist in physiological recordings made in humans, a *weak stationarity* may exist [9]. We evaluated weak stationarity as follows: each 64-s data segment was further divided into two 32-s segments. These two data sets were analyzed in two ways: (1) it was determined whether the average segment means deviated by more than 10% and (2) the variances of the two segments were compared for each subject with the paired *t*-test to determine whether the differences were statistically significant [8].

Statistical analysis

Values are expressed as mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA). The Fisher's LSD test was performed for multiple comparisons if the null hypothesis was rejected. The paired Wilcoxon test was performed to compare the respiratory rate. Spearman's correlation coefficients were calculated for power spectrum comparisons. $P < 0.05$ was considered statistically significant.

Results

The values obtained in each respiratory mode are shown in Table 1. Mean heart rate, mean systolic and diastolic pressure, and plasma catecholamines were essentially unchanged throughout the experiment. In the

Table 1. Measurements obtained during differing respiratory modes

Parameter	SR1	CR1	SR2	CR2
mHR (min^{-1})	64.2 ± 8.1	62.7 ± 7.7	62.8 ± 7.7	63.2 ± 8.8
mSBP (mmHg)	111.0 ± 8.7	109.7 ± 8.8	110.2 ± 9.8	112.0 ± 9.8
mDBP (mmHg)	61.3 ± 6.4	60.9 ± 6.5	60.6 ± 5.8	61.9 ± 5.3
Adr ($pg \cdot ml^{-1}$)	29.0 ± 9.9	25.9 ± 10.0	25.7 ± 11.5	25.6 ± 13.9
Nor ($pg \cdot ml^{-1}$)	227 ± 66	221 ± 85	210 ± 74	198 ± 71
mTV (ml)	597 ± 273	449 ± 127	691 ± 373#	468 ± 162§
mRR (min^{-1})	10.9 ± 2.6	15	9.4 ± 2.1*	15
LF ($sec^2 \cdot Hz^{-1} \cdot 10^{-3}$)	2.61 ± 2.36 (n = 10)[a]	0.74 ± 0.64	5.14 ± 5.06*# (n = 10)[a]	1.25 ± 1.41§
HF ($sec^2 \cdot Hz^{-1} \cdot 10^{-3}$)	3.02 ± 2.62 (n = 10)[a]	2.11 ± 1.57	5.48 ± 4.71 (n = 10)[a]	3.06 ± 2.92

Values are expressed as mean ± SD.
SR1, first spontaneous respiration; CR1, first rate-controlled respiration; SR2, second spontaneous respiration; CR2, second rate-controlled respiration; mHR, mean heart rate; mSBP, mean systolic blood pressure; mDBP, mean diastolic blood pressure; Adr, mean plasma adrenaline concentration; Nor, mean plasma noradrenaline concentration; mTV, mean tidal volume; mRR, mean respiratory rate; LF, integrated area under curve around low frequency; HF, integrated area under curve around high frequency.
[a] Number of subjects after inappropriate data were deleted.
* $P < 0.05$ *vs* SR1.
# $P < 0.05$ *vs* CR1.
§ $P < 0.05$ *vs* SR2.

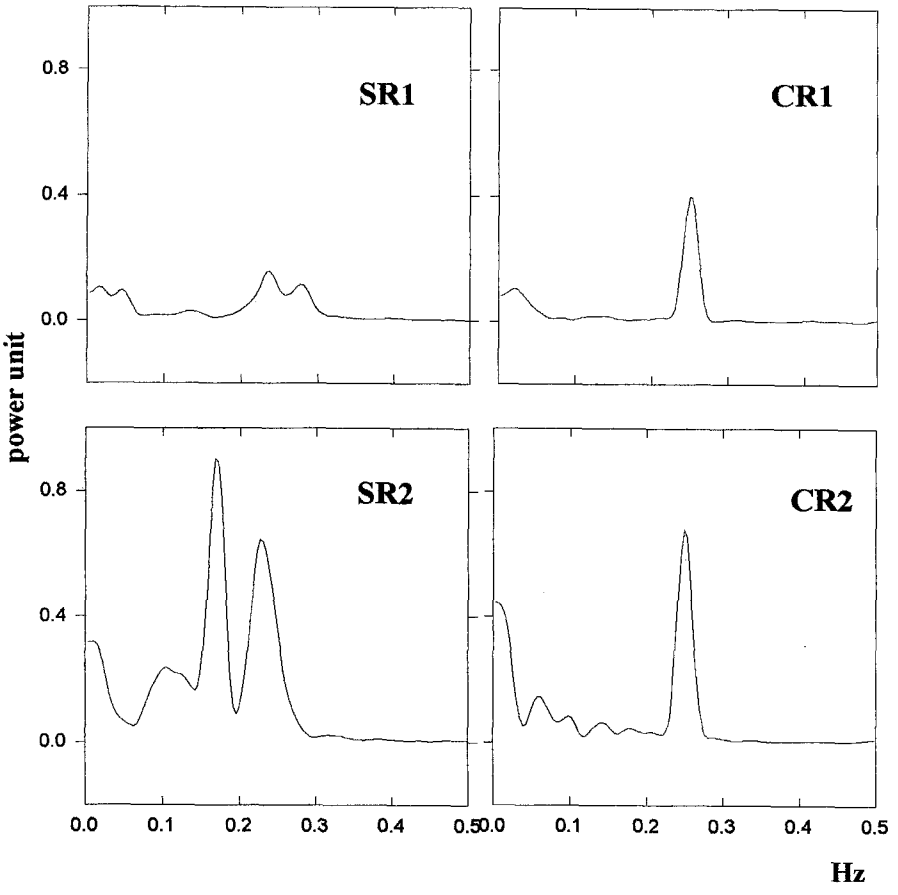

Fig. 1. Frequency domain analysis of heart rate (R–R interval) variability in one subject (no. 3) for four different respiratory measurements. *SR1*, the first spontaneous respiration; *CR1*, the first rate-controlled respiration; *SR2*, the second spontaneous respiration; *CR2*, the second rate-controlled respiration. *Power unit*, $sec^2 \cdot Hz^{-1} \cdot 10^{-3}$

second measurements, the mean tidal volume of spontaneous respiration was significantly greater than that of rate-controlled respiration, and the mean respiratory rate was less than that in the first measurements.

Concerning the frequency domain analysis, all the time series to be analyzed in this study satisfied the criteria for weak stationarity. However, we eliminated five data sets in the spontaneous respiration mode for

Table 2. Correlation coefficient (r) of power spectra between the first and the second measurement

Subject	SR1–SR2	CR1–CR2
1	...	0.73 ○
2	0.76 ○	0.82 ○
3	−0.05 ×	0.33 ○
4	0.86 ○	0.88 ○
5	...	0.88 ○
6	−0.16 ×	0.71 ○
7	0.85 ○	0.87 ○
8	0.81 ○	0.58 ○
9	...	0.88 ○
10	...	0.97 ○
11	0.71 ○	0.72 ○
12	...	0.74 ○
13	0.21 ×	0.50 ○
14	0.86 ○	0.95 ○
15	−0.24 ×	0.81 ○

○, two power spectra show a good correlation with P value < 0.05; ×, power spectra shows no correlation; dotted line, comparison not performed.

five subjects because the respiratory frequency of one or two measurements fell into the LF area. Consequently, it was not possible to distinguish the HF and LF respiratory peaks. After the inappropriate data were deleted, the LF power spectrum was increased significantly in the second measurements. Although the HF power was higher in the second than in the first measurements, the difference was not significant. Values were comparable in the rate-controlled respiratory mode.

Figure 1 shows the frequency domain analysis of the R–R interval from one subject during four different measurements. During rate-controlled respiration, the power spectra showed clear HF peaks (at ~0.25 Hz) and small LF peaks (at ~0.1 Hz) in both the first and second measurements. During spontaneous respiration, this subject showed two HF peaks in the first measurements. Two HF peaks and a small LF peak were also found in the second measurements, however, there was no correlation between the two spectra curves.

The correlation between the two respiratory measurements in the frequency domain analysis are shown in Table 2. No correlation between the two measurements of spontaneous respiration were found in four of the ten subjects. All the curves showed a good correlation in the rate-controlled respiration.

Discussion

The results indicate that spontaneous respiration should be avoided and that some respiratory control should be instituted when conducting frequency domain analysis of the R–R interval. Since frequency domain analysis of the variability of the R–R interval was first introduced by Sayer [1], it has been used to evaluate autonomic cardiac outflow to the heart. This approach provides quantitative information on the level of the sympathetic and the vagal cardiac neural outflow. Concerning methodology, there is no agreement about the control of respiration. Brown et al. [4] did a meta-analysis of the data in 147 articles published on frequency domain analysis of R–R interval variability and reported that about one-half of the studies involved the measurement of the respiratory rate, while about one-third involved control of the respiratory rate. Thus, many investigators have conducted studies using spontaneous respiration. Some investigators have advocated the control of respiration when studying the variability of the R–R interval, regardless of the method used [4,10,11].

The relationship between respiration and the power spectrum of the R–R interval has been studied [4,12]. These studies were based on the fact that such respiratory paramenters as respiratory rate, tidal volume, and end-tidal CO_2 each exert a major effect on the variability of the R–R interval [3,13]. In contrast, Pagani et al. [5] showed that voluntary control of breathing increased the power at respiratory frequencies, decreasing the power at low frequencies, and markedly blunting the increase of the LF component and the LF/HF ratio to tilt stimulation. Those investigators believe that these findings point toward a shift in the sympathovagal balance to the vagal component. Frequency domain analysis of the R–R interval time series is sometimes used for one-point diagnostic measurement and for monitoring autonomic nervous control of the heart, especially during anesthesia [14]. It was thus important to determine which mode of respiration, spontaneous or controlled, would be suitable for use in the power spectrum density analysis of the R–R interval.

The mean respiratory rate decreased from 10.9 ± 2.6 to 9.4 ± 2.1 breaths·min^{-1} in the two trials of spontaneous respiration. Data sets were deleted in five subjects because of the slow respiratory rates. Four subjects had a respiratory rate of less than 9 breaths·min^{-1} in the first measurement as did five subjects in the second measurement. Even though these data sets were eliminated, the LF power increased significantly in the second measurements. Correlation of the spectra were not found in the data sets of four subjects. These findings indicate that, in spontaneous respiration: (1) the respiratory rate is not always constant for the different measurements, (2) the respiratory peak is sometimes inseparable from the LF peak in frequency domain analysis, and consequently affects the LF power, and finally (4) the outcome of the power spectrum is not always constant. These observations suggest that the reproducibility of the R–R interval power spectrum is poor when the subject breathes freely.

The breathing pattern of healthy subjects is reportedly highly variable during brief recording sessions

[15,16]. A substantial percentage of breaths occur at frequencies between 9 and 20 breaths/min [17]. Novak et al. [11] reported that, at rest, the spontaneous slowing of respiration to the lower range is common, which consequently alter the LF component. These authors concluded that a false positive might result if the LH/HF ratio was considered as a marker of sympatho-excitation when alteration of respiratory patterns occur. High-frequency power is quite sensitive to the respiratory rate and tidal volume [4,12]. These findings are consistent with our observations that the LF power of spontaneous respiration always exceeded that of rate-controlled respiration and increased with slowing of the respiratory rate.

One might ask whether voluntarily controlling the respiration might influence LF power since mental stress alters sympatho-vagal balance, and subsequently affects the LF/HF frequency power ratio [18,19]. We carried out our measurements in a quiet room; the volunteers were supine, and the plasma levels of catecholamines showed no change throughout the experiment. Thus, we believed that the effect of mental stress was minimal.

Our subjects were under 35 years of age. The R–R interval variance and the magnitude of the baroreceptor cardiac reflex is decreased in subjects older than 35 years [20]. Hayano [21] showed that the response to tilt differs in those under the age of 35 and those over that age. Age should therefore be considered in interpreting the power spectrum, especially the LF, as the precise mechanism of the decreased LF fluctuation of the R–R intervals in older subjects is not clearly understood.

We did not investigate the optimal respiratory frequency for power spectrum analysis or the relationship between spectral power and tidal volume. Concerning the recommended frequency, any value above 0.15 Hz might be available. We used 0.25 Hz in accordance with Pagani's study [5] which showed the average spontaneous respiratory rate to be 14.4 breaths·min^{-1}. Other studies employed a respiratory frequency of around 0.25 Hz [4,8]. Few studies have evaluated the relationship between tidal volume and R–R interval power spectrum. Hirsch and Bishop [13] found that the magnitude of respiratory sinus arrhythmia is proportional to tidal volume. Brown et al. [4] calculated the R–R interval power spectrum with a different respiratory rate and tidal volume. However, in our study, the tidal volume was not controlled so it was not possible to determine its effect on the power spectrum. Additional study is required to define the relationship between vagal and sympathetic cardiac outflow and the variability of the R–R interval.

In summary, R–R interval variability was studied during spontaneous and rate-controlled respiration with frequency domain analysis. We conclude that the control of respiration is required to obtain a power spectrum of the R–R interval that can be interpreted clearly.

Acknowledgments. We thank the subjects who volunteered for this study. We also thank Hidetoshi Nagato and Toshihiko Sato for their advice on statistical analysis.

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