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Do the Oscillations of Cardiovascular Parameters Persist during Voluntary Apnea in Humans?

M. JAVORKA, I. ŽILA, K. JAVORKA, A. ČALKOVSKÁ

Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovak Republic

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Summary

The aim of this study was to ascertain the persistence of heart rate and blood pressure oscillations at the onset of voluntary apnea in humans and to assess the dependence of the fluctuations` parameters on the chemoreceptor activity. In 24 young subjects (10 males, 14 females, mean age 20.4 years) heart rate (represented by its reciprocal value – RR-intervals), systolic blood pressure (SBP) and diastolic blood pressure (DBP) during controlled breathing (CB) of atmospheric air and oxygen followed by apnea were recorded continuously. The cosine functions were then fitted by nonlinear regression analysis to the heart rate, SBP and DBP oscillations during CB and at the onset of apnea. The parameters of oscillations were different during atmospheric air breathing compared to oxygen breathing. During oxygen breathing there was an increase of the RR-interval oscillations – relative bradycardia and enhanced magnitude of respiratory sinus arythmia. During apnea, the base level of the blood pressure oscillations was higher after breathing of atmospheric air compared o oxygen breathing. At least one cosine-like wave oscillation was present at the onset of apnea in the heart rate, SBP and DBP and the second wave was present in all assessed parameters in at least 70 % of recordings. The oscillations in RR-intervals are, to some extent, independent of blood pressure oscillations. No significant gender differences were found either in the duration of breath holding or in the RR and SBP oscillations parameters.

Key words
Apnea • Respiratory sinus arrhythmia • Heart rate • Blood pressure • Oscillations

Introduction

Cardiovascular parameters, namely heart rate (HR) and blood pressure (BP), vary over a time span ranging from several seconds to the life time of an individual (Tazawa *et al.* 1999). Sophisticated mathematical analyses can reveal several patterns in these fluctuations and they can be utilized for diagnostic purposes e.g. for the assessment of baroreflex sensitivity (Honzíková *et al.* 1992, Fišer *et al.* 1993).

The influence of ventilation on the heart rate and blood pressure is well-known. Despite many past studies, the precise complex mechanisms of the respiratory sinus arrhythmia (RSA) are still debated.

RSA is primarily mediated by modulation of parasympathetic outflow. The mechanism of RSA can

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

generally be divided into central and peripheral components: *The central component* is based on the existence of a central link between the respiratory and cardiac rhythm generating neurons in the medulla. *The peripheral component* includes phasic alterations (by changes in venous return during respiratory movements) of the high and low pressure baroreceptors, vagal feedback from the pulmonary stretch and thoracic wall receptors, alteration of heart rate by local stretch of the sinus node etc. (Freyschuss and Melcher 1976, Bernardi *et al.* 1989, Honzíková 1992, Piepoli *et al.* 1997).

Previous experimental studies have shown that RSA-like fluctuations persist during breath holding in animals, e.g. in dogs, even after elimination of the peripheral RSA component. Horner *et al.* (1995) observed in five adult dogs that heart rate variability persisted at the onset of apnea (at least first cycle), and this variability had a periodicity similar to that observed during the preceding period of mechanical ventilation. These authors suggested that the memory of a preceding period of cyclic lung and chest wall movements may be present at the onset of central apnea and that neuronal networks of the respiratory system are capable of a short-term memory of preceding breathing movements and stimuli.

The oscillations of heart rate and peripheral blood pressure on the basis of beat-to-beat analysis *during apnea* have not yet been reported in humans. Therefore *the main aims* of our study were to study: 1) the existence or absence of rhythmic oscillations in heart rate and peripheral blood pressure during apnea in humans, 2) the dependence of parameters of these oscillations on chemoreflex activity.

Methods

Subjects

Studies were performed on 24 healthy volunteers (14 females, 10 males) aged 18.4-24.2 years (mean age 20.4±1.6) with the body mass index (BMI) in the range 14.6-30.7 kg/m^2 (mean BMI 21.4±3.7). The subjects were not taking any drugs, coffee and they did not smoke in the time preceding experiments. The subjects were informed about the protocol of the experiments but not about the purpose of the study.

Experimental protocol

Subjects were examined in quiet room in the morning (between 8.00 to 11.00 h) in the sitting position. Continuous recordings of RR-intervals, systolic (SBP) and diastolic (DBP) blood pressure were obtained during two phases of the experiment: 1) during controlled breathing (CB) and 2) during voluntary apnea

In the first phase, breathing was controlled for two minutes at a frequency of 10 cycles per minute (167 mHz) according to metronome pacing. The subjects were asked to maintain their tidal volume at 30 % of their previously determined vital capacity by visual observation of a pneumotachographic recording (Commet LBL 50, Bratislava, Slovak Republic).

The second phase started at the end of the first phase after normal expiration. The subjects were asked to hold their breath volitionally for as long as possible. During both phases, the subjects used nose clip.

This experiment was performed twice in every subject. During the first phase, the volunteers breathed atmospheric air (FiO_2 = 0.21 - atmospheric air breathing - AAB). During the second phase, the volunteers breathed pure oxygen (FiO_2 = 1.00) from a Douglas bag through a two-way valve (oxygen breathing - OB).

Data aquisition

The heart rate (HR) expressed by its reciprocal value of RR intervals, SBP and DBP were recorded simultaneously and noninvasively. The RR-interval time series were obtained using telemetric system (VariaCardio TF4, Sima Media, Olomouc, the Czech Republic). ECG signal (sampling rate 1000 Hz) from thoracic lead was transmitted to the computer for detection of R-waves and successive derivation of the RR-time series. Only a few premature beats and artifacts were identified automatically and corrected by linear interpolation with the previous and following beats.

SBP and DBP were measured by means of continuous noninvasive blood pressure monitor Finapres (Ohmeda 2300, USA) based on volume-clamp method (Peňáz 1969, 1973). The appropriate size cuff of Finapres was wrapped around the middle fallanx of the third finger of the left hand which was passively maintained at heart level to avoid errors due to hydrostatic pressure.

Data analyses

The series of RR-intervals, SBP and DBP were transferred into a computer for further analyses. Firstly, the real time was reconstructed from RR-interval series to obtain the time course of the measured parameters and, consequently, to ensure the computation of frequencies of periodic oscillations in Hertz (Hz) (not in cycles per

beat). Because we were focused on analyses of the short-term fluctuations, long-term fluctuations and/or trends, which occurred in records, had to be eliminated. Therefore, we subtracted the smoothed time series of RR-intervals, SBP, or DBP from original raw data. The process of smoothing was performed using LOWESS smoother (SYSTAT 6.0 for Windows, SPSS Inc., 1996) with tension set to 0.15. Thus, we obtained detrended time series for every parameter (three parameters) and for both experiments (AAB, OB) (six time series for every subject).

Furthermore, the time segments for nonlinear regression analysis (NLR) were selected. The first segment (CB segment) consisted of the last four wave-like oscillations at the end of the first phase, immediately before apnea (because of controlled frequency of breathing, this segment lasted 24 s (four-times period of breathing). In 7 recordings out of 48 (14 %) from 4 subjects, several parameters were not obtained due to technical problems (3 recordings), poor subjective control of breathing (1 recording), and lack of regular cosine-like waves (3 recordings). During the apnea, the first two waves (W1, W2) were analyzed separately, therefore the second and third segments consisted of the first and second wave (W1 and W2) after the onset of apnea at the beginning of phase two.

Then, the procedure of NLR was applied to three selected segments of RR, SBP, and DBP time series (CB, W1, and W2) separately. Process of curve fitting (NLR) was performed and parameters of oscillations were computed using Gauss-Newton algorithm for estimating nonlinear models. We used simple cosine wave model based on these equations:

$$RR_d = base_{RR} + amplitude_{RR} \cos((2\pi\ frequency_{RR}\ time) + \varphi_{RR}))$$

$$SBP_d = base_{SBP} + Amplitude_{SBP} \cos((2\pi\ frequency_{SBP}\ time) + \varphi_{SBP}))$$

$$DBP_d = base_{DBP} + amplitude_{DBP} \cos((2\pi\ frequency_{DBP}\ time) + \varphi_{DBP}))$$

for RR, SBP and DBP time series segments separately, where RR_d, SBP_d, and DBP_d were the measured variables (at corresponding times) after detrending, and the base, amplitude and frequency were computed parameters.

The base, amplitude, and frequency were assessed as parameters of oscillations in RR, SBP and DBP time series segments computed by the NLR process.

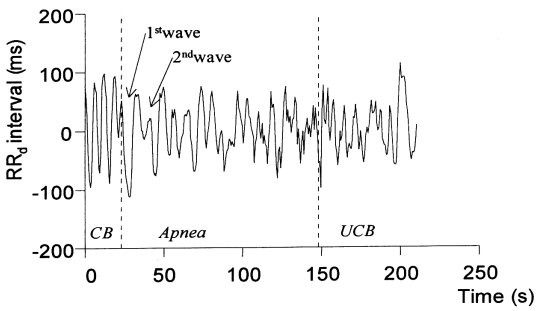

Fig. 1. *Recording from one subject after detrending process. RR_d intervals were computed as deviations of raw time series from fitted smoothed curve. Arrows indicate 1st and 2nd wave at the beginning of apnea. Vertical dashed lines separate analyzed time interval into three parts - CB (controlled breathing period), apnea and UCB (uncontrolled breathing period). Controlled breathing and first two waves during apnea were analyzed.*

The amplitude was the measure of the magnitude of oscillations, the frequency described the period of oscillations (the computed frequency was in Hertz and the period of oscillations (in seconds) can therefore be computed as the reciprocal value of the frequency). The base determined the value of RR (of SBP or DBP) around which a variable fluctuated: because of the detrending process, its value approximately equals zero. Therefore, we corrected the value of the base by adiing the mean of the corresponding smoothed time series segment. Further calculated parameter - mean corrected R^2 (MCR) - is the variability proportion of the obtained data explained by this model, and thus can be regarded as a measure of goodness of the curve fitting. Ideally, if this model can describe the all variability and, in our case, the constructed curve strictly followed a simple cosine wave, the MCR would equal one.

Statistical analysis

The results are given as mean ± S.D. Because of non-Gaussian distribution of variables (nonzero skewness, bimodal distributions of W1, W2 frequencies), the nonparametric tests were used. For within-subject comparisons (AAB vs. OB experiment, CB vs. W1 vs. W2 segments) Wilcoxon matched pairs signed rank test was performed. The inter-group differences (gender) were performed by Mann-Whitney U-test for two independent groups. Pearson's correlation coefficients were used for analyses of interdependency of selected parameters. A significance level of $p<0.05$ was used for all statistical tests. Values are presented as means ± S.D.

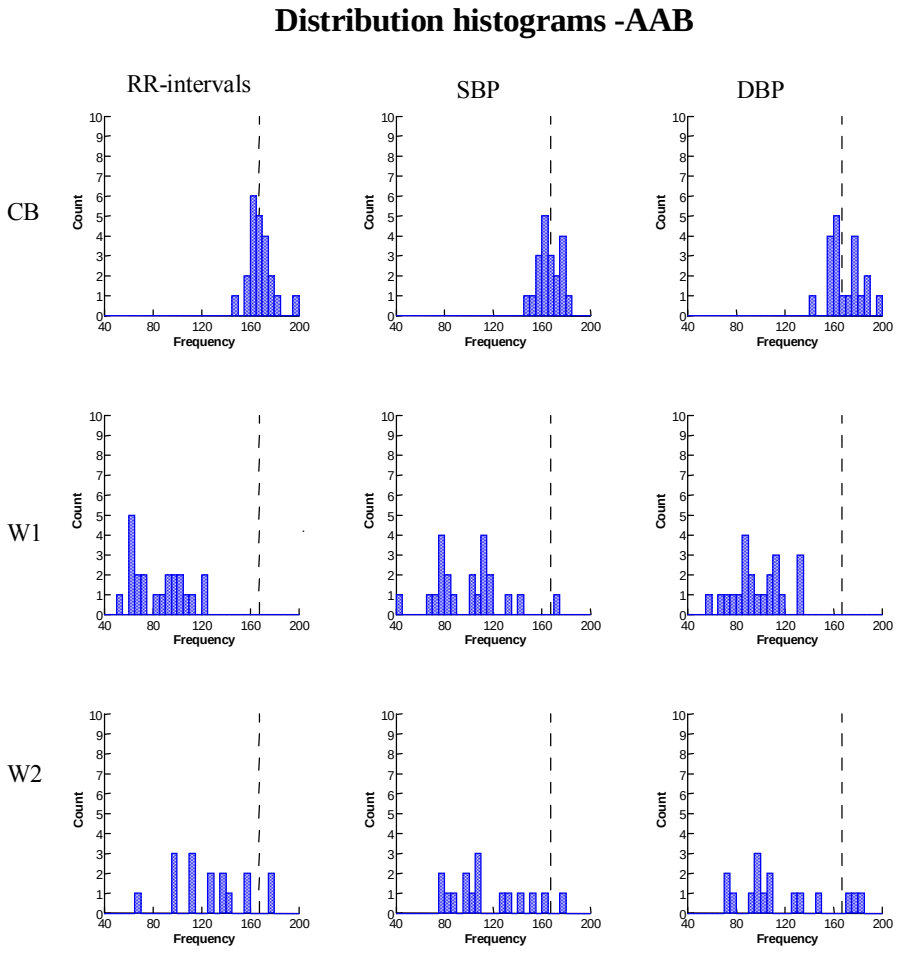

Fig. 2. *Distribution histograms of oscillation frequencies (observed during RR-intervals, SBP, DBP) during controlled breathing (CB), the first and second waves after the onset of apnea (W1, W2 respectively) in atmospheric air breathing (AAB) experiment. The frequencies are expressed in mHz.*

Results

The duration of apnea was significantly longer ($p<0.001$) after oxygen breathing (OB; 131±49 s, range 59-226 s), than after atmospheric air breathing (AAB; 45±19 s, range 16-102 s). There was no significant gender difference in the duration of breath holding and no significant correlation between the duration of apnea and age ($r = 0.323$ for AAB, $r = 0.039$ for OB) and between the duration of apnea and BMI ($r = –0.291$ for AAB, $r = 0.053$ for OB).

Occurrence of the oscillation waves

The first wave (W1) occurred in RR-intervals in 22 of 23 recordings (96 %), in SBP and DBP in all 22 recordings after AAB. After the oxygen breathing, the first waves were present in RR-intervals in all 24 recordings and in 20 out of 21 recordings (95 %) in SBP and DBP.

The second wave (W2) occurred in RR-intervals in 16 of 23 recordings (70 %), and in SBP, DBP in 16 of 22 recordings (73 %) after AAB. After oxygen breathing, the W2 was found in RR-intervals in 20 of 24 recordings (87 %), and in SBP, DBP only in 15 of 21 recordings (71 %).

Oscillation parameters during controlled breathing and in apnea

The frequencies of oscillations during CB were concentrated near the required frequency of breathing (167 mHz). The frequencies of W1 and W2 were scattered in the wider range of frequencies (43-181 mHz; Figs. 2 and 3).

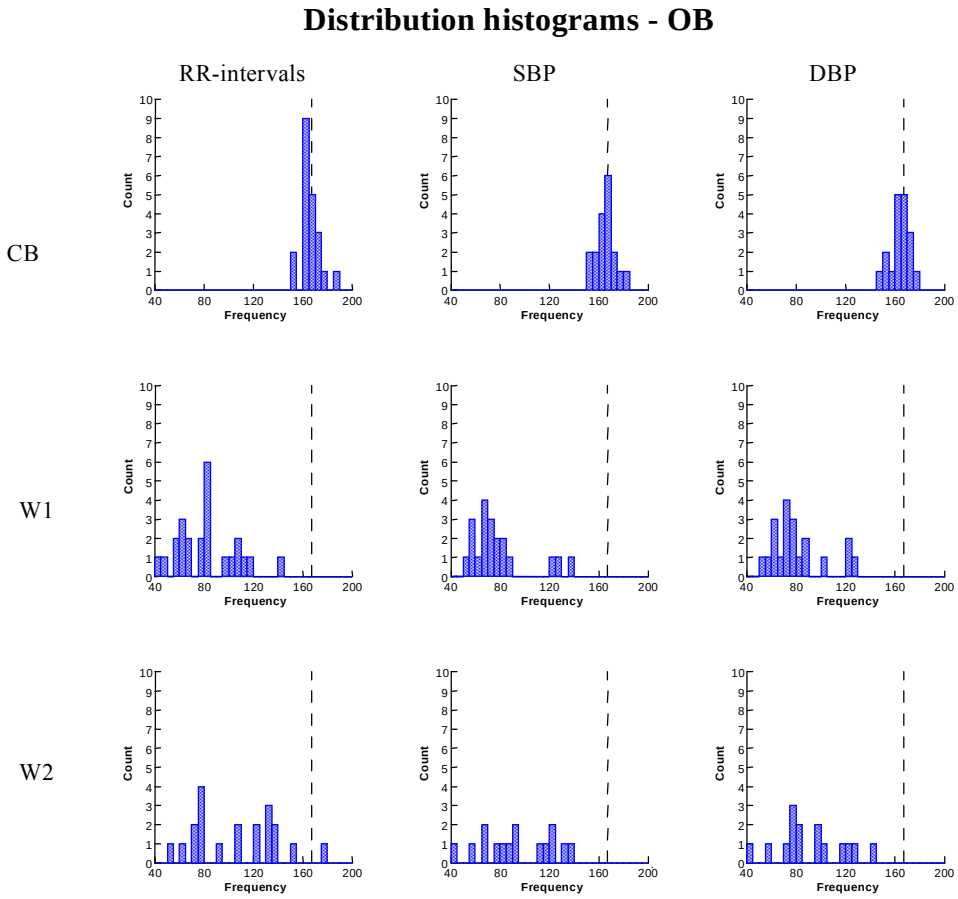

Fig. 3. *Distribution histograms of oscillation frequencies (observed during RR-intervals, SBP, DBP) during controlled breathing (CB), the first and second waves after the onset of apnea (W1, W2 respectively) in oxygen breathing (OB) experiment. The frequencies are expressed in mHz.*

Parameters during atmospheric air breathing (AAB) and description of the first two waves during subsequent apnea (Table 1)

RR-intervals: The RR base level value was decreased during W1, but it was increased in W2 compared to CB and W1. The amplitude of oscillations was markedly decreased in W1 and W2 in comparison to CB. No significant differences between W1 and W2 amplitudes were found. The frequency of W1 was significantly lower than the frequency of CB and the frequency of W2. The MCR parameter was significantly higher during CB than during W1 and W2.

Systolic blood pressure: The base of W2 was significantly higher than base of W1, but there was no significant difference in the SBP base between CB and W1. The W1 amplitude was decreased compared to the CB and W2 amplitude. The frequencies of W1 and W2 were lower than the frequency of CB. There were no statistically significant differences in MCR.

Diastolic blood pressure: The W2 base was significantly increased compared to the W1 base, but there was no significant difference between CB and W1. The amplitude of W1 was increased compared to the CB amplitude. The W2 DBP amplitude was significantly lower than the W1 amplitude. The frequencies of W1 and W2 were lower than the frequency of CB. There were no statistically significant differences in MCR.

Parameters during oxygen breathing (OB) and description of the first two waves during subsequent apnea (Table 2)

RR-intervals: The value of the RR base in CB was higher than the W1 value, but then subsequently returned to the W2 values. There were no statistically significant differences between CB and W2. The amplitude of oscillations significantly decreased from W1 to W2 however no significant difference between CB and W1, W2 amplitudes were found. The frequency of W1 was significantly lower than the frequency of CB. No significant differences in the MCR parameter values were found.

Table 1. Parameters of oscillations during controlled breathing period (CB) of atmospheric air and during the first two waves in the following apnea (W1,W2).

	AAB	CB	W1	W2
RR	Base (ms)	694 ± 85	649 ± 86 *	760 ± 107 * #
	Amplitude (ms)	66 ± 27	41 ± 16 *	42 ± 24 *
	Frequency (mHz)	170 ± 14	86 ± 21 *	128 ± 30 #
	MCR	0.790± 0.138	0.555± 0.192 *	0.621±0.196*
SBP	Base (mm Hg)	142 ± 18	142 ± 19	147 ± 19 *#
	Amplitude (mm Hg)	5.7 ± 2.3	5.3 ± 2.8	4.2 ± 1.9 *#
	Frequency (mHz)	171 ± 15	101 ± 28 *	116 ± 31 *
	MCR	0.737± 0.143	0.704±0.230	0.747±0.173
DBP	Base (mm Hg)	86 ± 15	87 ± 16	90 ± 16 *#
	Amplitude (mm Hg)	3.2± 1.4	4.0 ± 1.6 *	3.5 ± 1.4 #
	Frequency (mHz)	172 ± 15	99 ± 21 *	117 ± 3.7 *
	MCR	0.593± 0.205	0.720 ± 0.181	0.696 ± 0.139

** - significant differences CB: W1 and/or CB:W2; # - significant differences W1:W2*

Table 2. Oscillation parameters during controlled oxygen breathing (OB) period (CB) – and during the first two waves in the following apnea (W1,W2).

	OB	CB	W1	W2
RR	Base (ms)	758 ± 108	689 ± 80 *	757 ± 102 #
	Amplitude (ms)	78 ± 46	64 ± 22	42 ± 19 #
	Frequency (mHz)	172 ± 18	82 ± 24 *	107 ± 34 *#
	MCR	0.720±0.125	0.726 ± 0.178	0.672 ± 0.169
SBP	Base (mm Hg)	143 ± 18	137 ± 17 *	141 ± 15 #
	Amplitude (mm Hg)	5.3 ± 1.6	6.7 ± 3.0	4.6 ± 3.3 #
	Frequency (mHz)	172 ± 19	79 ± 24 *	95 ± 29 #
	MCR	0.575 ± 0.194	0.727 ± 0.175	0.794 ± 0.138*
DBP	Base (mm Hg)	86 ± 14	83 ± 13 *	85 ± 9 #
	Amplitude (mm Hg)	3.3 ± 1.9	4.4 ± 1.5 *	3.9 ± 1.9
	Frequency (mHz)	170 ± 19	82 ± 21*	93 ± 27 *#
	MCR	0.435 ± 0.185	0.690 ± 0.139*	0.773 ± 0.157*

** - significant differences CB:W1 and/or CB:W2; # - significant differences W1:W2*

Systolic blood pressure: The base decreased from CB value to the W1 value, and this decrease was followed by an increase during W2. No significant difference was found in SBP base between CB and W2. The amplitude decreased from W1 value to the value of W2, but there was no significant difference between CB and W1. The frequency of W1 was significantly lower than the frequency of CB and W2. MCR increased significantly from CB to W1. MCR was also significantly higher during W2 compared to the baseline CB value.

Diastolic blood pressure: The base decreased from the CB value to W1 value, and this decrease was followed by an increase in W2. Therefore, no significant difference was found in DBP base between CB and W2. The amplitude increased from CB to W1, but there was no significant difference between W1 and W2. The frequency of W1 was significantly lower than the frequency of CB and the frequency of W2. The MCR increased significantly from CB to W1 and W2 values.

Comparison AAB vs. OB (Tables 1 and 2)

During controlled breathing of atmospheric air the MCR parameter was significantly greater in all assessed parameters (RR-intervals, SBP, DBP) compared to breathing of oxygen. In addition, the base of the RR-interval oscillations was greater during oxygen breathing compared to AAB.

During apnea, significant differences were found in W1 and W2:

W1: RR-intervals: the base, amplitude and MCR were greater in apnea following oxygen breathing.

SBP and DBP: the base and the frequency were greater after AAB than after OB.

W2: RR-intervals: No statistically significant differences were found between both experiments.

SBP and DBP: In accordance with W1, the base and the frequency in SBP and DBP were higher after than OB.

Correlation of the heart rate and blood pressure oscillations in CB and W1 (Table 3)

CB: Not surprisingly, the fundamental frequencies of oscillations during the last four waves before apnea highly correlated between each other in both experiments.

W1: A significant correlation was observed between frequencies of W1 in RR-intervals and DBP after AAB. No significant correlation was found between frequencies in RR-intervals and SBP, although the correlation between frequencies of W1 in SBP and DBP was high in both experiments.

Gender differences

Gender differences were found only in the amplitude of DBP oscillations. This parameter was significantly higher in females than in males during CB with oxygen, as well as in W1 during apnea after OB.

Table 3. Pearson correlation of oscillation frequencies

A:				**B:**		
	Freq. RR	**Freq. SBP**	**Freq. DBP**	**Freq. RR**	**Freq. SBP**	**Freq. DBP**
Freq. RR	1.000			1.000		
Freq. SBP	0.950†	1.000		0.956†	1.000	
Freq. DBP	0.912†	0.916†	1.000	0.924†	0.952†	1.000
C:				**D:**		
	Freq. RR	**Freq. SBP**	**Freq. DBP**	**Freq. RR**	**Freq. SBP**	**Freq. DBP**
Freq. RR	1.000			1.000		
Freq. SBP	0.338	1.000		0.177	1.000	
Freq. DBP	0.688†	0.713†	1.000	0.173	0.880†	1.000

A: during controlled breathing of atmospheric air, B: during controlled breathing of oxygen, C: during the first wave after atmospheric air breathing, D: during the first wave after oxygen breathing

Values are Pearson correlation coefficients (r) of row vs. column variable; † $p<0.001$ (probability associated with corresponding correlation coefficient), RR - RR-interval; SBP - systolic blood pressure; DBP - diastolic blood pressure; Freq. - frequency; AAB - atmospheric air breathing; OB - oxygen breathing

Discussion

We have studied oscillations of heart rate (RR-intervals) and peripheral blood pressure during controlled breathing (air/oxygen) before and at the beginning of voluntary apnea.

a) Controlled breathing

Respiratory sinus arrhythmia (RSA)

The frequency of oscillations

The respiratory pattern has an important influence on the heart rate and blood pressure fluctuations. A negative correlation was found between respiratory rate and the magnitude of RSA (Snieder *et al.* 1997). Despite the findings that amplitude of RSA is greatest at a respiratory frequency of 6 breaths/min (0.1 Hz) (Honzíková 1992, Schmitz *et al.* 1995), we have chosen the respiratory frequency of 10 breaths/min (0.167 Hz) to clearly differ from the Mayer waves of 0.1 Hz frequency. The amplitude of RSA also correlates with tidal volume (Schmitz *et al.* 1995, Taha *et al.* 1995). We therefore controlled tidal volume of the examined subjects at the 30 % vital capacity level in all experiments.

In our experiments, during controlled breathing, the fundamental frequencies of the RR and BP oscillations, as assessed by NLR analysis using a simple cosine wave model were concentrated closely around the frequency of breathing (167 mHz). Only in 3 out of 48 recordings, NLR analysis failed to find oscillations during last four waves of controlled breathing before apnea. This indicates that during the controlled breathing of 10 breaths/min the respiration is a major determinant of oscillations in blood pressure as well as in RR-intervals.

A striking feature of virtually all studies about RSA is that they report large individual differences in the magnitude of RSA (for review see Schmitz *et al.* 1995). In accordance with this study, we found that the amplitude of CB fluctuations in RR-intervals during controlled breathing was in wide range (22-119 ms during normal atmospheric air breathing, and 21-210 ms during oxygen breathing).

There are many factors that can cause the large interindividual differences in the characteristics of RSA. Age is responsible for about 10-15 % of the variation in RSA magnitude; 15-20 % can be explained by individual differences in the respiratory rate (not during controlled breathing). Genetic factors can also be involved in about 30 %, and the external determinants (exercise, diet, stress) can explain about 65 % of the interindividual variations in the intensity of RSA (Snieder *et al.* 1997). We eliminated as many factors contributing to the variation of RSA as possible. All subjects breathed at a controlled frequency and tidal volume and the age of subjects was in narrow range of 18-24 years. External influences could not be eliminated, we therefore suggest that the wide range of the RSA amplitudes can be the result mainly of genetic and external factors (trained/untrained subjects etc.).

Blood pressure oscillations

Tracking of intraarterial pressure changes by non-invasive finger arterial pressure measurements (Finapres) is usually satisfactory even under conditions of strongly changing hemodynamics, although there can be usually slightly overestimated systolic blood pressure level and underestimated diastolic blood pressure level (Wesseling *et al.* 1985, Wesseling 1996). The amplitudes of oscillations in blood pressures (mostly SBP) during controlled breathing were also in wide range of values (for SBP 2.8-12.7 mm Hg during AAB and 3.0-8.4 mm Hg during OB). Because the amplitude of fluctuations in blood pressure could contribute to individual differences in the magnitude of RSA, it seems that the amplitude of respiratory related fluctuations in blood pressure could contribute to the interindividual variability of RSA.

b) Apnea

The apnea started after a quiet expiration (at the FRC level) to avoid changes in cardiovascular parameters due to intrathoracic pressure changes. Horner *et al.* (1995) observed in five adult dogs that the heart rate variability persisted at the onset of the apnea after disconnection of the mechanical ventilation. Furthermore, for at least one (but usually for two or three cycles) this variability had a periodicity similar to that observed during the preceding period of mechanical ventilation. We observed obvious RR-variability at the beginning of apnea, and NLR analysis revealed that at least one cosine-like wave was present in 96 % human subjects after AAB and in 100 % recordings after oxygen. Moreover, the second wave was found in RR-intervals in 70 % subjects after AAB and in 87 % recordings after oxygen. In similar proportion of recordings, the oscillations at the onset of apnea were also present in SBP and DBP.

The base level of oscillations

The base parameters reflect the basal level of heart rate or blood pressure. We found that base of RR-intervals decreased during the W1 compared to CB, at the onset of apnea in both (AAB, OB) experiments (tachycardic reaction) and increased significantly during the W2 compared to W1. This means that during the first approx. 10 seconds at the onset of apnea a tachycardia reaction started in subjects, however, it was later replaced by a decrease in heart rate. This decrease was bigger after AAB (only after atmospheric air breathing the base of W2 was significantly bigger than the base of CB) in comparison to oxygen breathing indicating the role of hypoxia in this response. These observations are in agreement with conclusions of Gross *et al.* (1976), who found bradycardia during apnea in normal subjects and tachycardia in subjects after resection of carotid bodies.

Similarly, in apnea after AAB, the base of SBP and DBP increased during W2 compared to W1 and CB. However, this increase was abolished after oxygen breathing (the bases of W2 in SBP and DBP after oxygen were not significantly different from those of CB). These

results suggest that chemoreceptors play a role in hypertensive reaction during apnea.

The frequency of oscillations in apnea

The waves (W1 and W2), during both (AAB, OB) experiments and in all cardiovascular parameters (RR, SBP, DBP) had frequencies scattered throughout a wide range (43-181 mHz). This interindividual variation indicates that several possible mechanisms or their combinations can be responsible for generating and modulating oscillations in cardiovascular parameters at the onset of voluntary apnea. First, these fluctuations can be explained by the concept of memory of a preceding period of cyclical lung and chest wall movements (Horner *et al.*1995). Second, part of the periodic fluctuations in blood pressure during apnea could be Mayer waves – 0.1 Hz oscillations in blood pressure. These oscillations are transferred *via* baroreflex into the heart rate variability and they are the major determinant of low frequency spectral component of the heart rate variability. Third, the closed-loop baroreceptor-mediated control of cardiac activity may be per se responsible for generating heart rate fluctuations of various frequencies (Cavalcanti 2000). Cavalcanti studied simple mathematical model of cardiovascular system based on delay differential equations and found that baroreflex control may induce low-frequency (0.1 Hz) and also high frequency (0.25 Hz) periodic oscillations without external influences (e.g. respiration).

Our results suggest several possible mechanisms and their combinations for generating rhythms during apnea. Careful analyses of W1 frequencies distributions (Figs 2 and 3) revealed that these distributions could be bimodal (particularly after AAB) with one peak in distribution close to 100 mHz and other close to 80 mHz. We suggest that Mayer waves may contribute to generating of the 0.1 Hz rhythms at the onset of apnea. The W1 waves with frequencies around 80 mHz have usually double-peak shape. It may be caused by fusion of the first two waves at the onset of apnea (by fusion of two consecutive waves with respiratory frequency (167 mHz), one wave, with half frequency can arise. Therefore, the concept of a memory in respiratory system can also be used for explanation.

For the study of RR-intervals oscillation dependency on blood pressure oscillations, we performed correlation analysis of oscillation frequency interdependency. During the last four waves before apnea, the frequencies of heart rate and blood pressure oscillations were highly correlated, because they were driven by one dominant oscillator (cyclic respiratory activity). However, in W1, significant correlations were observed only between SBP and DBP (in both - AAB/OB experiments), and between RR-intervals and DBP after AAB. These results suggest that oscillations of RR-intervals may not be generated by changes in blood pressure during apnea.

The amplitude of oscillations in apnea

RR intervals: The amplitude of W1 (during apnea) decreased significantly in AAB experiment, however, after oxygen this parameter only tended to the decrease, without statistical significance. The second cosine wave-like oscillation (W2) in RR-intervals during the apnea phase was significantly smaller than the magnitude of RSA. The big drop in amplitudes occurred between CB and W1 during AAB, and W1 and W2 during oxygen experiment.

Blood Pressure: The amplitude of DBP W1 during apnea was significantly greater than the amplitudes of DBP during CB of AAB and OB too. In all but one parameter, the amplitude of the blood pressure (SBP, DBP) oscillations decreased from W1 to W2 to the level not significantly different from CB amplitudes. Furthermore, the MCR of SBP and DBP oscillations after oxygen breathing progressively increased.

We suggest that the cessation of respiratory activity at the onset of apnea demasked the influence of other oscillator(s) with bigger amplitude, which caused blood pressure oscillations in a pattern similar to cosine wave. However, after the AAB, an increase in MCR was not observed. It seems that at the onset of apnea after the elimination of chemoreceptor influence, the oscillations in blood pressure are controlled by a single frequency oscillator.

A comparison of oscillation parameters between two experiments (AAB vs. oxygen breathing) showed that the RR-intervals base was bigger during controlled oxygen breathing, and the amplitude of RSA was larger during oxygen experiment than in AAB experiment. Thus, relative bradycardia with augmented RSA, the phenomenon typical for vagal predominance, was observed during controlled breathing of 100 % oxygen. It

seems that the vagal predominance occurs, when peripheral chemoreceptors are inactivated.

We conclude: 1) The parameters of oscillations are different during atmospheric air breathing compared to oxygen breathing. A wide range of oscillation frequencies exists. The oscillations in RR-intervals are, to some extent, independent of blood pressure oscillations. During oxygen breathing there was an increase of the base of RR interval oscillations – relative bradycardia and enhanced RSA. 2) During apnea, the primary reaction was tachycardia followed by bradycardia and hypertensive reaction in a few seconds. The base level of the blood pressure oscillations was higher in apnea after atmospheric air breathing compared to the breathing of oxygen. At least one cosine-like wave oscillation was present at the onset of apnea in RR-intervals and blood pressures (SBP and DBP), and the second wave was present in all assessed parameters at least in 70 % of experimental recordings. The mechanisms responsible for generating the oscillations during apnea need further elucidation.

Appendix

The cardiovascular parameters fluctuate at the first sight in erratic manner. These fluctuations shows great periodicity but also exhibit many irregularities (explained by non-linear deterministic and, to a lesser extent, stochastic components of underlying system). The information about both periodic and irregular fluctuations of the system can be extracted by analyses. A broad spectrum of rhythms with different frequencies and periods (seconds to months) has been found to characterize every biological function measured with sufficient density and duration (Halberg *et al.* 1989, 1990). The best known biological process, which affects cardiovascular parameters and generates their periodic behavior, is the respiration. In this work, we have focused on periodic oscillations of RR intervals and blood pressure caused by respiration and we tried to find out periodic oscillations at the onset of voluntary apnea.

There are several methods used for quantification of the oscillations, e.g. of the respiratory sinus arrhythmia (RSA). The RSA can be assessed in time domain: by determination of the RR intervals standard deviation (Du Plooy and Venter 1995), using peak-to-trough method (magnitude of RSA is expressed by the difference between the longest RR interval during heart rate deceleration and the shortest RR interval during heart rate acceleration phase (Snieder *et al.* 1997), similarly by computing of E:I ratio (ratio of the longest RR interval during expiration (E) and the shortest RR interval during inspiration (I) (Smith 1982)), the cross-correlation function (based on regression analysis to investigate the time shift between RSA and respiration (Brown *et al.* 1993), using the histogram analysis (two distributions of heart rate, one for inspiration, the other for expiration are compared and Mann-Whitney U-parameter is computed (Schmitz *et al.* 1995). Recently, new frequency domain methods have often been applied for assessment of heart rate and blood pressure variability. The most commonly used methods are based on Fast Fourier transform and autoregressive models - the magnitude of RSA and blood pressure oscillations caused by respiration are measured as the power of the high frequency component of the variability spectrum (Blues and Pomfrett 1998, Dinh *et al.* 1999).

Investigation of RSA in clinical settings should warrant rapid assessment of the time course of RSA changes as result of drug administration, changes in the breathing pattern or intervention procedures (Dinh *et al.* 1999). Spectral analysis (particularly Fast Fourier Transform), which is the method of choice, assumes that there are no changes during the period of recording and requires the stationarity of the signal (Brown *et al.* 1993). In our experiments, the insertion of necessarily short period of apnea in a recording introduces the nonstationarity into signal and therefore the assumption of Fast Fourier Transform is no longer valid. Such nonstationary time series can be analyzed by time-varying spectral analysis algorithms (Wigner-Ville transform (Mansier *et al.* 1996, Piepoli *et al.* 1997), wavelet transform (Pichot *et al.* 1999)), or by nonlinear regression analysis (Horner *et al.* 1995, Dinh *et al.* 1999).

The main principle of NLR is the fitting of curve to measured data so that the parameters of this nonlinear function can be studied. To obtain a quantitative description of the heart rate variations we assumed that oscillations would follow simple cosine wave model. As described before (Dinh *et al.* 1999), the heart rate during a breath can be reasonably well fitted by sinusoidal (or cosinusoidal) curve in man, and its amplitude can be used as an index of RSA intensity. However, Horner *et al.* (1995) prefer to use models with the fundamental and the first harmonic of a sine wave, because heart rate data during mechanical ventilation in dogs fit relatively poorly to a simple sine-wave function. Our results agree with both papers: we suggest that heart rate and blood pressure

oscillations during controlled breathing can be relatively well modeled by simple cosine function (high values of MCR). However, visual inspection of residuals (differences between observed and predicted values) showed, that residuals were not random (white noise), but often oscillated in a periodic manner around zero. When these residuals were taken for NLR analysis with cosine wave model, they usually had frequencies that are close to or equal the first harmonic frequency (double fundamental frequency). For the purpose of our study, we used simple cosine wave model because of its simplicity and ability to describe the fundamental periodic oscillation observed.

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Abbreviations

AAB	Atmospheric air breathing
BMI	Body mass index
CB	Controlled breathing
DBP	Diastolic blood pressure
HR	Heart rate
MCR	Mean corrected R^2 – measure of goodness of curve fitting
NLR	Nonlinear regression analysis
OB	Breathing of oxygen
RSA	Respiratory sinus arrhythmia
SBP	Systolic blood pressure
W1	The first wave at the onset of apnea
W2	The second wave at the onset of apnea

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Reprint requests

Michal Javorka, M.D., Department of Physiology, Jessenius Medical Faculty, Comenius University, Malá Hora 4, 037 54 Martin, Slovak Republic.