Determination of the Frequency Bands for Heart Rate Variability: Studies on the Intact and Isolated Rabbit Hearts

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Abstract

There are many different methods for diagnosis of cardiac disorders. One of them is spectral analysis of heart rate variability (HRV). Its frequency bands are standardized only for human hearts. The present study is focused on definition of frequency bands in isolated hearts of New Zealand rabbits and on analysis of changes of these bands caused by myocardial ischemia. The results of the study show that HRV of isolated hearts differs from that of intact hearts and the changes depend on the experiment phase (control, ischemia, and reperfusion). In particular, the limit between LF (low frequency) and HF (high frequency) bands is not constant in ischemic periods and reaches the same value in each reperfusion period. Thus, it is necessary to take into consideration anatomical and physiological differences between intact and isolated hearts when analysis of HRV is used for studying the heart function.

1. Introduction

In experimental cardiology, isolated hearts of certain species are used to study phenomena occurring in heart tissue. The isolated heart is a very good model that in particular allows examining myocardial work changes induced by various cardiac diseases representing serious problem in developed countries [1].

Pathological changes of cardiac function may be detected by different methods. One of them is spectral analysis of heart rate variability (HRV) caused by influence of the parasympathetic and sympathetic nerves on the heart. The HRV contains periodic components, which correspond with peaks placed in specific bands of its spectrum [2]. For humans, these frequency bands are standardized as follows [2]: Ultra Low Frequency: less than 0.003Hz; Very Low Frequency: 0.003 – 0.05Hz; Low Frequency: 0.05 – 0.15Hz; High Frequency: 0.15 – 0.4Hz. However, equivalent guidelines are still missing for animal hearts [3]. Many authors use standards of the human HRV frequencies bands in their studies on the animal isolated hearts, despite the fact that there are other oscillations of HRV spectrum present in isolated heart, although there is no general consensus about their origin.

Currently there are several approaches to definition of the HRV frequency bands for the animals. The first one assumes determination of bands based on oscillations at measured spectra using values proposed for humans. The second one uses empirical information of the HRV spectrum character [3]. The latter can be used for both intact and isolated hearts of any species. This makes it suitable for studying the changes of heart function for example during the ischemia, and differences between intact and isolated hearts function that are in particular important for understanding the origins of HRV in isolated hearts.

2. Methods

2.1. Experiments

Twelve New Zealand rabbits were included in this study. The experiments were performed in accordance with the guidelines for animal treatment approved by local authorities and conformed to the EU law.

The ECGs of the intact nonischemic hearts of five rabbits were recorded by 4 skin surface electrodes placed on the bodies of animals in such a way that the resulting records were comparable with those of isolated hearts (see below). The record of 5 minutes duration was performed with SEIVA system.

Seven rabbits underwent general anesthesia with i.m. injection of xylazin and ketamin. The heart was rapidly excised, the aorta cannulated and the heart was placed in a bath, filled with Krebs-Henseleit solution (1.25mM Ca^{2+}, 37°C). It was retrogradely perfused on Langendorf apparatus in the mode of constant perfusion pressure (85mmHg) [4]. The experiments were performed according to protocol summarized in Fig.1. The perfusion started with stabilization and was stopped after the control period. Stopping flow of the solution into the

heart caused s.-c. global or flow ischemia (phases Ischemia1, 2, and 3 in Fig.1). Resumption of the perfusion restored heart function (Reperfusion1, 2, and 3 in Fig.1).

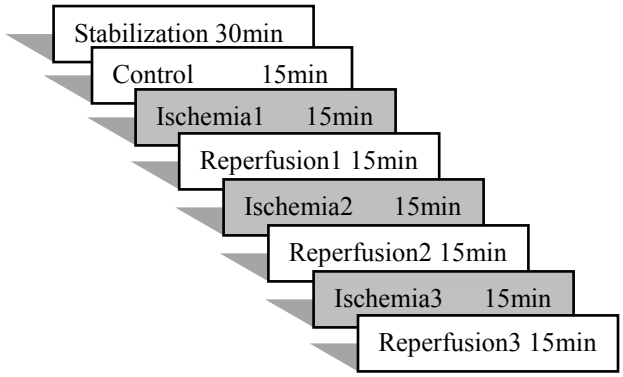

Figure 1. Experimental protocol in isolated hearts

The ECG signals were recorded by touch-less method with the leads in orthogonal system [4, 5]. The recording system consists of three Ag-AgCl disc electrodes which are placed in the walls of the bath.

The measurements were made during each phase of the experiment (see Fig.1). The 12bit analog-to-digital conversion was done with a sampling frequency fs=2000Hz, which is sufficient for the correct R-waves detection. The 5-minutes long parts were extracted from the whole ECG signals at the beginning of each corresponding phase.

All recorded signals were fragmented into one-minute segments for further off-line processing. Thus, 25 and 245 ECGs were selected for intact and isolated hearts, respectively.

2.2. Spectral analysis of HRV

The non-stationary character of the RR intervals duration is described by term HRV. In present paper, the RR series are computed on the basis of R-peak positions. For the EEGs of the intact hearts, R-peak positions are detected manually. For the EEGs of the isolated hearts, they are marked via own designed R-wave detector. The accuracy of the detecting R-waves is controlled manually.

Then, RR series trend components are removed by smoothness prior detrending procedure with the regularization parameter λ=800 using algorithm published previously [6]. This pre-processing procedure is carried out using special software (Kubios HRV version 2.0 [7]). The remainder processing steps are realized in MATLAB 7.0 (The MathWorks, Inc.).

After detrending, the data are interpolated by piecewise cubic Hermit interpolation and resampled with frequency fres=15Hz to obtained equidistant RR-series suitable for further analysis.

The FFT based periodogram is used to estimate a power spectrum (PS) of each one-minute long RR-series. Hann window is chosen as a window function.

The frequency bands are defined in accordance with the procedure proposed in [3]. This is an empirical method which can be applied independently of the studied species. The main idea of the method is that the special spectral parameters required for the spectral band definition are calculated from the accumulated power spectrum (APS) of the HRV. The APS is computed as follows:

$$APS(n) = \frac{\sum_{i=1}^{n} PS(i)}{\sum_{i=1}^{N} PS(i)} \quad (1)$$

where PS(i) and PS(N) are the PS estimated at frequency f(i) and Nyquist frequency, respectively.

The parameters defined from the APS are shown in Fig.2 and described in [3] in detail.

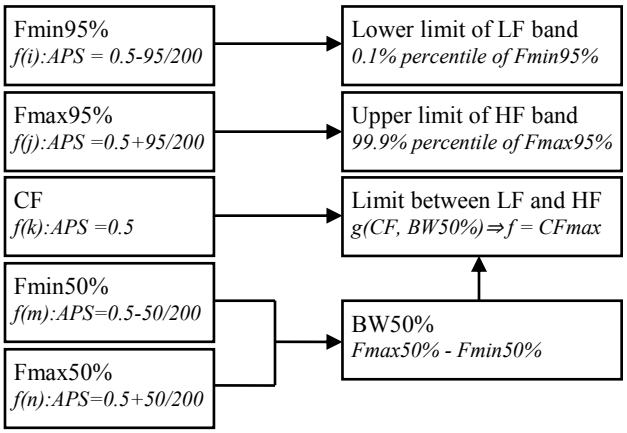

Figure 2. Spectral parameters calculated from APS

In Fig.2:

- CF - central frequency,
- Fmin95% - minimum frequency at 95% of power,
- Fmax95% - maximum frequency at 95% of power,
- Fmin50% - minimum frequency at 50% of power,
- Fmax50% - maximum frequency at 50% of power,
- BW50% - bandwidth at 50% of power.

3. Results

The HRV of both intact and isolated hearts included in the present study have a different character of the power spectra: there are the spectra with one, more or no dominant oscillations.

The two types of the PS obtained for *intact* rabbit hearts are shown in Fig.3 (top). As it can be seen, there is either one (in LF band) or more oscillations (in LF and HF bands) in these PS.

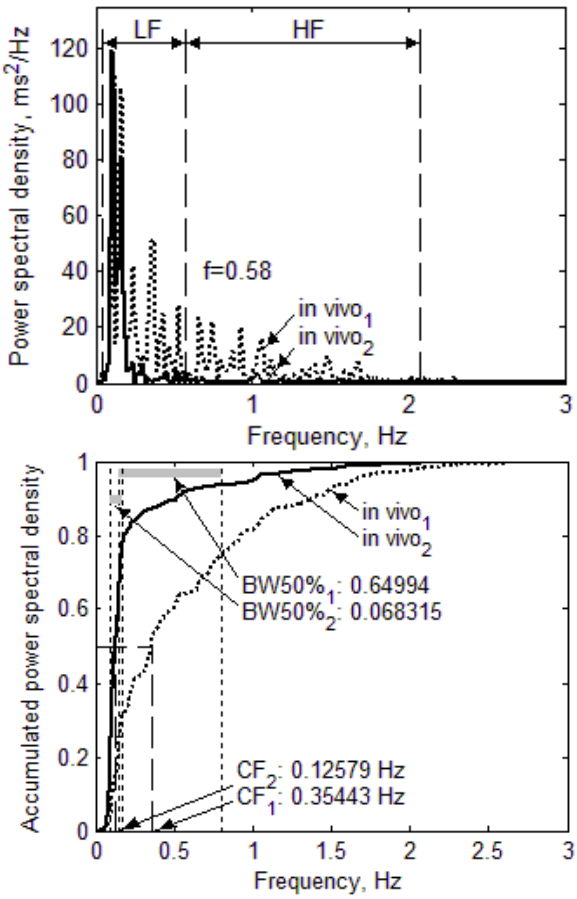

Figure 3. Definition of the parameters for two intact hearts: the PS (top), the APS and spectral parameters (bottom).

The relationship between the PS and computed parameters for two intact hearts is also presented in Fig3. In the case of one dominant spectral component (in $vivo_2$ in Fig.3), the BW50% is small and the CF corresponds to the location of oscillation. If the obtained PS has more than one oscillation (in $vivo_1$ on the Fig.3), the BW50% is high. This is in accordance with the results obtained in [3].

In control period, the PS of four *isolated* hearts out of seven obtains the peak at about 1.5Hz. This yet disappears during the first ischemic phase and does not occur in following phases of the experiment (see Fig.4).

Parameters calculated from the APS are used for definition of the LF and HF bands of HRV. Particularly, the border between them is estimated from the parabola fitted by using the relationship CF against BW50%. The estimation of the limit frequency for intact hearts and control phase of experiment for all isolated hearts are shown in Fig.5 (top).

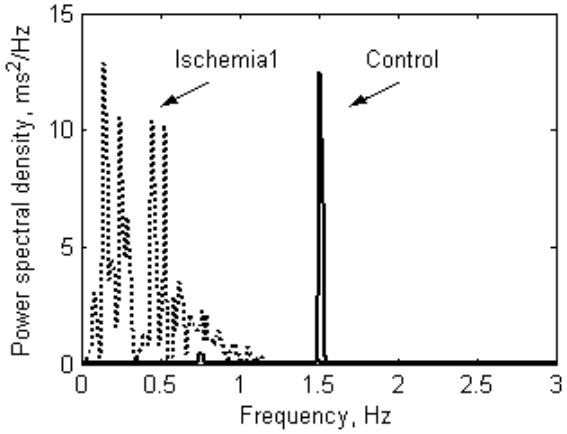

Figure 4. Power spectra of the isolated rabbit heart

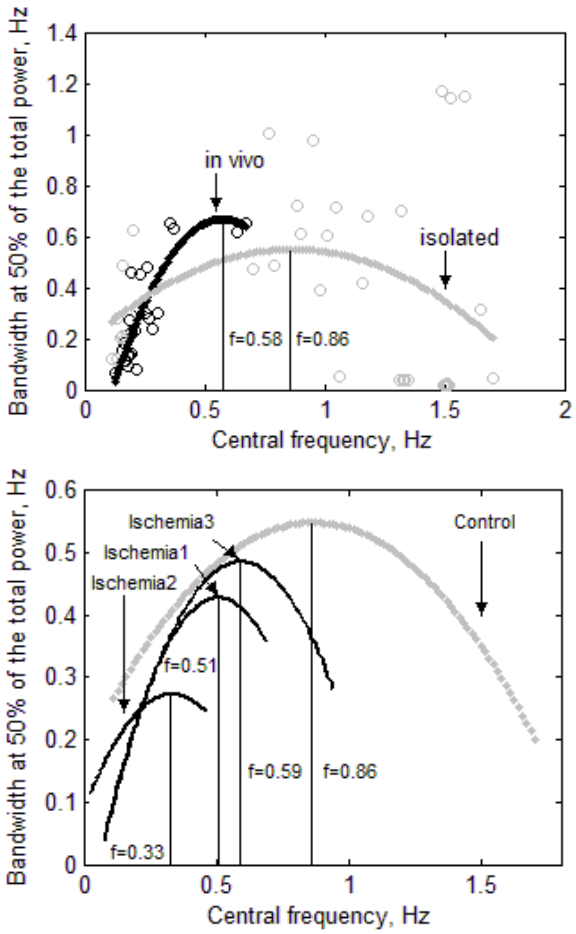

Figure 5. Definition of the limit between LF and HF bands: for all intact hearts and isolated hearts in control phase of experiment (top), for isolated hearts in control and ischemic periods of experiment (bottom)

The fitted parabola has its maximum (*CFmax* on the Fig.2) at the frequency which is a desired border between LF and HF bands (*f* on the Fig.5).

The global ischemia influences the heart performance.

This can be seen in Fig.5 (bottom). The shape and position of the parabola and as a result the values of the frequency bands obtained in the ischemic periods of the experiment are different from those for the control period.

In the case of the reperfusion periods, parabola has the constant shape and only shifts vertically which has no effects on the defined bands border.

The results of the bands definition are summarized in Table 1.

Table 1. Frequency bands defined for intact and isolated rabbit hearts

	Low frequency, Hz	High frequency, Hz
Intact hearts:	0.04 - 0.58	0.58 - 2.07
Isolated hearts:		
Control	0.03 - 0.86	0.86 - 1.91
Ischemia1	0.03 - 0.51	0.51 - 1.41
Reperfusion1	0.03 - 0.51	0.51 - 1.87
Ischemia2	0.03 - 0.33	0.33 - 1.24
Reperfusion2	0.03 - 0.51	0.51 - 1.24
Ischemia3	0.03 - 0.59	0.59 - 1.43
Reperfusion3	0.03 - 0.51	0.51 - 1.58

As it can be seen, the bands for the isolated hearts are different from those for intact hearts. The upper limit of the HF band and the limit between LF and HF bands for isolated hearts change during the experiment. In the two reperfusion phase, there is a trend of return of these values to those in control period (Reperfusion1 and Reperfusion2 in Table 1). This may be explained by phenomenon of ischemic preconditioning [8] which is described as a protection of the myocardium against the tissue injury during the brief repeated ischemic and reperfusion periods. The values of bands borders for the third cycle (Ischemia3-Reperfusion3 in Table 1) show that after two ischemic periods the preconditioning is not so powerful as at the beginning of the experiment; it does not allow to restore the effectiveness of mechanisms occurring in the heart to the original level.

4. Conclusions

The present study shows that the HRV spectral bands of the isolated heart are different from those of the intact heart because of their morphological and functional differences. This must be taken into account when the HRV analysis of isolated heart is used for studying the influence of various factors on the heart function.

It is ascertained that myocardial ischemia leads to changes in the character of the HRV spectrum of isolated rabbit hearts: the high-frequency oscillations presented in some spectra in the control phase disappear during ischemia and after that the spectra acquire the character of random fluctuations. These changes may be detected by means of APS of the HRV.

Thus, the pattern of the HRV spectrum of isolated heart is determined by mechanisms which differ from the phenomena occurring in intact heart. These mechanisms are still unknown and represent very important topic for future studies.

Acknowledgements

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References

[1] Lloyd-Jones D, et al., on behalf of the American Heart Association Statistic Committee and Stroke Statistic Subcommittee. Heart disease and stroke statistic - 2010 update. A report from the American Heart Association, Circulation 2010; 121: e1-e170.

[2] Bernston G, et al. Heart rate variability: Origins, methods, and interpretive caveats. Psychophysiology 1997; 34: 623-648.

[3] García-González MA, Fernández-Chimeno M, Escorihuela RM, Capdevila L, Castro RJ. An Empirical Methodology for the Definition of Frequency Bands for Spectral Analysis of Heart Rate Variability in Animals: Application to Sprague-Dawley Rats, World Congress on Medical Physics and Biomedical Engineering 2009; 25/4: 449-452.

[4] Nováková M, Moudr J, Bravený P. A modified perfusion system for pharmacological studies in isolated hearts. In Analysis of Biomedical Signals and Images. 15th Biennial International Eurasip Conference Biosignal 2000. Vyd. 1. Brno: Vutium Press, Brno University of Technology, 2000. ISBN 80-214-1610-6, s. 162-164. Brno, 2000.

[5] Kolářová J, Fialová K, Janoušek O, Nováková M, Provazník I. Experimental methods for simultaneous measurement of action potentials and electrograms in isolated heart. Physiol. Res. 59 (Suppl. 1): S71-S80, 2010.

[6] Tarvainen MP, Ranta-aho PO, Karjalainen PA. An advanced detrending method with application to HRV analysis. IEEE Trans Biomed Eng 49: 172-175, 2002.

[7] Niskanen JP, Tarvainen MP, Ranta-aho PO, Karjalainen PA. Software for advanced HRV analysis, Computer Methods and Programs in Biomedicine 2004; 76(1): 73-81.

[8] Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology, Physiological Reviews 2003; 83:1113-1151.

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