Determination Of Frequency Bands For Heart Rate Variability In Isolated Rabbit Heart

Ronzhina M 1, Janoušek O 1, Kolářová J 1, Nováková M 2, Fialová K 2, Provazník I 1
1 Brno University of Technology, Czech Republic, 2 Masaryk University, Czech Republic
xronzh00@stud.feec.vutbr.cz

Spectral analysis of heart rate variability (HRV) is one of the effective methods for diagnosis of cardiac disorders. However, the frequency bands of HRV are standardized only for human hearts. The paper presents the study focused on definition of frequency bands in isolated hearts of White New Zealand rabbits and on analysis of changes of these bands caused by myocardial ischemia. The results of this study are the frequency bands of HRV (LF - the low frequency band and HF - the high frequency band): LF=(0.03-0.86)Hz and HF=(0.86-1.91)Hz, LF=(0.03-0.51)Hz and HF=(0.51-1.41)Hz, LF=(0.03-0.54)Hz and HF=(0.54-1.87)Hz for the control, ischemic and reperfusion period respectively.

1 Introduction

Cardiac disorders represent serious problem in developed countries, where these types of diseases take human life approximately each minute [1]. The heart rate variability (HRV) serves as a powerful tool for diagnosis of these disorders. Heart rate is controlled by continuous drive of the heart by the sympathetic and parasympathetic nerves.

In intact heart, periodic components of HRV tend to aggregate within several frequency bands [2]. For humans, these frequency bands were standardized as follows [2]: High Frequency: 0.15 – 0.4Hz, Low Frequency: 0.003 – 0.04Hz, Very Low Frequency: 0.003 – 0.04Hz, Ultra Low Frequency: less than 0.003Hz.

However, standardization of frequency bands was proposed only for human hearts. For animal studies equivalent guidelines are still missing [3]. Two approaches are used to solve this inconvenience: use of frequency bands proposed for humans and determination of frequency band based on oscillations at measured spectra. The second approach – with evidently better results – was introduced by González [3] who proposed an empirical method how to define the frequency bands and tested it on Sprague-Dawley rats. This method can be used for any animal species.

The frequency bands discussed above are based on intact working heart, heart in situ. However in studies of the heart electrophysiology, the experiments with isolated hearts of animals are widely used. There are other oscillations of HRV present in isolated heart, although there is no general consensus about their origin.

The aim of this study was to define frequency bands for isolated heart of White New Zealand rabbits and to analyze changes of these bands caused by myocardial ischemia.

2 Methods

2.1 Experiment

Seven 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 rabbits underwent general anaesthesia 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 Ca²⁺, 37°C). It was retrogradely perfused according to Langendorff in the mode of constant perfusion pressure (85mmHg) [4].
The perfusion started with stabilization for 30 minutes. The following 15 minutes were used as a control period. After the control period the perfusion was stopped, which caused a global ischemia. Then perfusion was recommenced. The last two phases of the experiment lasted for 15 minutes.

The ECG signals were recorded by touch-less method with the leads in orthogonal system [4, 5]. The recording system consists in three Ag-AgCl disc electrodes which are placed in the walls of the bath. The measurements were made during each phase of the experiment (control, ischemic and reperfusion period). The 12 bit analog-to-digital conversion was done with a sampling frequency $f_s = 2000\text{Hz}$. These recording parameters are required for the correct R-waves detection.

The 5-minutes long parts were extracted from the whole ECG signals at the beginning of the corresponding phase. These parts were fragmented into one-minute segments for further off-line processing. Thus, 15 ECG signals were selected in each rabbit.

### 2.2 Experimental data processing

The term HRV is used for describing variations of the RR intervals. In this work, the RR series were computed on the basis of R-peak positions which were marked via own designed R-wave detector. The accuracy of the detecting R-waves was controlled manually.

The RR series were detrended by smoothness prior detrending procedure with the regularization parameter $\lambda = 800$ using algorithm published previously [6]. This preprocessing procedure was carried out using a special software (Kubios HRV version 2.0, The Biosignal Analysis and Medical Imaging Group at the Department of Physics, University of Kuopio, Kuopio Finland [7]). The next steps of the processing were realized in MATLAB 7.0 (The MathWorks, Inc.)

The obtained RR-series are not equidistant which makes them unsuitable for further analysis. Therefore, after detrending the data were interpolated by piecewise cubic Hermite interpolation and resampled with frequency $f_{res}=15\text{Hz}$.

Then, a power spectrum ($PS$) of each one-minute long RR-series was estimated with FFT based periodogram method using a Hann window.

For the definition of frequency bands the procedure proposed by González was used [3]. This empirical method is universal: it is independent from the studied animal species. Briefly, 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:

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

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

The following parameters are defined:

- central frequency ($CF$): the frequency at which $APS=0.5$,
- minimum frequency at 95% of power ($Fmin95\%$): the frequency $f(n)$ at which $APS(n)=0.5-95/200$ (it means that at this frequency $APS$ crosses the 0.025 threshold),
- maximum frequency at 95% of power ($Fmax95\%$): the frequency $f(k)$ at which $APS(k)=0.5+95/200$,
- minimum frequency at 50% of power ($Fmin50\%$): the frequency $f(l)$ at which $APS(l)=0.5-50/200$,
- maximum frequency at 50% of power ($Fmax50\%$): the frequency $f(m)$ at which $APS(m)=0.5+50/200$,
- bandwidth at 50% of power ($BW50\%$): $BW50\% = Fmax50\% - Fmin50\%$. 

The results of the power spectrum cumulating and parameters definition are presented in Fig. 1.

Fig 1. Computation of the spectral parameters without band definition. Estimated power spectrum and accumulated power spectrum (left and right, respectively).

As can be seen, the signals included in the present study have a different character of the power spectra: there are the spectra with one or two dominant oscillators or with no dominant...
oscillators. Figure 1 shows the relationship between the computed indices and the obtained power spectra for one of the rabbit hearts for different phases of experiment: Fig. 1a) for reperfusion period, Fig. 1b) for control period, and Fig. 1c) for ischemic period. In the case of one dominant spectral component (Fig. 1a)), the $BW50\%$ is small and the $CF$ corresponds to the location of oscillator. If the obtained power spectrum has two oscillators (Fig. 1b)), the $BW50\%$ depends on the distance between them and the $CF$ is the mean value of their frequencies. In the case of a power spectrum with no dominant oscillator (Fig. 1c)), the $BW50\%$ is high. The results confirm those proposed in [3].

The obtained parameters are used for definition of the frequency bands, namely the 0.1% percentile of $F_{min95\%}$ for the lower limit of the LF band and the 99.9% percentile of $F_{max95\%}$ for the upper limit of the HF band definition.

Figure 2 shows the estimation of the limit between the LF and the HF bands for control phase of experiment for all rabbit hearts. The 35 input values (five values of the $CF$ against the $BW50\%$ for each heart – the blue dots in the graph on the Fig.2) were used for fitting of the parabola whose maximum occurs at the frequency which is a desired limit.

![Fig 2. Estimation of the limit between LF and HF bands for control phase for all rabbits: 'f' is the frequency corresponding to the maximum of the parabola (the red dotted line)](image)

3 Results

Frequency bands for each phase of the experiment were determined on the bases of the above described algorithm. The results are summarized in the Table 1:

<table>
<thead>
<tr>
<th>Phase of experiment</th>
<th>Low Frequency, Hz</th>
<th>High Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 - control period</td>
<td>0.03 - 0.86</td>
<td>0.86 - 1.91</td>
</tr>
<tr>
<td>F2 - ischemic period</td>
<td>0.03 - 0.51</td>
<td>0.51 - 1.41</td>
</tr>
<tr>
<td>F3 - reperfusion period</td>
<td>0.03 - 0.54</td>
<td>0.54 - 1.87</td>
</tr>
</tbody>
</table>

Tab 1. Frequency bands defined for White New Zealand rabbit isolated hearts

As can be seen, the upper limit of the HF band and the limit between LF and HF bands change during the experiment. In reperfusion phase there is a trend of return of these values to those in control period. This can 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 (one cycle in our case).

4 Discussion

The pattern of the HRV spectrum of isolated hearts is determined by mechanisms that differ from the phenomena occurring in intact hearts. These mechanisms are still unknown.
The frequency bands for spectral analysis of HRV in isolated hearts of small animals have not been reported yet. Many authors in their studies on isolated hearts are guided by the standards for human intact hearts. This, however, is hardly acceptable because of significant anatomical and physiological differences. Results of the present work show, for example, that the upper limit of the HRV spectrum in isolated hearts of White New Zealand rabbit goes beyond 1 Hz (see Tab.1). There is a dominant oscillator at a frequency of more than 1 Hz in some spectra (see Fig. 1b)). The reasons for the existence of the oscillation at these frequencies are unknown.

Myocardial ischemia leads to changes in the character of the HRV spectrum of isolated rabbit hearts. Even if there are high-frequency oscillations present in control phase (see Fig. 1b)), they disappear during ischemia and reperfusion and the spectrum acquires the character of random fluctuations (see Fig. 1c)). These changes may be detected by calculating the spectral parameters (see above).

5 Conclusions
The method described in this paper was used for determination of the borders between HRV frequency bands suitable for New Zealand rabbit hearts. Experiments were performed as myocardial ischemia study. The frequency bands were successfully determined for control, ischemic and reperfusion phases of the experiment. Changes at the upper limit of the individual bands and at the border between LF and HF bands were revealed during the transition from one phase of the experiment to another.

Acknowledgement
This work was supported by the grant projects of the Grant Agency GACR 102/07/1473, GACR 102/09/H083, MSM 0021622402 and MSM 0021630513.

References