Heart rate variability parameters in isolated rabbit hearts

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Ischemia in seven New Zealand isolated rabbit hearts was studied by analysis of 35 standardized parameters conventionally used for heart rate variability (HRV). Aassessment of HRV was based on analysis of consecutive normal R-R intervals during control period, three ischemia periods and three reperfusion periods, each of 5 minutes duration. Variation of heart rhythm persists even in isolated, completely denerved hearts. Two parameters - HF peak and SD2 – may be used as marker of ischemia in isolated hearts.

1 Introduction

Over 17 million people die in the world each year on account of cardiovascular diseases [1]. Predisposition for lethal arrhythmia is associated with either increased sympathetic or reduced vagal activity [2]. Relationship between these activities and heart condition may be quantitatively described by heart rate variability (HRV). It represents variations of heart rhythm and is often used as a diagnostic tool for myocardial ischemia analysis. However, not only innervation of the SA node is responsible for control of the heart rhythm – control mechanisms of heart rate are more complex. For reliable interpretation of HRV, it is necessary to distinguish parameters which reflect extracardiac mechanisms (parasympathetic, sympathetic, humoral, stretch-induced, and others) from parameters which reflect exclusively changes in the heart itself.

Respiratory sinus arrhythmia is believed to be mediated by vagal activity, whereas lower frequency oscillations are jointly mediated by parasympathetic and sympathetic systems [3]. When the heart is denerved, there is no consensus in the literature how HRV is affected. Sands et al. [4] used power spectrum analysis of HRV in human cardiac transplant recepients and reported that heart rate fluctuations in humans in the frequency band from 0.02-1.0Hz are related primarily to autonomic modulation of sinus node activity. Bernardi et al. [5] studied intrinsic mechanism regulating HRV in the transplanted and intact heart during exercise and concluded that at peak exercise a non-autonomic mechanism, probably intrinsic to the heart muscle, may determine heart rate fluctuations in synchrony with ventilation in both groups of studied hearts. Hrushesky et al. [10] quantified respiratory sinus arrhythmia and found that an individuals with a transplanted heart had resting RSA values the same as healthy subjects. Blinks [6] studied effect of increasing right atrial pressure in isolated dog's and rabbit's hearts and presented that increase in right atrial pressure may increase the heart rate by more than 50%. These findings indicate that some autonomic mechanisms of heart may control heart rate. Nearest to our present study is Frey's article [3] where isolated, denerved rabbit hearts were analysed. (Frey used white ELCO rabbits, whereas we use white New Zealand rabbits.) Part of his work is a review where Frey concluded with concordance to our study that nonautonomic mechanisms may control heart rate in the experimental set-up.

The aim of this paper was to study whether global ischemia causes significant changes in HRV in isolated heart - i.e. under conditions when extracardiac mechanisms cannot affect state of the heart. Standard parameters recommended by Task Force [2] were evaluated.

2 Methods

All experiments followed the guidelines for animal treatment approved by local authorities and conformed to the EU law. Seven New Zealand rabbits were included in the study. Their isolated hearts were perfused according to Langendorff in the mode of constant perfusion pressure (85mmHg) [7]. In deep anaesthesia with xylasin and ketamin, the heart was excised and fixed on perfusion apparatus filled with Krebs-Henseleit (K-H) solution (1.25mMCa^{2+}, 37°C) and placed in a bath. The hearts were stabilized for 30 minutes. The experiment consisted of seven phases. The first 15 minutes after stabilization were used as control period. Then, global ischemia periods and reperfusion periods were repeated three times, each of them lasting 15 minutes (see scheme in Fig. 1). Global ischemia was achieved by complete stopping of perfusion.

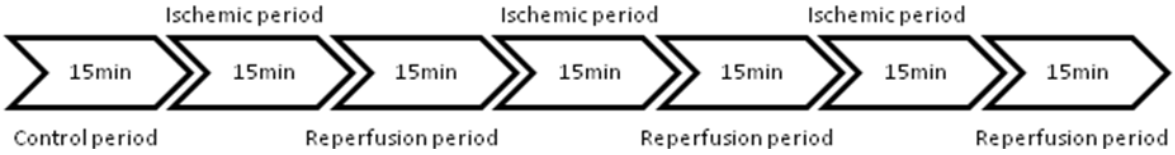

Fig 1.Experimental scheme

The ECG signal was measured by touch-less method [7, 11]. Briefly, three Ag-AgCl disc electrodes in three orthogonal directions x, y, and z are placed in the walls of the bath which is a part of the perfusion system. Each isolated rabbit heart used in this study was positioned in the same way in the bath.

ECG signals were recorded by data acquisition multifunction card PCI-6111E (National Instruments, USA) with sampling frequency f_s = 2000Hz. The accuracy of the R-wave occurrence time estimates is usually required to be 1-2ms, and thus the sampling frequency of the ECG should be at least 500-1000Hz. If the sampling frequency of the ECG is less than 500Hz, the errors in R-wave occurrence times can cause critical distortion to HRV analysis results, especially to spectrum estimates [8]. In this study sampling frequency was adequate for further analysis in spectral domain. ECG signals were acquired by designed application in LabView 7.1 software (Texas Instrument, 2008). The 12 bit analogue to digital conversion was used. The digital signal was stored on a hard disk for off-line processing. Three ECG signals with duration approximately two hours were recorded. Afterwards, seven 5-minutes long parts were extracted from correspond experiment phase in Matlab R2006a (MathWorks, 2006). Recommended duration of analyzed ECG signals is 5 minutes [2], therefore this period was chosen. HRV parameters were computed from RR series interpolated with cubic spline method and resampled at f_{res} = 30Hz. Slow trends were removed by detrending procedure based on smoothness priors regularization with regularization parameter λ=3000. Proportional evaluation between control period and other (ischemic and reperfusion) periods was used. Comparison of values from seven sets of data, obtained from seven different rabbits, cannot be done by absolute values comparison because there is variability between rabbit characteristics as body mass, age, etc. Therefore proportional measurement was used. Value in control period was always considered as 100%. Value in ischemic and reperfusion period was expressed as percentage change against control period. Simplified scheme of procedure is shown in Fig. 2.

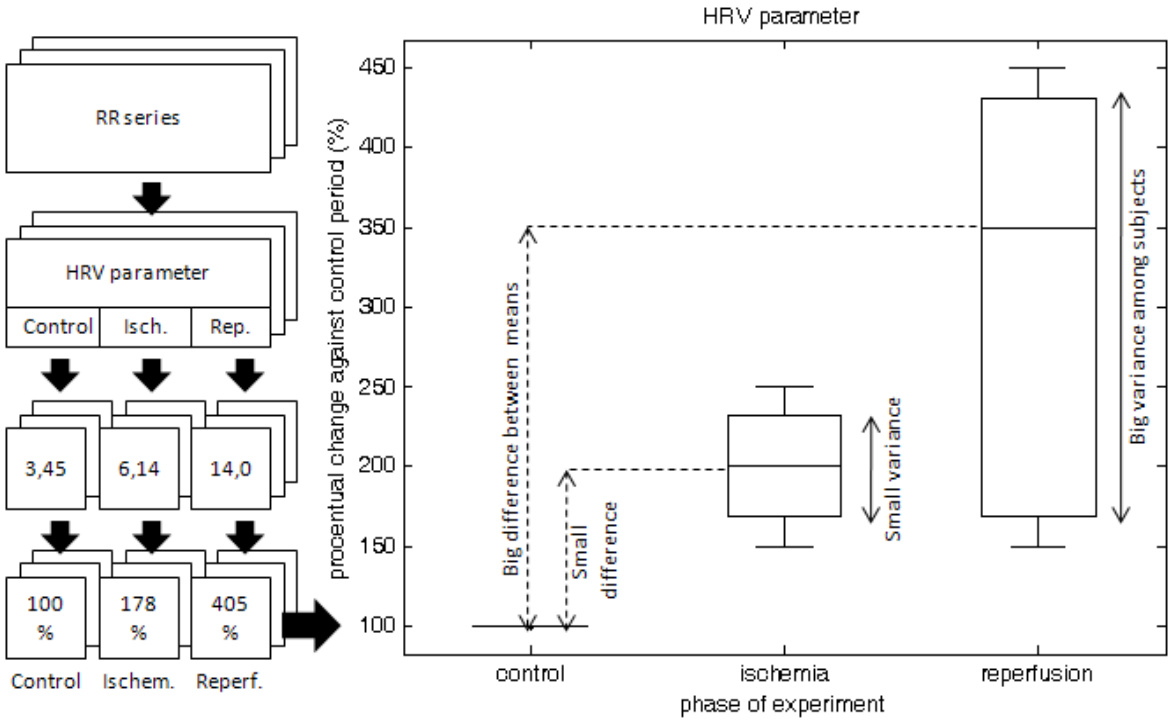

Fig 2.Simplified scheme of evaluation of HRV parameters (left part) and it's proportional comparison (right part)

Seven box-plots were drawn for each HRV parameter. Two box-plot characteristics were used in each box-plot for evaluation of ischemia and reperfusion influence:

1. **Difference between control mean and ischemia (resp. reperfusion) mean**. High difference between control mean and ischemic (resp. reperfusion) mean means that some intracardial mechanism may influenced state of the heart during ischemia (resp. reperfusion).
2. **Range of box for ischemia (resp. reperfusion) period.** Except ischemia, some random mechanism can influence examined HRV parameter. Random behaviour manifests itself by large range of box. Small range of box reveals that examined HRV parameter is significantly influenced only by ischemia (resp. reperfusion).

Valid marker of ischemia has two main characteristics. They are (a) small variance and (b) big difference between means. Table 1 shows decision rules for finding of good ischemia (reperfusion) marker.

		Difference	
		Small	Big
Variance	Small	☹	☺
	Big	☹	☹

Legend:

☺ = good marker – significantly big difference between states of heart and homogeneity between experiments.

☹ = useless marker

Tab 1. Decision rules for finding of good ischemia (reperfusion) marker.

A number of parameters can be used for HRV evaluation. A set of commonly used and standardized parameters was recommended [2] and 35 of them were used in this paper. They are summarized in Table 2; detail description can be found in [2] and [9].

	Measure	**Unit**	**Description**
Time domain	RR	[ms]	The mean of RR intervals
	STD RR (SDNN)	[ms]	Standard deviation of RR intervals
	HR	[1/min]	The mean heart rate
	STD HR	[1/min]	Standard deviation of instantaneous heart rate values
	RMSSD	[ms]	Square root of the mean squared differences between successive RR intervals
	NN50		Number of successive RR interval pairs that differ more than 50 ms
	pNN50	[%]	NN50 divided by the total number of RR intervals
	HRV triangular index		The integral of the RR interval histogram divided by the height of the histogram
	TINN	[ms]	Baseline width of the RR interval histogram
Frequency domain	Peak frequencies	[Hz]	VLF, LF, and HR band peaks frequencies (evaluated both by FFT spectrum and AR spectrum)
	Absolute powers	[ms^2]	Absolute powers of VLF, LF, and HF bands (evaluated both by FFT spectrum and AR spectrum)
	Relative powers	[%]	Relative powers of VLF, LF and HF bands (evaluated both by FFT spectrum and AR spectrum)
	Normalized powers	n.u.	Powers of LF and HF bands in normalized units (evaluated both by FFT spectrum and AR spectrum)
	LF/HF		Ratio between LF and HF band powers. (evaluated both by FFT spectrum and AR spectrum)
Nonlinear	SD1, SD2	[ms]	The standard deviation of the Poincaré plot perpendicular to (SD1) and along (SD2) the line-of-identity
	ApEn		Approximate entropy
	SampEn		Sample entropy

Tab 2. Table of evaluated HRV parameters (adopted and modified from [2])

3 Results

Thirty-five HRV parameters were analyzed. All of these parameters are widespread and commonly used in HRV assessment. Their use was standardized by Task Society [2]. Analyzed parameters are summarized in Table 2 and it can be found in [2] and [9]. Results are presented as procentual change against control period (not absolute values) because this way is most meaningful. It allows comparing data from individual animal's models which are naturally different each from other. Results are shown in a form of box-plot in Fig. 3 and Fig. 4. Parameters which (a) significantly changed in ischemic (resp. reperfusion) period against control, and (b) simultaneously have small variance among all seven subjects, are significantly influenced by intracardiac mechanisms. Two standardized HRV parameters match up these criteria very well: HF peak (describes maximum of spectra in frequency band 0.15 – 0.4 Hz) and SD2 (describes shape of Poincare's plot).

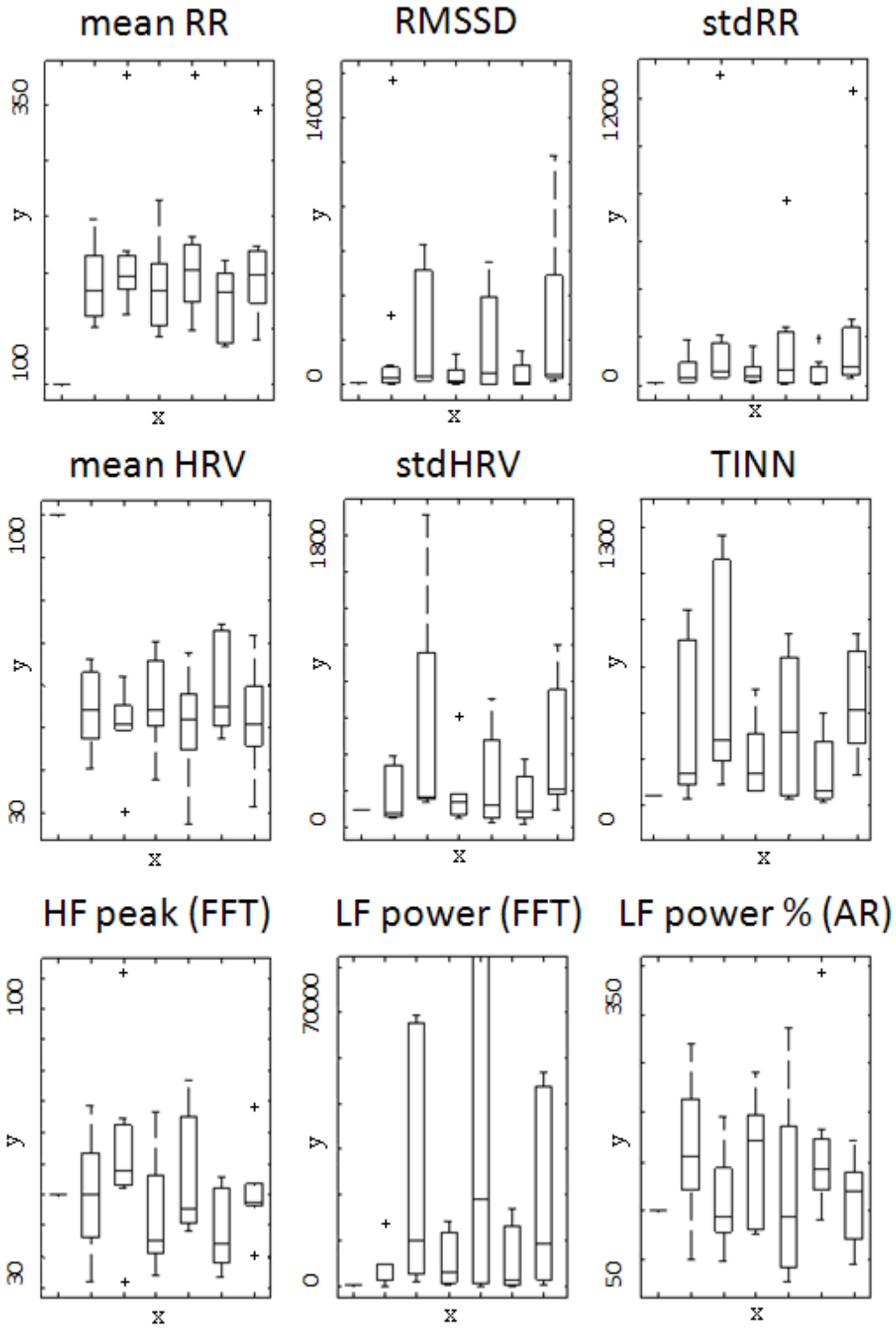

Fig 3. HRV parameters at control period and three ischemic and reperfusion periods. X-axis: Phase of experiment in following order: Control – Ischemia 1– Reperfusion 1 – Ischemia 2 – Reperfusion 2 – Ischemia 3 – Reperfusion 3; Y-axis: Procentual change against control period (%).

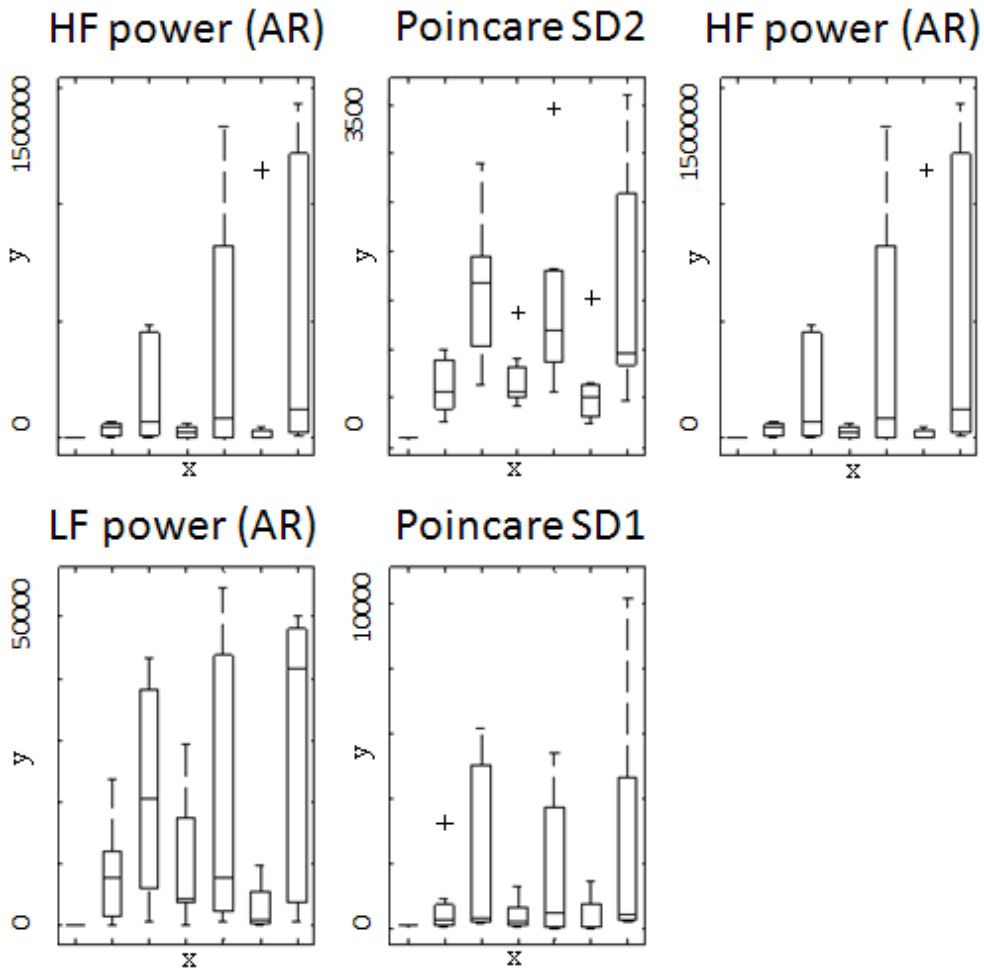

Fig 4. HRV parameters at control period and three ischemic and reperfusion periods. X-axis: Phase of experiment in following order: Control – Ischemia 1– Reperfusion 1 – Ischemia 2 – Reperfusion 2 – Ischemia 3 – Reperfusion 3; Y-axis: Procentual change against control period (%)

4 Conclusions

We found - concordantly with Frey [3] - that HRV persists in isolated rabbit hearts. Not only innervation of the heart is responsible for changes in heart rate variability, but intracardiac mechanisms have to be taken into account in HRV analysis for ischemia diagnosis as well. HRV parameters have dissimilar sensitivity for ischemia exposure.

Myocardial ischemia and reperfusion of isolated heart affect HRV parameters in a manner which can be described by certain well known and standardized HRV parameters.

There are two HRV parameters which significantly reveal intracardiac mechanism of influencing of HRV. The first is HF peak which describes maximum of spectra in high frequency band, and the second is SD2 which describes shape of Poincare's plot.

Acknowledgement

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