The Effect of Cardiac Filling on Heart Rate Variability in Rabbit Isolated Heart

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Abstract

Presence of heart rate variability (HRV) in isolated hearts is widely recognized; however, its mechanisms are still subject of debate. One of the possible explanations is that mechanoreceptors in cardiac tissue affect HRV. In order to evaluate possible dependence of HRV on heart mechanoreceptors activated during left ventricle filling, the HRV parameters in two perfusion modes of isolated heart were compared: Langendorff and working heart mode. Ten New Zealand rabbit isolated hearts were perfused (Krebs-Henseleit, 37°C, 85 mmHg) in Langendorff mode and consecutively in working heart mode (8 cmH_2O preload, 60 cmH_2O afterload). A total of 27 HRV parameters in time, frequency, geometric, and non-linear domain were computed. No significant differences (Wilcoxon signed rank test, $p < 0.05$) were found among all studied HRV parameters between Langendorff and working heart mode. The study confirms the presence of heart rate fluctuation in isolated hearts in both Langendorff and working heart modes. Results of statistical analysis show that heart rate fluctuation is irrespective to mechanical stimulation of heart during atrium and ventricle filling.

1. Introduction

Presence of heart rate variability (HRV) in isolated hearts is widely recognized; however, its mechanisms are still subject of debate. One of the possible explanations is that mechanoreceptors in cardiac tissue affect HRV. This hypothesis is supported by the fact that mechanical stimulus can induce changes of heart rhythm [1, 2]. In order to evaluate effect of mechanical stimulation of isolated heart during atrium and ventricle filling, HRV parameters were compared between Langendorff and working heart modes.

2. Method

Ten New Zealand rabbit isolated hearts (both sex) were studied. All experiments followed the guidelines for animal treatment approved by local authorities and conformed to the EU law.

2.1. Isolated hearts

In deep anaesthesia with xylasin and ketamine, the hearts were excised and perfused in Langendorff mode (constant pressure of 85 mmHg) and consecutively in working heart mode (8 cmH_2O preload, 60 cmH_2O afterload) with Krebs-Henseleit solution (in mM: NaCl 118, $NaHCO_3$ 24, KCl 4.2, KH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 5.5, Taurine 10, and $CaCl_2$ 1.2). The Krebs-Henseleit solution was continuously oxygenated with 95% O_2 and 5% CO_2 and solution temperature was stabilized at 37°C in order to avoid temperature effect on RR intervals. The isolated heart itself was submerged in Krebs-Henseleit solution in bath tempered (37°C) with water jacketed system. The details of perfusion system have been published previously [3]. Stabilization of temperature was secured by closed-loop of temperature sensor and feedback controlled thermostat, resulting in precision under 0.1°C.

2.2. Data acquisition

Electrograms of 5 minutes duration were recorded in Langendorff and working heart modes. The 30 minutes long stabilization period preceded recording in Langendorff mode to avoid effect of heart excision and cannulation.

Electrograms were recorded by touch-less method [4] with sampling frequency of 10 kHz and with 12-bit resolution (PCI-6111E, National Instruments) by custom software (LabView 2010). Electrograms degraded by noise were excluded from further processing.

R-peaks were automatically detected by own R-wave detector (Matlab R2013a, MathWorks) and carefully reviewed by human. Tachograms were then obtained from RR series.

2.3. Preprocessing

Preprocessing of RR interval was applied on recorded data with aim to reduce analysis errors. The three preprocessing steps were performed on RR series: detrending, resampling and ectopic beat correction. Detrending using wavelet transform alleviated any non-stationarities within RR series using smoothness prior approach. Resampling at 10 Hz ensured equidistant sampling of RR series requiring for correct calculation of frequency domain parameters. Ectopic beat correction process removed any kind of false R-peak detection or cardiac ectopy, which were longer than 20% of RR mean or quadruple of RR standard deviation.

2.4. HRV parameter evaluation

A total of 27 HRV parameters in time, frequency, geometric, and non-linear domain were computed from RR series. In order to facilitate comparison of results, the parameters recommended by Task Force [5] were computed. Time domain parameters include *RR mean, RR median, SDNN, NN50, pNN50,* and *RMSSD*. Geometric domain parameters include triangular index *HRVTi* and triangular interpolation of histogram *TINN*. Frequency domain parameters include total amount of power *aTotal* and powers in standardized frequency bands *aVLF, aLF, aHF* with their normalized correlates *nLF, nHF* and its ratio *LF/HF*. Peak frequencies in each band were computed as *pVLF, pLF* and *pHF*. The frequency band were defined according to the table 1.

Table 1. HRV frequency bands.

Band name, abbreviation	Frequency range [Hz]
Very low freq. (VLF)	0 – 0.04
Low frequency (LF)	0.04 – 0.15
High frequency (HF)	0.15 – 0.4

Estimating of power spectrum density were performed using both non-parametric fast Fourier transform and parametric autoregressive modelling. Autoregressive modelling were performed using Burg model with the 16^{th} order. Since there was no significant difference between parameters computed by these two methods, only result from autoregressive modelling are shown in Table 3. Nonlinear parameters include Poincaré diagram identifiers *SD1* and *SD2*, sample entropy *SampEn* and scaling exponents of detrended fluctuation analysis *alpha, alpha1* and *alpha2* with breakpoint scale equal to 10.

Analysed HRV parameters are summarized in table 2. All HRV parameters were estimated by HRVAS software [6] using Matlab (Matlab R2009, MathWorks).

Table 2. HRV parameters with short description.

HRV parameter	Short description of HRV parameter
RR mean	mean of RR interval
RR median	median of RR interval
SDNN	standard deviation of RR intervals
NN10	number of successive differences that are greater than 10 milliseconds
pNN10	percentage of successive differences that are greater than 10 milliseconds
RMSSD	root mean square of successive differences of RR intervals
HRVTi	triangular index
TINN	triangular interpolation of the RR interval histogram
aVLF	amount of power contained within a frequency band 0 - 0.04Hz
aLF	amount of power contained within a frequency band 0.04 - 0.015 Hz
aHF	amount of power contained within a frequency band 0.15 - 0.4 Hz
aTotal	total amount of power
pVLF	percentage of power within a frequency band 0 - 0.04 Hz
pLF	percentage of power within a frequency band 0.04 - 0.15 Hz
pHF	percentage of power within a frequency band 0.15 - 0.4 Hz
nLF	normalized amount of power within a frequency band 0.04 - 0.015 Hz
nHF	normalized amount of power within a frequency band 0.15 - 0.4 Hz
LF/HF	ratio of LF to HF
peakVLF	peak frequency within the 0 - 0.04 Hz
peakLF	peak frequency within the 0.04 - 0.15 Hz
peakHF	peak frequency within the 0.15 - 0.4 Hz
SD1	standard deviation perpendicular to the Poincaré diagram line of identity
SD2	standard deviation along to the Poincaré diagram line of identity
SampEn	sample entropy
alpha	scaling exponent of detrended fluctuation analysis
alpha1	short term scaling of detrended fluctuation analysis
alpha2	long term scaling of detrended fluctuation analysis

3. Results

The effect of atrial filling on HRV was quantified by Wilcoxon signed rank sum test for the two pairs of

experimental conditions: Langendorff mode and working heart mode.

No significant differences ($p < 0.05$) were found among all studied HRV parameters, as calculated from electrograms during perfusion in two different modes.

Comparison of HRV parameters between Langendorff and working heart mode are shown in table 3.

Table 3. Comparison of HRV parameters for Langendorff and working heart mode.

HRV parameter	Unit	Langendorff mean ± std	Working heart mean ± std
RR mean	ms	363.3±66.08	374.88±69.7
RR median	ms	362.21±65.93	373.95±71.4
SDNN	ms	6.93±3.79	11.61±12.6
NN10	count	1.1±2.23	1.8±2.49
pNN10	%	0.14±0.29	0.21±0.25
RMSSD	ms	4.55±5.53	5.73±6.86
HRVTi	ms	8.6±5.27	7.69±5.67
TINN	ms	17.83±13.76	18.04±16.29
aVLF	ms2	13.35±24.47	89.69±198.65
aLF	ms2	4.35±10.2	30.45±82.64
aHF	ms2	3.98±8.19	14.82±29.77
aTotal	ms2	21.67±38.81	134.96±231
pVLF	%	72.31±27.1	67.27±27.64
pLF	%	14.24±10.31	14.95±16.14
pHF	%	13.46±20.1	17.79±20.79
nLF	%	0.69±0.23	0.62±0.31
nHF	%	0.31±0.23	0.38±0.31
LFHF		7.55±11.65	6.02±7.86
peakVLF	Hz	0.01±0.01	0±0
peakLF	Hz	0.07±0.02	0.06±0.02
peakHF	Hz	0.22±0.08	0.22±0.07
SD1	ms	3.22±3.9	4.04±4.86
SD2	ms	8.66±5.04	15.7±17.34
SampEn	-	0.54±0.45	0.53±0.29
alpha	-	1.11±0.48	1.06±0.44
alpha1	-	0.84±0.53	0.72±0.28
alpha2	-	1.19±0.52	1.19±0.47

4. Discussion

HRV is the result of the joint effects of several physiological factors, both extracardiac and intracardiac. Extracardiac factors affecting HRV seem to be well published and widely accepted, but intracardiac factors are subject of state-of-the-art research. Current existing literature supports the presence of intracardiac mechanisms of HRV [7]. Effect of intracardiac mechanisms is especially evident from studies of transplanted hearts and *ex-vivo* experimental studies [8, 9].

This study confirms the presence of heart rate fluctuation in isolated hearts in both Langendorff and working heart modes.

We further focused on mechanical stimulation of isolated heart associated with heart filling in the working heart mode.

The effect of mechanical stretch stimulus has been previously studied in isolated heart model. It has been proven that increasing right atrial stretch by increasing perfusion pressure increases HRV [1, 2]. Mathematical simulation exhibits small increase of HRV variation induced by simulated atrial load [10]. The mechanical stretch of heart during inspirium may affect HRV, since cardiac transplant patients have resting RSA values similar to healthy subjects, although there is no nervous system controlling their heart [9].

Two possible mechanisms explaining how mechanical stimuli can influence HRV have been published.

The mechanical stimulus may affect the open probability of mechanically gated ion channels in sinoatrial node. Mechanical stimulus therefore may produce pacemaker shifts and alter HRV. This effect may be further enhanced due to sinoatrial node functional and anatomic inhomogeneity, since its cells differ in terms of responsiveness to ions [11].

The mechanical stimulus may affect electrotonic interactions between gap junctions. Electrotonic interactions have been found to play a significant role in beating rate irregularity [12]. HRV variability may therefore be affected by changes in gap junction channels characteristic [13].

Those effects may by combined with each other or they may amplify non-mechanical cell characteristics. In order to minimalize undesirable side effects (such as effect of temperature or pH changes) the temperature was stabilized and closed-loop controlled and hearts were continually oxygenated in this experiment.

The result of this study is that HRV does not differ in the heart perfused on Langendorff mode and working heart mode.

It can be concluded that either heart mechanoreceptors´ contribution to HRV is insignificant, or the heart compensatory mechanisms work in the same manner both in Langendorff as well as in working heart modes.

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