The Effect of Voltage-Sensitive Dye di-4-ANEPPS on Heart Rate Variability in Langendorff-Perfused Isolated Rabbit Heart

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Abstract

Optical mapping of heart electrical activity in Langendorff-perfused isolated hearts is based on voltage-sensitive dyes, of which the most commonly used one is di-4-ANEPPS. Prolongation of action potential duration by di-4-ANEPPS was reported in isolated cells; however, this phenomenon was not observed in the whole heart model.

In order to search for other adverse phenomena of di-4-ANEPPS use, its effect on heart rate variability (HRV) was investigated during staining and washout periods in five New Zealand White rabbit isolated hearts.

Time domain, frequency domain and non-linear HRV parameters (21 in total) revealed that there are no significant changes between control, staining and washout periods. Voltage-sensitive dye di-4-ANEPPS does not affect HRV when applied into the isolated heart coronary system. It can be concluded that di-4-ANEPPS may be safely used in studies combining optical mapping and HRV analysis.

1. Introduction

Voltage sensitive dyes provide a fast and accurate approach for recording of electrical activity from single cardiac cell to the whole heart. Due to their fast response and consistent potentiometric response the most utilized voltage sensitive dye in the field of electrocardiology is di-4-ANEPPS [1]. It is known that di-4-ANEPPS causes prolongation of action potential duration in isolated cells, but this phenomenon has not been described in the whole organ yet [2]. In order to disclose any di-4-ANEPPS-induced changes in the system controlling heart rate, the effects of di-4-ANEPPS on heart rate variability during staining and washout were investigated.

2. Methods

Five isolated New Zealand rabbit hearts (both sex, average body mass: 2.86±0.19 kg) were studied. All experiments followed the guidelines for animal treatment approved by local authorities and conformed to EU law.

2.1. Isolated hearts

In deep anaesthesia with xylasin and ketamin, the hearts were excised, fixed on Langendorff apparatus and perfused (constant pressure 85 mmHg) with Krebs-Henseleit solution (in mM: NaCl 118, NaHCO$_3$ 24, KCl 4.2, KH$_2$PO$_4$ 1.2, MgCl$_2$ 1.2, glucose 5.5, Taurine 10, and CaCl$_2$ 1.2). The solution was continuously oxygenated with 95% O$_2$ and 5% CO$_2$. The hearts were stabilized in a thermostatically-controlled (37°C) bath for 30 minutes; the ECG signals were recorded after this stabilization period.

2.2. di-4-ANEPPS

Stock solution 2 mM of di-4-ANEPPS (Molecular Probes, Eugene, USA) was diluted in perfusion solution up to final concentration of 2 μM. The 25 minutes long loading procedure via coronary system (instead of bolus injection) provides enough time for di-4-ANEPPS molecules binding on phospholipid cell membranes. The same period was used for washout.

2.3. ECG recording

ECG signals were recorded (PCI-6111E, National Instruments) by touch-less method [3-5] with sampling frequency of 2000 Hz and with 12-bit resolution.

ECG signals degraded by noise were excluded from further processing. R-peaks were automatically detected by own R-wave detector (Matlab R2013a, MathWorks) and reviewed by human.

2.4. HRV parameters

Tachograms were created from ECG records as follows: 5 x 5-minutes of control period, 5 x 5-minutes of di-4-ANEPPS loading, 5 x 5-minutes of di-4-ANEPPS washout. Trends in tachograms were removed by smoothness prior method before further processing.

HRV parameters recommended by Task Force society [6] were computed by Kubios HRV software [7]. Time domain, frequency domain and non-linear parameters were analysed, namely: mean (RR mean) and standard deviation (RR std) of RR interval, root mean square of successive differences of RR interval (RMSSD), number of successive RR intervals differing more than 100 ms (NN100), baseline width of the RR histogram evaluated through triangular interpolation (TINN), power (VLF power) and peak (VLF peak) of very low frequency (0–0.04 Hz), power (LF power) and peak (LF peak) of low frequency (0.04–0.15 Hz), power (HF power) and peak (HF peak) of high frequency (0.15–0.4 Hz), standard deviations of the points perpendicular to the line-of-identity (SD1) and along the line-of-identity (SD2) of Poincaré diagram, approximate (ApEn) and sample (SampEn) and Shannon (ShanEn) entropy of RR series, short-term (DFA alpha 1) and long-term (DFA alpha 2) fluctuation of RR series computed by detrended fluctuation analysis, slope of regression curve computed by correlation dimension (CorDim D2), and determinism (RPA det), and reccurence (RPA rec) of reccurence plot analysis of RR series.

3. Results

Total number of 21 HRV parameters were statistically tested. Trends of the most interested ones are visualised further.

3.1. Statistical test of HRV parameters

The effect of di-4-ANEPPS on HRV was quantified by Wilcoxon rank-sum test for those two pairs of experimental conditions: (1) control – loading and (2) loading – washout. The last 5-minutes long segments of each 25-minutes long experimental phases were used for HRV parameters comparison in order to secure sufficient time for evolution of some potential changes caused by the dye. No statistically significant difference of HRV parameters between experimental conditions was found. Results are summarized in Table 1.

Although there are no statistically significant differences in HRV parameters in both control-loading and loading-washout pairs of experimental phases, the remarkable trends appear in some HRV parameters. Those trends are shown below.

Table 1. Wilcoxon rank-sum test p-values of RR series differences in control–loading and loading–washout periods.

HRV parameter	cont. – load. p-value	load. – wash. p-value
RR mean	0.095	0.421
RR std	0.420	0.222
RMSSD	0,548	0,310
NN100	0.841	0.413
TINN	0.214	0.167
VLF peak	0.143	0.444
LF peak	0.286	0.850
HF peak	0.587	0.380
VLF power	0.420	0.420
HF power	0.690	0.095
LF power	0.690	0.310
SD1	0.548	0.310
SD2	0.310	0.222
ApEn	0.420	0.841
SampEn	0.690	0.690
DFA alpha 1	0.421	0.548
DFA alpha 2	0.690	0.151
CorDim D2	0.841	0.444
RPA ShanEn	1.000	0.690
RPA det	0.420	0.841
RPA rec	0.547	1.000

3.2. Trends of HRV parameters

Duration of RR interval sharply prolongs during the first three minutes of di-4-ANEPPS loading, although RR interval duration rises slowly during the whole experimental period. The slope of regression line is twice higher (k = 0.017) in loading period as compared to control (k = 0.009) and washout (k = 0.008) periods, as shown in Figure 1.
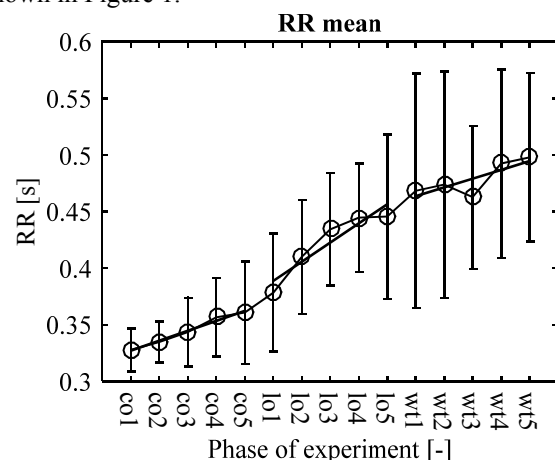

Figure 1. Mean RR interval in control (co), loading (lo) and washout (wt) phase of experiment.

Presence of free di-4-ANEPPS molecules in the heart tissue increases number of successive RR interval longer than 100ms; it may be seen in Figure 2. The usage of 100ms (NN100) instead of 50ms (NN50) commonly used in human studies is reasonable since there are differences between human and rabbit heart and also experimental conditions differ. [8].

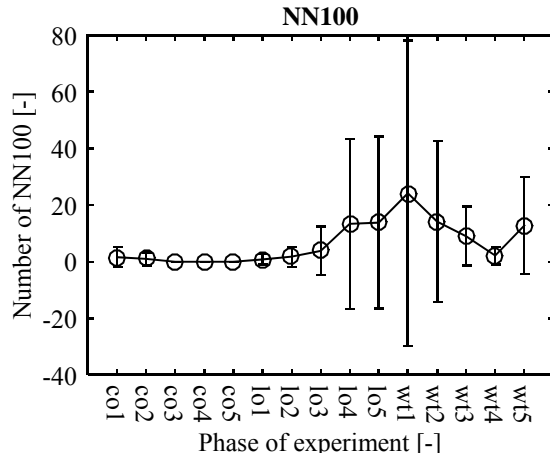It can be seen that NN100 returns back to control values after removing of unbounded di-4-ANEPPS molecules from the heart tissue by washout procedure.

Figure 2. Number of NN100 in control (co), loading (lo) and washout (wt) phase of experiment.

The short-time variation of tachograms increases in the loading period approximately after 15 minutes of loading. The same amount of short-time variability persist in tachograms even after di-4-ANEPPS washout. It can be seen from SD1 curve in Figure 3. The same curve can be seen for RMSSD (not shown).
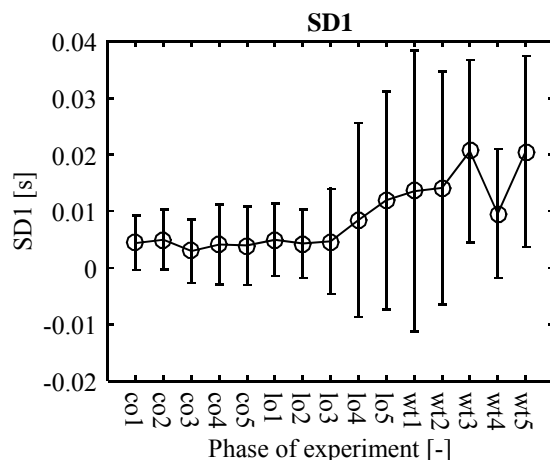

Figure 3. Poincaré parameter SD1 in control (co), loading (lo) and washout (wt) phase of experiment.

The long-time variation of tachograms slowly increases during both loading and washout period, as shown by SD2 curve in Figure 4.
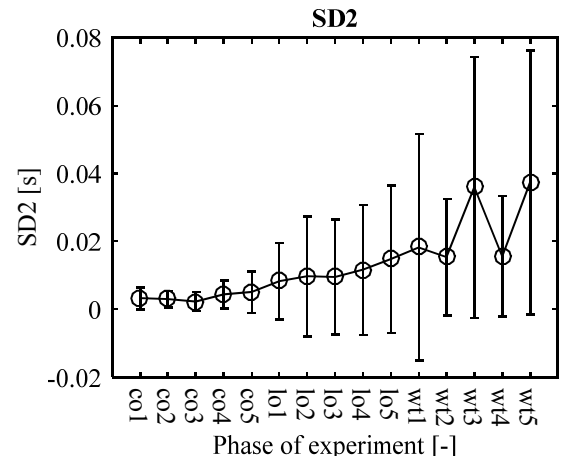

Figure 4. Poincaré parameter SD2 in control (co), loading (lo) and washout (wt) phase of experiment.

Total power of spectrum increases in all three frequency bands (HF, LF, VLF) during di-4-ANEPPS loading. The same holds for washout period, as shown in Figure 5.

While the loading does not change the ratio of power in frequency bands in comparison with control period, the washout decreases power in VLF band and increases power in HF band.
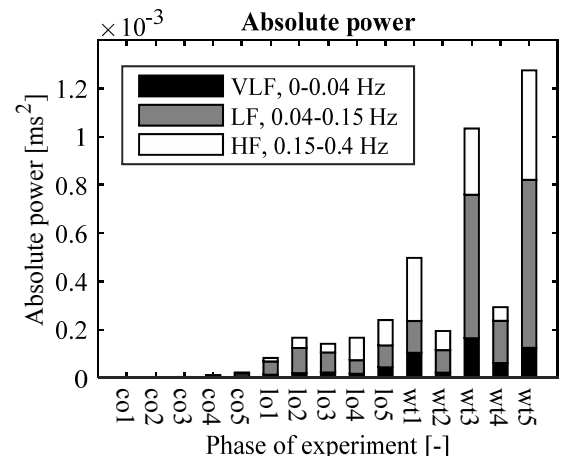

Figure 5. Power spectrum in VLF, LF and HF frequency bands in control (co), loading (lo) and washout (wt) phase of experiment.

The complexity of the tachogram rapidly increases due to di-4-ANEPPS loading. The effect of di-4-ANEPPS on underlying system controlling the heart rate is irreversible, as can be observed from curve of correlation
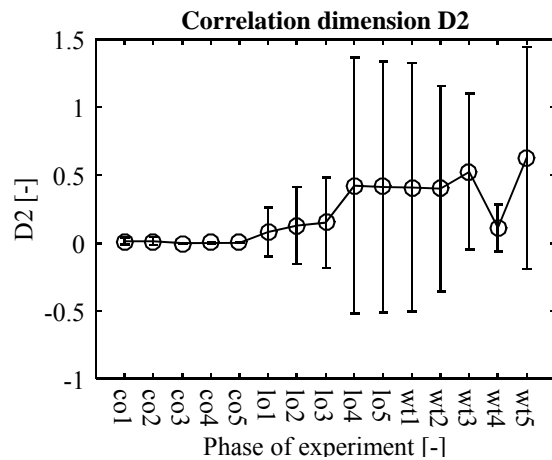
dimension parameter D2 in Figure 6.

Figure 6. Correlation dimension D2 in control (co), loading (lo) and washout (wt) phase of experiment.

4.   Discussion

The effect of di-4-ANEPPS loading and washout on heart rhythm was analyzed using 21 HRV parameters evaluation. The presence of di-4-ANEPPS molecules in the heart tissue affects heart rhythm, but the degree of di-4-ANEPPS influence is not statistically significant.

The most evident effect of di-4-ANEPPS on heart rhythm is prolongation of RR interval, which is in accordance with previous studies [9, 10]. The prolongation of RR interval is gradual and irreversible. Moreover, the single occurrence of rapid prolongation (more than 100ms) is found during loading only.

Presence of di-4-ANEPPS molecules also increases both short-time and long-time heart rate variability and irreversibly increases a complexity of underlying mechanisms controlling the heart rate.

It should be noted as limitation of this study that (a) the heart rhythm may be significantly affected after di-4-ANEPPS illumination by excitation light due to photodynamic effect, (b) the isolated heart heart rhythm differs from *in-vivo* one, (c) rabbit heart is robust against di-4-ANEPPS affection [3], whereas it may not be true for other small mammalian heart.

Acknowledgements

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