The effect of voltage sensitive dye di-4-ANEPPS on the RT/RR coupling in rabbit isolated heart

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Abstract

Voltage sensitive dye (VSD) di-4-ANEPPS is ordinary used for optical measurement of action potential in experimental cardiology. In this work, the effect of the dye on the RT/RR coupling measured in electrograms (EG) recorded on rabbit isolated hearts was studied. The results were compared with the control experiments without VSD administration. The comparison was performed in preparation stage of the experiment that consisted of three phases: stabilization (S), VSD loading (L), and washout (W).

The median and interquartile range ($Q_{0.75}$-$Q_{0.25}$) of the difference in RR, QT and RT/RR between the beginning and the end of the phases were computed and compared between the both groups. Δ(RT/RR), in S, L and W, was -0.095, 0.061; -0.093, 0.043; -0.027, 0.039 in di-4-ANEPPS-treated group and -0.127, 0.071; 0.006, 0.016; -0.001, 0.025 in control group. The significant difference ($P<0.01$) was found between two groups in L phase. Additionally, there was the significant difference in ΔRR [ms] which was 120.9, 74.8 in di-4-ANEPPS-treated group and 6.94, 19.28 in control group in the same phase.

The significant difference in Δ(RT/RR) between both groups shows a possible effect of di-4-ANEPPS on the RT/RR coupling which is mainly caused by the prolongation of RR interval during loading with the dye.

1. Introduction

Voltage sensitive dye (VSD) di-4-ANEPPS is considered as the most suitable dye for optical measurement of the action potential (AP) in isolated animal heart [1], as was shown by Kolarova et al. [2]. Unfortunately, there are not many studies showing the effect of the dye on the isolated heart function, especially on the electrophysiology. Fialova et al. [3] compared the effect of di-4-ANEPPS on the heart rate in different species and showed that the dye causes rapid heart rate decrease in case of rat and guinea pig compared with slow and long-term decrease in rabbits. According to the results of Larsen et al. [4], di-4-ANEPPS significantly modulates cardiac impulse propagation in guinea pig isolated hearts.

The effect of di-4-ANEPPS on continually measured RR and RT intervals in isolated rabbit hearts was studied in this work.

2. Methods

Sixteen rabbits divided into group T (with di-4-ANEPPS; n = 10) and C (without di-4-ANEPPS; n = 6) were included in this study; all experiments followed the guidelines for animal treatment approved by local authorities and conformed to EU law. The isolated hearts were placed onto the Langendorff perfusion system and appended to the perfusion cannula via aorta. The Krebs-Henseleit (K-H) solution (NaCl, 118 mM; $NaHCO_3$, 24 mM; KCl, 4,2 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.25 mM; glucose, 5.5 mM) was bubbled by gas mixture (95% O_2 and 5% CO_2) during the whole experiment and used for heart perfusion. According to applied perfusion method, the K-H solution flows only through the coronary system and no through the heart chambers. The measuring protocol lasted aproximately 120 minutes and consisted of two stages: preparatory stage (lasting minimally 60 minutes) and experimental stage. In group T, the heart stabilization and VSD loading followed by washout (each phase 20 minutes long) were done during preparatory stage. In group C, the heart was stabilized by the K-H solution only but the preparation stage was imaginary divided into three phases as well as in treated group. Experimental stage was the same for both groups global ischemia and reperfusion alternated three times. Since this study was focused on the analysis of the VSD effect on the heart function, probably recognizable mainly during the dye loading, only preparation stage was further analyzed. Six Ag-AgCl electrodes were used for measurement of EG in three orthogonal directions x, y, and z. The AP was measured simultaneously in group T. All signals were recorded by acquisition card (National Instruments) with frequency rate of 2000 Hz and 16 bit resolution. The whole processing was made in off-line mode using MATLAB (R2014b, MathWorks, MA). In this study, only the analysis of EG is presented. The positions of R wave and the end of T wave were subsequently detected by the own automatic detection methods. The R wave detection was based on wavelet transform. The simple wavelet db1 with scale 32 was chosen from MATLAB wavelet library. For detection of the end of T wave the isoline regression method was used. RT intervals were detected in one EG channel per experiment only; the channel with the most prominent T wave was used. An example of RR and RT interval courses for one experiment from group T is shown in Fig. 1.

3. Analysis

The median value (Me) and interquartile range ($IQR=Q_{0.75}-Q_{0.25}$) of RR, RT and RT/RR were computed from the window 1 minute long at each second minute. The computing was proved during the first 10 minutes of each preparation phase only. The values were computed separately for each group of experiments and the graphic interpretation of one observed parameter (RT/RR) is presented in Fig. 2. The values for the second and last minute of each phase of preparation were consequently used for computing of the difference of the observed parameters (RR, RT, RT/RR) between the end and the beginning of phases, as shown in Table 1 and Table 2. Since the test of processed data distribution showed that the data do not come from the family with the normal distribution, the median and interquartile range were chosen for the expression of the results. The significance of the difference in observed parameters between both tested group was computed according to the Kruskal-Wallis statistical test.

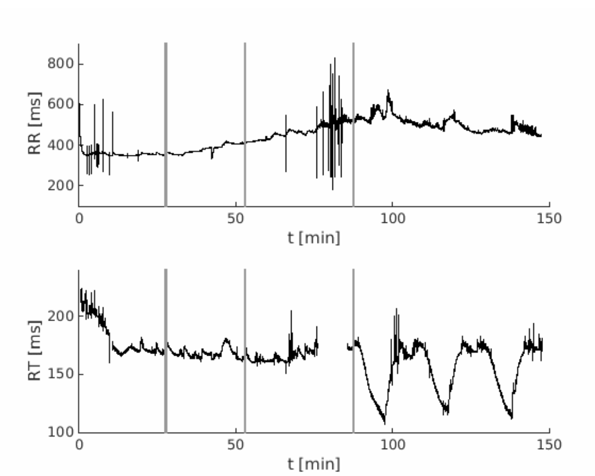

Figure 1. Examples of the time courses of RR and RT for an experiment with di-4-ANEPPS. The gray lines show the ends of individual phases: from the left stabilization, VSD loading, washout.

Table 1. Median (Me) and interquartile range (IQR) of RR, RT and RT/RR over all experiments in each group for different phases of experiment (stabilization – S, VSD loading – L, washout – W, experimental phase – E). 0, 2 – the beginning and the 2nd minute of the preparation phase.

Parameter	Group	Phases of experiment											
		S2		L0		L2		W0		W2		E0	
		Me	IQR	Me	IQR	Me	IQR	Me	IQR	Me	IQR	Me	IQR
RR [ms]	T	332.6	60.1	360.5	52.3	375.5	66.0	490.5	113.5	488.4	133.7	518.1	62.7
	C	302.8	50.2	312.8	6.9	319.0	6.1	334.5	13.5	338.2	19.1	350.2	34.3
p		0.068		0.002		0.010		0.002		0.003		0.001	
RT [ms]	T	180.9	19.6	174.6	24.9	182.1	26.2	178.1	18.1	176.3	7.4	181.8	27.8
	C	173.8	43.2	147.2	8.1	147.0	17.5	152.5	19.1	151.8	17.5	158.0	8.1
p		0.602		0.013		0.009		0.009		0.010		0.007	
RT/RR [-]	T	0.559	0.101	0.482	0.083	0.475	0.079	0.370	0.063	0.379	0.052	0.351	0.075
	C	0.605	0.065	0.460	0.047	0.449	0.049	0.464	0.052	0.436	0.037	0.451	0.015
p		0.117		0.278		0.233		0.009		0.007		0.009	

Table 2. Median (Me) and interquartile range (IQR) of ΔRR, ΔRT and ΔRT/ RR over all experiments in each group computed between different phases of experiment (L0-S2 – difference between the beginning of VSD loading and the 2nd minute of stabilization, W0-L2 – difference between the beginning of washout and the 2nd minute of VSD loading, E0-W2 – difference between the beginning of experimental phase and the 2nd minute of washout).

		Phase of experiment					
Parameter	Group	L0-S2		W0-L2		E0-W2	
		Me	IQR	Me	IQR	Me	IQR
ΔRR [ms]	T	20.55	58.85	120.93	74.77	23.50	114.35
	C	30.10	69.83	6.94	19.28	15.39	36.09
p		1.000		0.007		0.588	
ΔRT [ms]	T	-11.56	35.35	2.64	21.89	3.88	14.89
	C	-24.40	24.73	6.26	10.15	4.30	12.97
p		0.465		0.448		0.637	
ΔRT/RR [-]	T	-0.095	0.061	-0.093	0.043	-0.027	0.039
	C	-0.127	0.071	0.006	0.016	-0.001	0.025
p		0.076		0.001		0.059	

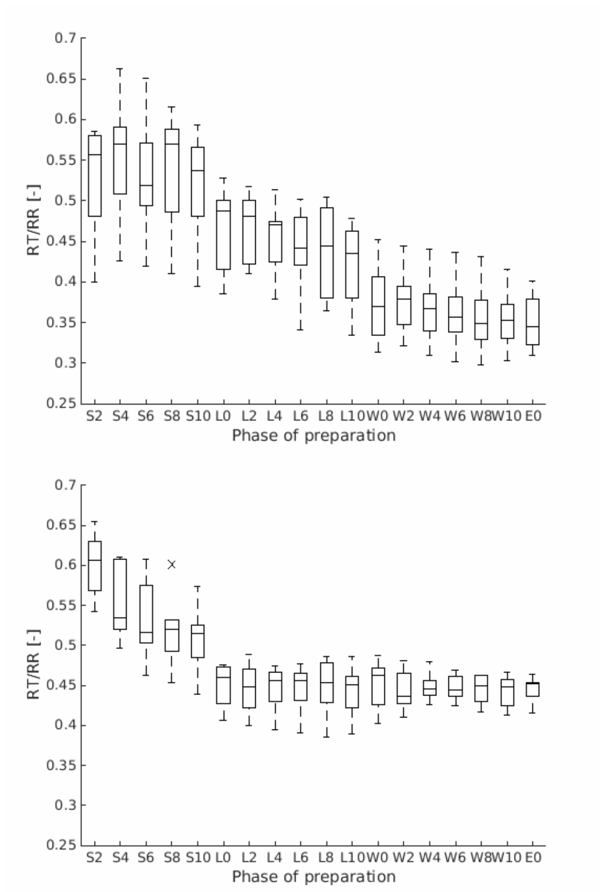

Figure 2. Boxplots of RT/RR coupling computed over all di-4-ANEPPS-treated (top) and control experiments (bottom). S-stabilization, L-VSD loading, W-washout. The number at X axis describes the time within individual phase when the median was computed (0 – the phase beginning; 2 – the 2nd minute of the phase, etc).

4. Discussion

As shown in Table 1, di-4-ANEPPS administration resulted in significant change in the length of RR interval compared to the control group of experiments . Besides the heart rate decrease (or RR interval prolongation) described by Fialova et al. [5], the interquartile range analysis shows that the VSD probably affected the heart rate stability too. It should be mentioned that the differences between both group in median and interquartile range appeared during loading and persist even after washout. Thus, the values of differences at the end of washout do not return to the values at the end of stabilization phase (L0). The above mentioned is not valid for RT interval where the average value of IQR computed over all phases is 20.7 ms for treated group and 18.9 ms for control group, respectively (see Table 1). The longer RT interval in case of treated group is probably caused by the longer RR interval only. Since the maximal percentage change of RT compared to the end of stabilization (L0) is 4.3% in treated group and 7.3% in control group, respectively, assuming the 2nd minute of stabilization is not involved into computation, the courses of RT can be considered as stable and eventual changes of RT/RR coupling should be attribute to the change of RR. Whereas the results for RR interval duration show the significant difference between tested groups already at the end of stabilization that could be caused by short phase of stabilization the statistical analysis of RT/RR coupling reaches the significant difference between groups at the end of VSD loading and moreover it persist to the beginning of experimental stage where the RT/RR coupling in treated group is significantly lower than at the end of stabilization. The results mentioned in Table

2 just confirm the fact that the significant prolongation of RR commonly with RT/RR coupling decrease occurred in treated group compared to control during the phase of loading (W0-L2). It is shown in Fig. 2 that RT/RR coupling in control experiments stays stable from the end of stabilization as compared to treated group where the RT/RR coupling reaches a different value for each phase of preparation stage. It is also shown that the average value of RT/RR coupling over the washout is significantly lower in treated group of animals compared to controls.

5. Conclusion

VSD di-4-ANEPPS administration affects primarily the RR interval duration and the washout of isolated heart by the perfusion solution does not cause the return to the state at the end of stabilization. Together with the RR interval change di-4-ANEPPS causes the change of RT/RR coupling but it is probably a secondary effect because there was not confirmed any direct effect of di-4-ANEPPS to the RT interval duration.

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