Effect of the Repeated Global Ischemia and Reperfusion on the RR and QT Interval in Isolated Rabbit Heart

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Abstract. *This study was focused on determination of the QT/RR coupling during the repeated global ischemia and reperfusion in isolated rabbit hearts perfused according to Langendorff. In addition, the effects of these alternating states on heart function were compared between groups with and without administrated voltage-sensitive dye di-4-ANEPPS. The results showed the reverse relationship between RR and QT during ischemia as compared to reperfusion. Moreover, the heart response on the global ischemia in form of the RR prolongation was more pronounced in experiments without voltage-sensitive dye.*

Keywords: isolated rabbit heart, global ischemia, QT/RR coupling.

1. Introduction

The global ischemia effect of which was studied in this work is complete stop of coronary perfusion and therefore cannot be studied in human heart. On the other hand, the knowledge of the heart response to this pathophysiologic state may be of high importance since it might serve for early prediction of the life-threatening situations. Therefore, the animal models are used for such experiments. There is a positive coupling between QT and RR in humans under physiological conditions [1]. A trace of the reverse QT/RR coupling was studied during the stress measurement [2], [3] and during the coronary flow arrest. The aim of this study was to follow the effect of alternating phases of coronary flow arrest and full coronary flow on the heart function.

2. Subject and Methods

Eleven rabbits divided into group A (with voltage-sensitive dye di-4-ANEPPS; n = 8) and N (without di-4-ANEPPS; n = 3) 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 was bubbled by gas mixture (95%O_2 and 5%CO_2) during the whole experiment and used for heart perfusion. In this method of perfusion the solution flows only through the coronary system and no through the heart chambers. The measuring protocol lasted 120 minutes and consisted of two stages: preparatory stage (lasting 60 minutes) and experiment itself. In group A, the heart stabilization and voltage sensitive dye loading followed by washout was done during preparatory stage. In group N, the heart was stabilized by the K-H solution only. The experiments in both groups were divided into seven sections: control (constant perfusion with

K-H), ischemia I (I1; no perfusion), reperfusion I (R1; constant perfusion), ischemia II (I2; no perfusion), reperfusion II (R2; constant perfusion), ischemia III (I3; no perfusion), and reperfusion III (R3; constant perfusion). While the duration of ischemia and reperfusion was set strictly (10 minutes), the length of control section (at the end of preparatory stage) was about 2 minutes. Three Ag-AgCl electrodes were used for measurement of ECG in three orthogonal directions x, y, and z. Along with the ECG the action potential was measured. For the action potential measurement the optic method using the voltage sensitive dye was employed in group A. All signals were recorded by acquisition card (National Instruments) with frequency rate of 2000 Hz and 12 bit resolutions. The channels were filtered by the low-pass filter with the cut-off frequency 250 Hz. The R detection was based on wavelet transform and was performed in all available leads. For detection of the end of T wave the channels were filtered in the pass band from 0.3 to 35 Hz and the isoline regression method was consequently used for exact detection. The QR interval was considered constant and was given as mean level from manually detected QR intervals from all parts of experiment. RT intervals were detected in the one ECG channel per experiment only - the channel with the best shape of T wave was used. An example of RR and QT interval courses for one experiment from group A is shown in Fig. 1. The mean levels of RR and QT intervals were computed from the window of 40 beats at the end of all phases. The absolute values of RR and QT intervals were normalized to values in the resting state (nRR, nQT). The differences ΔnRR (ΔRR) and ΔnQT (ΔQT) between the ends of main sections (I1, R1, I2, R2, I3, R3) and the end of stabilization were computed over all experiments and both groups.

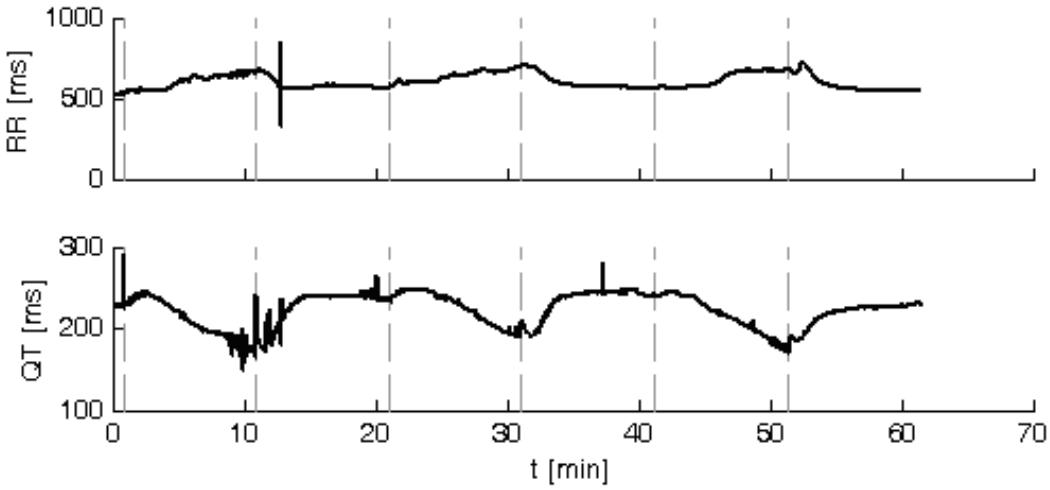

Fig. 1. An example of the RR and QT interval courses for experiment number 4 from group A. The gray dashed lines represent the beginnings of the sections.

3. Results

The mean ± STD values [ms] of RR and QT intervals at the ends of individual sections together with the differences between the values in main sections and the resting state in stabilization section computed over all experiments in each group are summarized in Table 1. The differences are supplemented by the statistical significance computed using the T-test and the ratio ΔQT/ΔRR. For clarity, the normalized differences were used for boxplots shown in Fig. 2. The values of ΔnRR and ΔnQT separately for sections ischemia and reperfusion were consequently fitted by lines over individual experiments and the mean ± STD of their slopes were for following comparison listed in Table 2.

Table 1. Mean ± STD of RR and QT interval at the ends of individual sections and their difference to the value in stabilization over all experiments and both groups. The marks in column ΔRR and ΔQT indicate the statistical significance: NS – non significant, * 0.05 >= p , ** 0.01 >= p, *** 0.001 > p.

Section (group)	RR [ms] Mean ± STD	ΔRR [ms] Mean ± STD	QT [ms] Mean ± STD	ΔQT [ms] Mean ± STD	ΔQT/ΔRR Mean ± STD
S (A)	514 ± 82	-	207 ± 19	-	-
I1 (A)	821 ± 118	307 ± 161 **	184 ± 29	-23 ± 22 *	-0.15 ± 0.15
R1 (A)	529 ± 76	15 ± 106 NS	208 ± 23	0.51 ± 8.49 NS	0.11 ± 0.13
I2 (A)	793 ± 120	279 ± 151 **	181 ± 29	-26 ± 23 *	-0.21 ± 0.25
R2 (A)	529 ± 86	16 ± 71 NS	207 ± 30	-0.59 ± 13.71 NS	0.23 ± 0.18
I3 (A)	745 ± 117	231 ± 132 **	177 ± 18	-30 ± 16 **	-0.21 ± 0.19
R3 (A)	558 ± 100	44 ± 80 NS	207 ± 29	-0.40 ± 14.43 NS	0.41 ± 0.45
S (N)	345 ± 23	-	162.2 ± 5.0	-	-
I1 (N)	617 ± 68	273 ± 91 NS	140 ± 12	-22 ± 7 *	-0.09 ± 0.02
R1 (N)	345 ± 24	0.46 ± 3.11 NS	158.2 ± 3.3	-4.0 ± 2.1 NS	-1.05 ± 1.69
I2 (N)	610 ± 67	265 ± 90 NS	123.0 ± 4.7	-39 ±5 **	-0.17 ± 0.07
R2 (N)	354 ± 13	9.0 ± 16.9 NS	166 ± 13	3.9 ± 12.4 NS	0.51 ± 0.34
I3 (N)	708 ± 32	363 ± 9 ***	101 ± 16	-62 ± 16 *	-0.17 ± 0.05
R3 (N)	341 ± 11	-3.3 ± 14.9 NS	159.4 ± 6.6	-2.7 ± 6.2 NS	0.29 ± 0.46

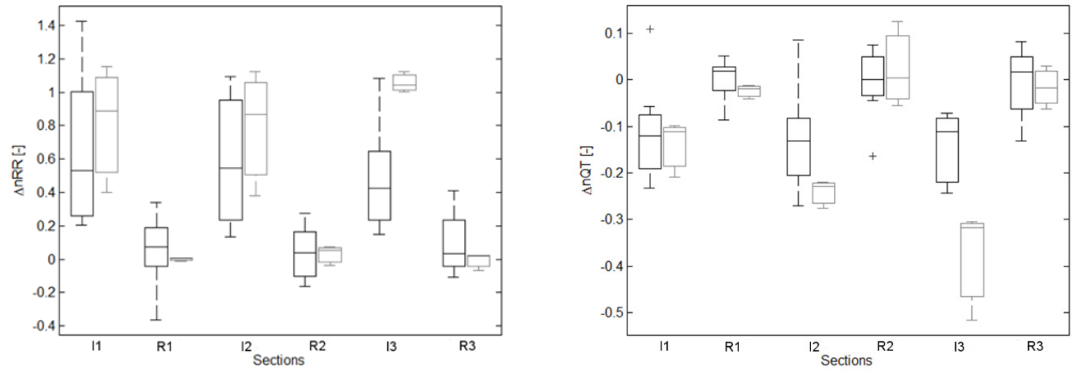

Fig. 2. The differences ΔnRR and ΔnQT between the ends of main sections and the end of stabilization over all experiments in each group. The black boxplots for group A and the gray boxplots for group N.

Table 2. The slope of the line fitted through the values of ΔnRR and ΔnQT separately for section of ischemia and reperfusion over individual experiments.

			Group A		Group N	
			Ischemia	Reperfusion	Ischemia	Reperfusion
Slope	ΔnRR	Mean±STD	-0.086 ± 0.123	0.021 ± 0.108	0.122 ± 0.134	-0.004 ± 0.022
	ΔnQT	Mean±STD	-0.015 ± 0.032	-0.003 ± 0.031	-0.120 ± 0.062	0.004 ± 0.015

4. Discussion

The standard QT/RR coupling, i.e. QT interval prolongation with increasing RR interval duration, is violated by the sections of the global ischemia. As shown in Fig. 1, QT interval shortened with elongating RR during these sections. The following reperfusion, during which the flow of perfusion solution was restored, caused the gradual return to the standard QT/RR coupling. The alternating sections of ischemia and reperfusion caused analogous changes of

RR and QT in both tested groups, but the rate of RR and QT reaction on the section transitions differed. There was a difference in the mean values of RR and QT at the end of the stabilization section between both groups, too. The mean values of RR and QT were greater for group A, meaning that the administration of voltage sensitive dye caused significant prolongation of both intervals. Therefore, only the changes computed from the normalized values (ΔnRR and ΔnQT) could be compared over the groups. The absolute values of ΔnRR and ΔnQT in group N were greater than in group A. This implied to stronger reaction of nRR and nQT on section transitions in group N. In addition, the slopes summarized in Table 2 showed almost opposite trend of ΔnRR and ΔnQT for both groups, especially the slopes computed for repeated ischemia. While in group A the impact of repeated ischemia on ΔnRR and ΔnQT is similar (ΔnQT) or even decreased (ΔnRR), in group N the impact is increased.

5. Conclusions

The standard QT/RR coupling, i.e. QT prolongation with increasing RR, was violated by the perfusion arrest in both tested groups. The differences between groups A and N were observed in the mean values of RR and QT at the end of stabilization section and in the rate of reaction of ΔnRR and ΔnQT on the perfusion arrest. The administration of voltage sensitive dye di-4-ANEPPS caused prolongation of RR and QT together with the heart function attenuation expressed as smaller reaction of ΔnRR and ΔnQT on the perfusion arrest. These results are in concordance with Fialova et al [4].

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