Evaluation of Repeated Global Ischemia in Isolated Rabbit Heart

[1,2]M. Ronzhina, [3]V. Olejníčková, [3]T. Stračina, [1]T. Potočňák, [1,2]O. Janoušek,
[1,2]P. Veselý, [1,2]J. Kolářová, [2,4]M. Nováková, [1,2]I. Provazník

[1]Brno University of Technology, Brno, Czech Republic,
[2]International Clinical Research Center – Center of Biomedical Engineering,
St. Anne´s University Hospital Brno, Brno, Czech Republic,
[3]Masaryk University, Brno, Czech Republic,
[2]International Clinical Research Center – Animal Center, St. Anne´s University
Hospital Brno, Brno, Czech Republic
Email: ronzhina@feec.vutbr.cz

Abstract. *The paper is focused on evaluation of morphology changes of electrograms recorded from isolated rabbit hearts during experiments with short repeated global ischemia with and without prior loading of the heart with voltage-sensitive dye di-4-ANEPPS. It is shown that degree and time course of certain morphology changes in these two cases are quite different and must be taken into account.*

Keywords: Isolated rabbit heart, repeated global ischemia, electrogram, voltage-sensitive dye

1. Introduction

Animal models are often used to study the changes of heart electrical activity caused by myocardial ischemia. Rabbit biomodel is quite useful tool because of the similarity of rabbit cardiac physiology to that of human. Conventional ECG technique and optical recording of action potential using voltage-sensitive dye (VSD) can be successfully employed simultaneously in such studies. However, it is known that some VSDs, such as frequently used di-4-ANEPPS, may affect cardiac tissue of various species [1]. Consequently, results of analysis of ECG data recorded during experiment with VSD application must be carefully interpreted taking into account possible signal changes related to VSD side effects.

In this study, the time course of changes caused by global repeated ischemia has been investigated in EGs recorded from isolated rabbit hearts without chemical intervention and undergoing loading with di-4-ANEPPS. Four morphological indexes calculated from EGs have been evaluated using the ischemic changes sensor to describe their capacity to detect the ischemic changes in both cases.

2. Methods

The animal experiments were performed in accordance with the guidelines for animal treatment approved by local authorities and conformed to the EU law. The isolated hearts of twelve New Zealand rabbits perfused with Krebs-Henseleit solution (1.25mM Ca^{2+}, 37°C) according to Langendorff in the mode of constant perfusion pressure were used [2].

The electrograms (EGs) were recorded simultaneously by touch-less method using the orthogonal system which includes three pairs of Ag-AgCl disc electrodes (see Fig. 1a) placed in the bath wall. The sampling frequency of 2 kHz was used for the correct detection of QRS complexes.

EGs were recorded according to two protocols. In the first one (see Fig. 1b), VSD di-4-ANEPPS was added to the perfusate to record left ventricular action potential by optical method simultaneously with EG (optical part of recording system is not shown in Fig. 1a).

After stabilization period (20 min), heart was loaded with di-4-ANEPPS for 20 minutes. After washout (15 min) and control (5 min), three consecutive periods of ischemia and reperfusion (10 min each) were carried out. More detailed information about experimental setup and data recording according to this protocol can be found in [2]. In the second type of experiments, only EGs were recorded in isolated hearts. Thus, dye loading and washout periods were excluded from the second experimental protocol (see Fig. 1c). Control and three ischemia-reperfusion repetitions (of the same duration as in the first experimental setup) were followed by 30 minutes long stabilization period.

a)

ST	DL	WT	CO	I1	R1	I2	R2	I3	R3
20 min	20 min	15 min	5 min	10 min	10 min	10 min	10 min	10 min	10 min

b)

c)

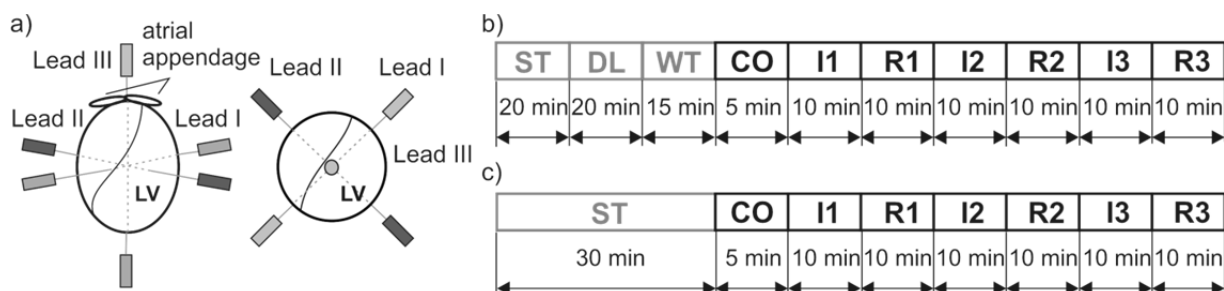| ST		CO	I1	R1	I2	R2	I3	R3
30 min		5 min	10 min	10 min	10 min	10 min	10 min	10 min

Fig. 1.   Electrograms recording: a) orthogonal system of electrodes, b) protocol of experiments with voltage-sensitive dye di-4-ANEPPS application, c) protocol of experiments without di-4-ANEPPS application. LV – left ventricle, ST – stabilization, DL – loading with di-4-ANEPPS, WT – washout, CO – control, I1, I2, I3 – ischemia, R1, R2, R3 - reperfusion.

EGs recorded using lead II (depicted with dark grey colour in Fig. 1a) during control and ischemia-reperfusion periods (depicted with black in Fig. 1b and Fig. 1c) were used for further analysis. The low-frequency baseline wander was suppressed using Lynn's filter with cut-off frequency of 0.5 Hz. QRS complexes were then detected automatically with the wavelet based detector and verified manually. The beginning of QRS, J point, and the end of T wave were manually detected to calculate four *indexes*: duration of QJ interval (QJ width), position of maximum deviation of JT interval with respect to the J point (J-Tmax width), level of JT interval at J+10ms (JT10 level), and maximum deviation of JT interval (J-Tmax).

The ischemic changes sensor (ICS) previously introduced by García et al. [3] was then used to describe the indexes changes caused by repeated short-term global ischemia. The ICS describes the changes in EG indexes during ischemic (or reperfusion) period in relation with background noise represented with the standard deviation of a certain index in control period. The ICS was estimated for all indexes for every 10 seconds from the beginning of the first ischemic period to the end of the third reperfusion period.

3. Results

The example of typical time courses of indexes calculated from EG recorded during control period and first ischemia-reperfusion repetition and corresponding EGs are shown in Fig. 2. Progress of morphological changes in lead II EG during global ischemia can be divided into following parts: 1) widening of QJ interval from the 1st second of ischemia and often inversion of T wave (B in Fig. 2), 2) shifting of the T wave to the QRS complex (is associated with QT shortening) (B-C in Fig. 2), 3) changes in JT (elevation) and inverting of T wave back to the positive values at the 5th minute of ischemia (C in Fig. 2), 4) overlapping of depolarization and repolarization phases of EG after 5-7 minutes of ischemia (D in Fig. 2). In reperfusion, the backward changes occur (E-F in Fig 2). These changes are carried out more quickly than in ischemia and thus EG morphology at the 3rd minute of reperfusion is almost the same as in control period (A in Fig. 2).

Time courses of mean ICS calculated from their absolute values among six experiments (for experiments with and without VSD application individually) during repeated ischemia are presented in Fig. 3.
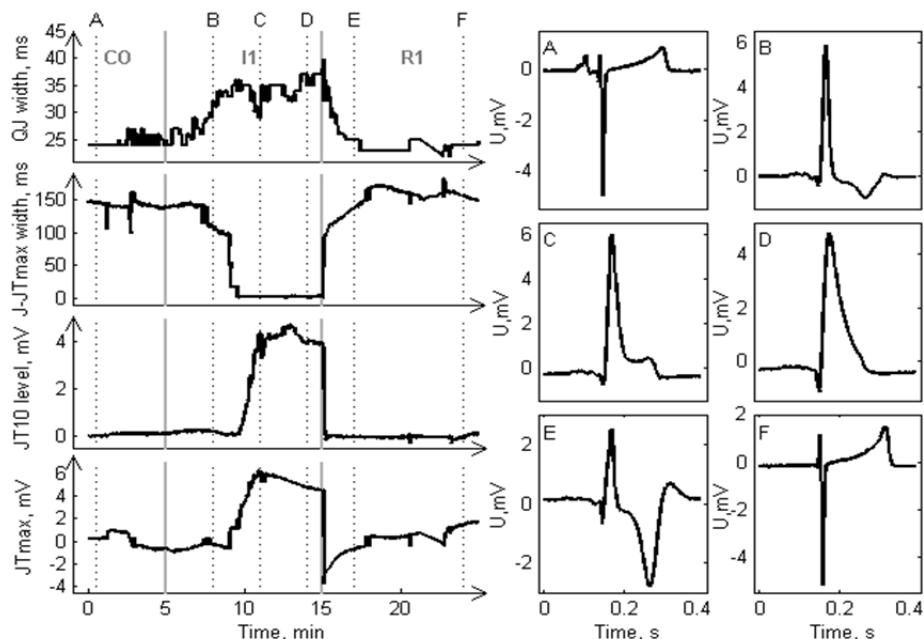

Fig. 2. EG morphology changes (lead II) caused by global ischemia (experiment without application of di-4-ANEPPS): time courses of EG indexes calculated from control to the end of the first reperfusion period (left), corresponding EG courses (right). CO – control, I1, R1- first ischemic and reperfusion period, respectively.
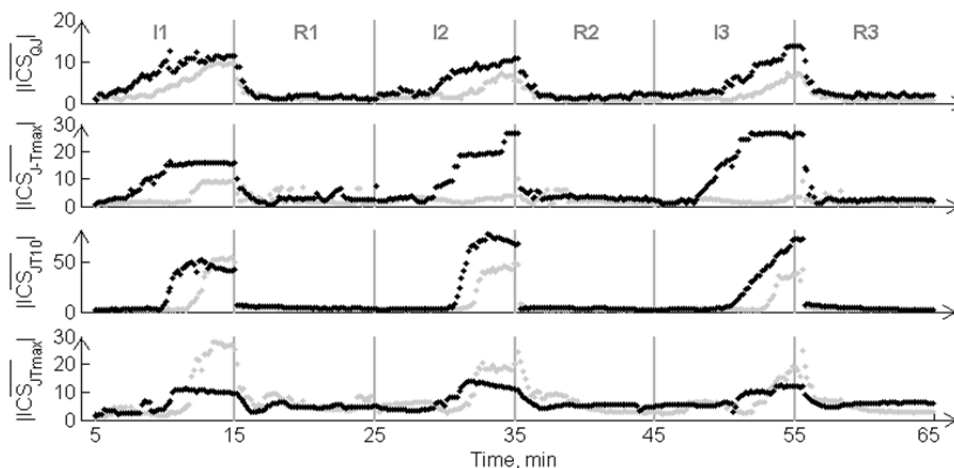

Fig. 3. Mean of absolute values of ICS indexes among six experiments with (light-grey) and without (black) application of di-4-ANEPPS during three ischemia-reperfusion repetitions. I1, I2, I3 – ischemic periods, R1 ,R2 ,R3 – reperfusion periods.

It is obvious that indexes calculated from data recorded according to the first protocol except for J-Tmax show larger values of mean ICS, indicating that these indexes have larger capacity to detect ischemic changes in such data. For both data sets, mean ICS values of all indexes calculated for different ischemia-reperfusion repetitions are quite similar. QJ and J-Tmax calculated from the first data set have the highest ICS value during the 1st ischemia, whereas J-Tmax and JT10 calculated from the second data set have the highest ICS during 2nd and 3rd ischemia. Moreover, J-Tmax of the data recorded with SVD application does not show marked changes in its mean value during the 2nd and 3rd ischemia.

Mean ICS calculated for different indexes during the 1st ischemia-reperfusion repetition are presented together in Fig. 4 to further evaluation. During the first half of ischemia, the larger ICS values are characteristic for QJ and J-Tmax and for QJ and J-Tmax calculated from the first and second data set, respectively. It implies a faster response of these indexes to ischemia. Moreover, the changes occur earlier in the second case. In both cases, JT10 has the largest ICS value than other indexes from the 5th minute of ischemia. Moreover, ICS for J-Tmax is much more noticeable and ICS for J-Tmax is slightly higher than other ICS. It is obvious from Fig. 3 and Fig. 4 that the changes in indexes during ischemic periods are slower than the return of indexes to their control values at the beginning of reperfusion.
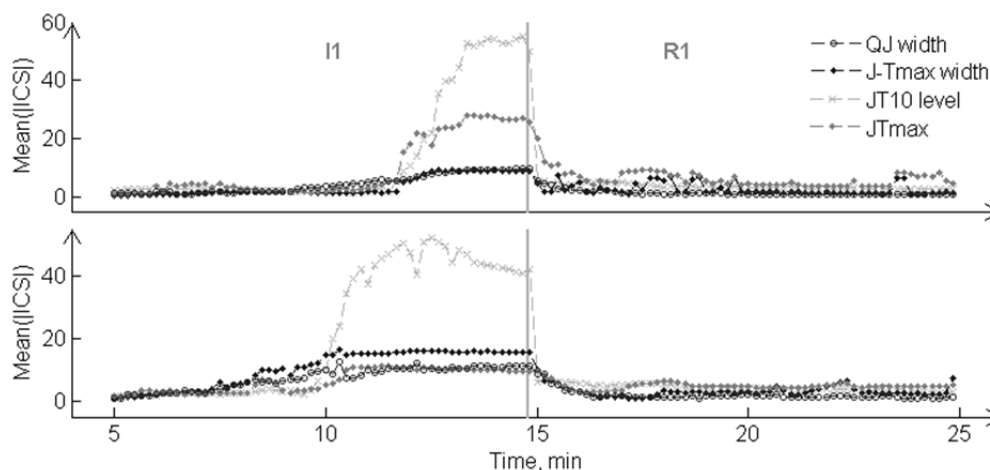

Fig. 4. Time course of mean ICS indexes (absolute values among six experiments) during the first ischemia-reperfusion repetition for experiments with (top) and without (bottom) application of di-4-ANEPPS. I1, R1 – first ischemic and reperfusion period, respectively.

4. Conclusions

The degree and time course of ischemic changes of some indexes calculated from EG recorded in experiments with and without di-4-ANEPPS application are quite different. These differences are probably due to effect of VSD on myocardial tissue and must be taken into account. In both cases, the changes in JT part of EG are delayed with respect to changes in QJ part for approx. 2 minutes. All ischemic changes are fully reversible in both cases.

Acknowledgements

References

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