Influence of Ischemia and Reperfusion Duration on Left Ventricular Depolarization in Isolated Rabbit Hearts Registered by Optical Method

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Abstract

The influence of ischemia and reperfusion duration on left ventricular depolarization is studied from electrograms (ECG) and monophasic action potentials (MAPs) recorded by optical method.

Left ventricular depolarization is evaluated by activation time (AT), which is defined as the distance from the earliest QRS deflection to the MAP upstroke and which describes time duration of depolarization propagation.

The excitement conduction in ischemic heart takes longer time in comparison to non-ischemic myocardium. Speed of impulse conduction decreases with ongoing ischemia, and thus the activation time increases proportionally to ischemia duration. During reperfusion, speed of impulse conduction increases immediately with the first minute of reperfusion and remains at the same value till the end of examined period.

1. Introduction

Electrical and mechanical activity of the heart has been repeatedly studied since the very first days of existence of cardiology. Cardiac contraction is triggered by electrical impulses generated by specialized tissue – conductive system of the heart. They originate from sinoatrial (SA) node and are transmitted via atrioventricular (AV) node and His-bundle to Purkyne fibers and from them to contractile tissue. Any change of action potential generation or its conduction in conductive system or within working myocardium or any change of electrical or mechanical properties of cardiac tissue appear as a change in conduction of depolarization in the heart muscle and – as a consequence – as a change in the shape and time intervals of electrocardiogram (ECG) and in the heart muscle performance.

The speed of impulse conduction via cardiac tissue can be evaluated from activation time (AT), measured from ECG and MAPs synchronously recorded on the surface of the left ventricle [1].

In our laboratory, continuous synchronous records of ECG and MAPs are registered in isolated mammalian hearts perfused according to Langendorff. These recordings are used in studies of various cardiovascular phenomena, such as ischemia and reperfusion.

2. Methods

The influence of ischemia and reperfusion duration on left ventricular depolarization is studied from electrograms (ECG) and monophasic action potentials (MAPs).

ECG is recorded by touchless method on isolated hearts perfused according to Langendorff and MAPs are acquired by optical method based on voltage-sensitive dye (VSD) di-4-ANEPPS (Molecular Probes, Oregon, USA). Three orthogonal ECG leads and MAPs are followed at control conditions and during three consecutive phases of ischemia and reperfusion. This experimental protocol represents the same situation on myocardium as during so-called preconditioning.

2.1. Animal experiments

Six isolated New Zealand rabbit hearts are included in this study. The hearts are perfused at Langendorff set-up. The isolated heart is pleased into temperature controlled bath filled with Krebs-Henseleit solution and heart coronary system is filled through aorta. The hearts are perfused at the constant pressure of 85 mmHg. Experimental conditions are kept stable in all experiments (37°C, 1.25mM Ca^{2+}). The performance and viability of spontaneously beating heart is controlled during the whole experiment via recording of three electrocardiographic leads.

Each experiment consisted of five phases: heart isolation, control perfusion, staining with the dye, dye washout and MAPs recording. The last part of experiment in the presented study consisted of three consecutive periods of ischemia and reperfusion, 10 minutes each. Duration of control, staining and washout was 20 minutes for each period. For details of

experimental protocol, see Table 1.

Table 1. An overview of experimental protocol.

	experimental period								
	control	VSD staining	VSD washout	ischemia I	reperfusion I	ischemia II	reperfusion II	ischemia III	reperfusion III
duration [minutes]	20	20	20	10	10	10	10	10	10
ECG recording	✔	✔	✔	✔	✔	✔	✔	✔	✔
MAP recording				✔	✔	✔	✔	✔	✔
time	→								

All experiments followed the guidelines for animal treatment approved by local authorities and conformed to the EU law.

2.2. Recording setup

Three orthogonal ECG signals and MAPs are recorded simultaneously during ischemia and reperfusion in isolated rabbit hearts perfused according to Langendorff.

The ECG signals from orthogonal leads are recorded from Ag-AgCl electrodes positioned on the inner surface of the bath in Langendorff apparatus.

MAPs are recorded by optical method via fluorescence measurement from the heart surface at appointed place. The principle of optical measurement of MAP lies in an application of voltage-sensitive dye (VSD), di-4-ANEPPS in our experimental set-up, into the heart tissue. Optical signal therefore depends on cardiac action potential. MAPs should be recorded continuously during whole experiment.

Simple optical setup, presented in Figure 1, includes 150W halogen light source with built-in IR filter, which prevents a preparation from heating and a band-pass filter (560nm ± 30nm), which selects light at excitation maximum of the used dye. The light goes through bifurcated fiber cable, which ensures two different ways of light. The fiber cable source light leads to the heart surface and the emitted light from heart to optical sensor. The emitted fluorescent light is converted to an electric signal by a photodiode detector and amplified by a two-stage operational amplifier. Changes of intensity of the detected light correspond to changes of the membrane potential (e.g. MAP).

The preamplified MAP signals are digitized with the use of a LabView compatible data acquisition multifunction card PCI-6250 (National Instruments, USA) with 16 bits dynamic range and at rate of 2000 samples per second [2].

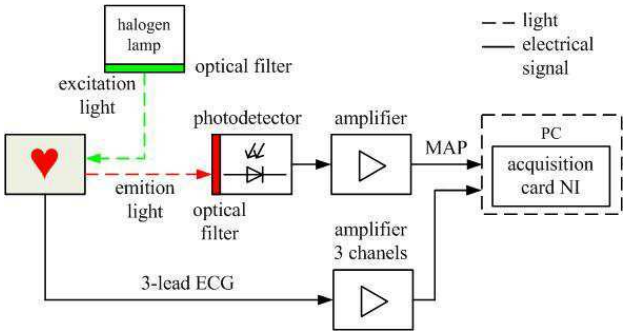

Figure 1. Block diagram of the simple recording setup.

2.3. Analysed data

During all monitored periods, ECG and MAP records are analyzed in each consecutive minute of all three periods of ischemia and reperfusion. Representative recording of ECG during one heart cycle and corresponding MAP is shown in Figure 2. Activation time (AT) should be measured from simultaneous recordings. AT is defined as the time measured from the earliest QRS deflection to the MAP upstroke. Action potential duration (APD) is duration of MAP from upstroke to 90% repolarization. Repolarization time (RT) is equal to the sum of AT and APD [3].

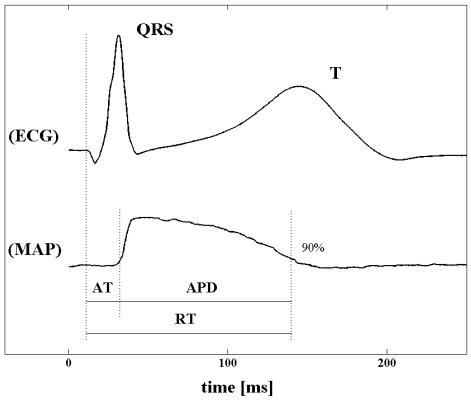

Figure 2. Simultaneous recording of ECG and MAP

The speed of impulse conduction via cardiac tissue can be evaluated from AT. AT represents the time necessary for spread of depolarization from atrioventricular (AV) node to the place of MAP recording, to be specific on the left ventricle epicardium (Figure 3). MAPs are recorded by optical method with optic fiber, which provides for MAPs registration from area of 200μm in diameter. The propagation of depolarization through the ventricular cardiac muscle is depicted in Figure 3 (red line).

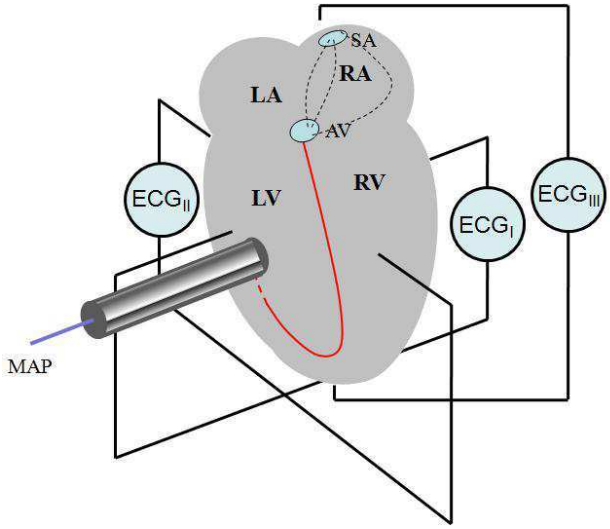

Figure 3. Schematic illustration of the heart, left atrium (LA), right atrium (RA), left ventricle (LV), right ventricle (RV), sinoatrial node (SA), atrioventricular node (AV), 3 leads ECG, optical probe for recording of MAP.

ECG signals are processed by R-peak detector to determine the onset of segments with the whole MAP. Then, MAP for each minute of particular experimental period is obtained sample-by-sample averaging of all MAPs recorded in the course of the examined minute.

3. Results

Evolvement of AT measured from ECG and MAP during ischemia periods is shown in Figure 4.

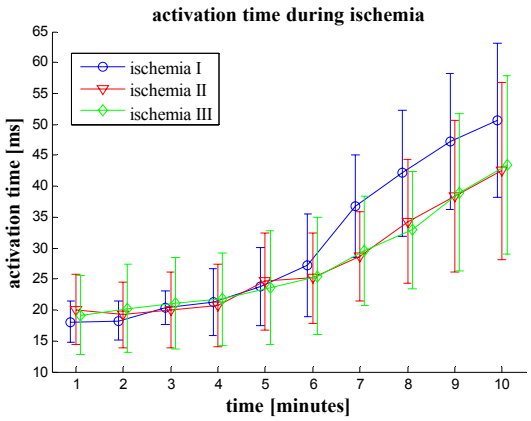

Figure 4. Activation time during three consecutive ischemia periods (interrupted by three consecutive periods of reperfusion of the same duration).

AT measured at the beginning of the first minute of ischemia I is the shortest one and it is dramatically changing during ischemia. During ischemia I, AT lengthened more than during next two repeating period of ischemia (II and III).

During reperfusion periods, AT shortens immediately within the first minute and it remains at the same value till the end of the examined period. The behaviour of AT during reperfusion is shown in Figure 5.

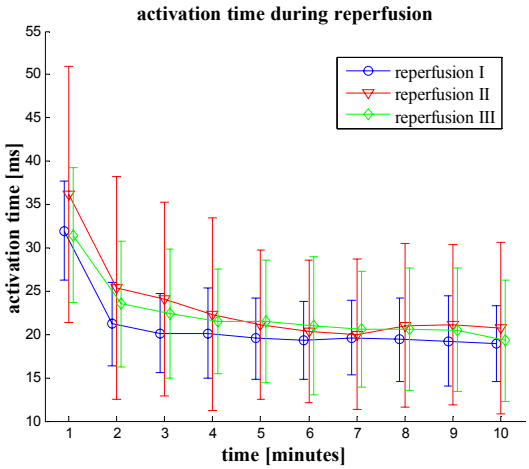

Figure 5. Activation time during three consecutive reperfusion periods (following always corresponding ischemia period).

The speed of impulse conduction via cardiac tissue can be calculated from AT and distance from AV node to the place of MAP recording, which is approximately 20 mm for rabbit hearts (see red line in Figure 3). In our experiments, speed of impulse conduction in rabbit heart tissue is 0.2-0.8 m/s for trajectory of 20mm. The above mentioned values are the mean values that include the propagation within the cell as well as the delays at the gap junctions [4]. Speed of impulse conduction decreases with ongoing ischemia and increases with ongoing reperfusion.

The change of propagation of depolarization within cardiac tissue results for instance in the change of the shape of ECG recording as it was discussed earlier [5].

4. Discussion and conclusions

The study of influence of ischemia on speed of impulse conduction through isolated rabbit hearts perfused according to Langendorff is possible due to uninterrupted long-time MAP and ECG recordings. The method of loading the heart tissue with VSD used in our laboratory enables us to record MAPs continuously throughout the whole experimental period, at least for 60 minutes [6]. MAPs recorded in this way from isolated rabbit hearts are comparable with recordings obtained by classical microelectrode technique. Indisputable advantage of this approach is the possibility to follow

electrophysiological changes during long periods of time, such as repeated ischemia-reperfusion phases representing a classical preconditioning model.

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