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RECORDING OF MONOPHASIC ACTION POTENTIALS DURING ISCHEMIA – REPERFUSION BY OPTICAL METHOD

NOVÁKOVÁ M.[1], BARDOŇOVÁ J.[2], PROVAZNÍK I.[2]

[1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic,
[2]Department of Biomedical Engineering, Brno University of Technology, Brno, Czech Republic

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A b s t r a c t

The present paper deals with new possibility how to study ischemia and reperfusion in rabbit isolated heart perfused according to Langendorff. Electrogram was recorded by touch-less method and coronary flow measured throughout the experiment. Moreover, monophasic action potentials (MAPs) were obtained by optical probe using voltage-sensitive dye di-4-ANEPPS. Each experiment consisted from four successive periods: stabilization (control), staining with the dye, washout of the dye, flow ischemia, reperfusion. During ischemic period, typical changes of electrogram were observed and they were accompanied by corresponding changes of MAPs. In conclusion, MAPs recorded by optical method can be used as a tool for studying ischemia – reperfusion changes in isolated rabbit hearts. However, since the effects of the dye to tissue itself are under intensive investigation and not fully elucidated yet, it is necessary to be somewhat cautious about the results obtained in this way.

K e y w o r d s

Isolated rabbit heart, Ischemia, Reperfusion, Monophasic action potential, Electrogram

INTRODUCTION

Cardiovascular diseases are the most common cause of morbidity and death in the Western countries. Prominent diagnosis among them is ischemic heart disease (or coronary heart disease) which – as a rule – is caused by a narrowing of a branch of the coronary artery (stenosis) by atherosclerotic process; thus, the blood supply to the heart muscle, and consequently the heart functions, become limited. The high incidence of this diagnosis is a reason for performing numerous studies – experimental and clinical – focused on causes, diagnosing and treatment of ischemic damage of myocardium.

Simple, but very reliable and prolific in results experimental model for studying both regional and total ischemia is isolated heart perfused according to Langendorff. Various parameters can be followed during periods of ischemia and reperfusion, such as electrogram (analogy to electrocardiogram recorded from the body surface), coronary flow, left-ventricular pressure, composition of perfusate, etc. Information about electrical activity of the heart is usually obtained from electrogram, which

represents summary from electrical field generated by the whole organ. It can be enriched by recording of monophasic action potentials (MAPs) which gives the experimenter additive information – about electrical changes of very small part of cardiac muscle (one cardiac cell in ideal set-up).

The golden standard for MAPs recording is microelectrode technique. Recently, optical method for MAPs recording with use of voltage-sensitive dyes was introduced. The aim of this paper was to compare ischemic changes in electrogram with MAPs recorded by optical method in isolated rabbit hearts and to decide whether this novel method is suitable for studying ischemia and reperfusion in this experimental model.

MATERIALS AND METHODS

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

In the study the hearts of 14 adult New Zealand rabbits of both sexes were included. The body weight of animals varied from 2200 to 3150 (average 2835 ± 263gr).

The animal was deeply anaesthetized by ketamin (60mg/kg of body mass) and xylazin (2mg/kg of b.m.), artificially ventilated and the chest opened. Then the heart was excised with a sufficiently long segment of ascending aorta and placed in a preparation bowl with a cold (5°C) Krebs-Henseleit solution of the following composition: NaCl, 118 mM; $NaHCO_3$, 24 mM; KCl, 4.2 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.2 mM; glucose, 5.5m M and Taurine, 10m M. Then, the aorta was cannulated and the heart fixed on the perfusion set-up modified for pharmacological studies (*1*).

All experiments were carried out at the temperature 37°C. The hearts were perfused with Krebs-Henseleit solution of above mentioned composition at constant perfusion pressure of 80mmHg. The solution was oxygenated with 95% O_2 and 5% CO_2. The hearts were allowed to beat spontaneously. After approximately 30 minutes of stabilization (control period), the cardiac tissue was stained with voltage-sensitive dye di-4-ANEPPS (amino-naphthyl-ethenyl-pyridinium) diluted in Krebs-Henseleit solution to the concentration of 2μM (stock solution in DMF, 2mM). The tissue was perfused with this mixture for 20–25 minutes. Then, excess of the dye was washed out for the same period of time as the staining. After the washout, the heart was ready for recording of optical APs. All hearts exhibiting any arrhythmias during stabilization (control period) were discarded.

During the whole experiment, electrogram was recorded and mean coronary flow monitored. The electrogram was recorded by the touch-free method (*2*). Six silver-silver chloride disc electrodes (4 mm in diameter) are placed on the inner surface of the bath in which the heart is placed during the experiment. Electrical signals are recorded from three orthogonal bipolar leads (X, Y, and Z). The signals are amplified and digitised at a sampling rate of 500 Hz by a three-channel, 16-bit AD converter. The maximum amplitude of recorded signals varies usually between 100 μV and 500 μV, depending on the heart. The mean coronary flow was measured every fifth minute during the whole course of experiment.

MAPs were recorded by optical method using a voltage sensitive dye di-4-ANEPPS (*3*). The recording system (*Fig. 1*) employs a flexible bifurcated fiber cable with seven optical fibers – six illumination fibers positioned in a circle and one detection fiber positioned in the center of the cable (*4*). Fibers (protected by a silicon inner tube and a flexible chrome plated brass outer tubing) are of diameter of 200 μm and are designed for wavelengths from 350 to 1100 nm. The used fiber optics makes the system flexible so the experimenter is able to scan action potentials from various sites of the preparation with almost no mechanical constraint. Optical probe is softly attached to the preparation to suppress motion artifacts without a need of focusing. The "input" end of the cable with six illumination fibers is

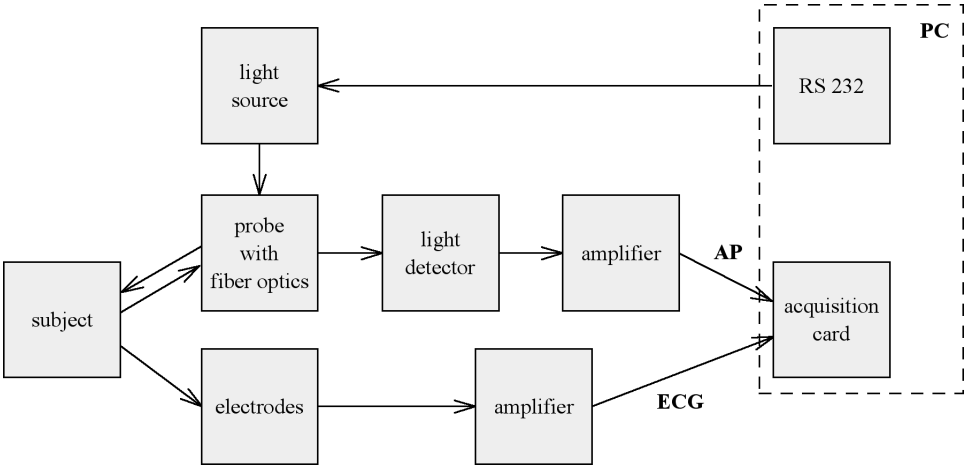

Fig. 1
The block diagram of the acquisition system. The excitation light is generated by a light source Intralux DC-1100 with a 150W tungsten-halogen lamp. The light is led by flexible fiber optics to the sample. Fluorescent light is emitted by voltage-sensitive dye present in the sample (e.g. isolated heart) and led back by the parallel fiber optics. The emitted light hits a photodiode detector. An electrical signal from the detector is amplified and digitized.

connected to light source. The "output" end with the detection fiber is connected to a light detector that senses the beam of emitted light. The changes in dynamic of transmembrane potential result in amplitude modulation of the emitted light. This is detected by a photodiode detector, filtered and processed by the data acquisition card. The digital signal is stored on a hard disk for further off-line processing (noise suppression, visualization and analysis). The signal is acquired by a LabView compatible data acquisition multifunction card and the data acquisition is controlled by subroutines of a software package LabView (National Instruments, USA).

Typical experiment consisted from five parts: control (stabilization) period, staining, washout, flow ischemia (flow of perfusate into the coronary system was decreased to zero) of various duration and reperfusion (of the same length). In this study, three successive periods of ischemia and following reperfusion duration of 10 minutes each were introduced.

RESULTS

At the end of stabilization (control period), synchronous recording of electrogram and MAPs was started (*Fig. 2A*). Spot with strong signal was found on the free wall of either ventricle and it has not been changed throughout the experiment. Electrograms recorded during periods of ischemia and reperfusion showed the typical picture of ischemia and the corresponding MAPs recorded by optical method behaved in the same way as MAPs obtained by classical microelectrodes (as known from literature), e.g. shortening of MAPs was observed during ischemia.

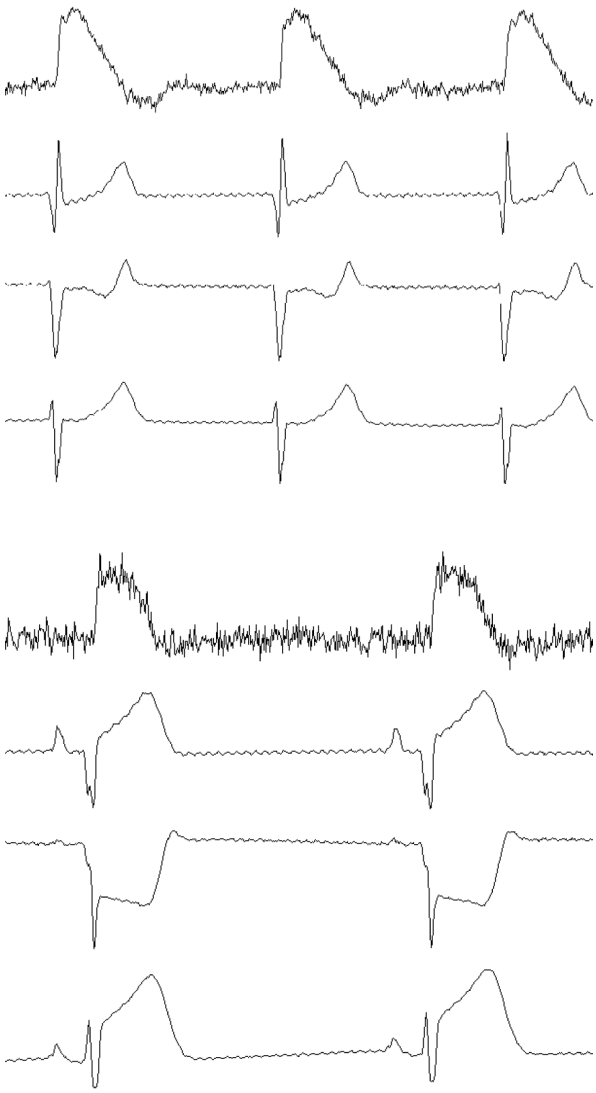

Fig. 2
Original recording of monophasic action potentials (top) and electrogram from 3D bipolar leads (bottom) in rabbit isolated heart at the end of control (stabilization) period (A) and during ischemia (B).

These changes were reversible during washout period. For typical recording during ischemia see *Fig. 2B.*

Detailed analysis from raw recordings (as seen in *Fig. 2*) is somewhat difficult since not always good signal-to-noise ratio can be obtained and the recorded signals are messed up with noise. Another problem is motion artifacts which affect the quality of recorded optical signal. In our laboratory, only mechanical constraint is used in order to diminish motion of the stained heart during measurement. In order to improve the quality of recorded optical signals as well as electrograms, at least ten successive MAPs and corresponding electrograms were averaged and used for further processing. An example of averaged signals during various phases of the experiment can be seen in *Fig. 3*.

Changes of mean coronary flow are summarized in *Fig. 4.* Measuring of coronary flow in this experimental set-up is not very precise and gives only supplementary information. It has been done merely in order to follow up the preparation and be sure that our model reacts in proper way. Although in first minutes of reperfusion after flow ischemia a clear tendency of hyperemia can be observed, the overall data are somewhat inconsistent and the changes insignificant. The values are summarized from all experiments and normalized to the end of control perfusion at the end of washout from staining, immediately before application of ischemia. For better clarity, the standard deviations are shown in the figure though it is not usually done in case of normalized values.

DISCUSSION

Voltage-sensitive dyes undergo changes in their electronic structure, and consequently their fluorescence spectra, in response to changes in the surrounding electric field. This optical response is sufficiently fast to detect transient potential changes in excitable cells (*5*). They have been introduced mainly to experimental cardiology in order to record action potentials by optical probe in a wide variety of cardiac preparations – from isolated heart to single cardiac cells. Simultaneous recordings of transmembrane potential by optical and microelectrode techniques have validated the high fidelity of optical MAPs (compared with microelectrode recordings). It demonstrated that optical MAPs recorded the classic features of MAPs from various parts of the conductive system and working myocardium (*6*). Voltage-sensitive dyes provide a powerful new technique for measuring membrane potential in systems where – for reasons of scale, topology, or complexity – the use of electrodes is inconvenient or impossible, e.g. in the presence of external electric fields – uninterrupted and artefact-free recording during pacing stimuli and defibrillation shocks or recording of high-resolution maps of cardiac repolarization (*7*).

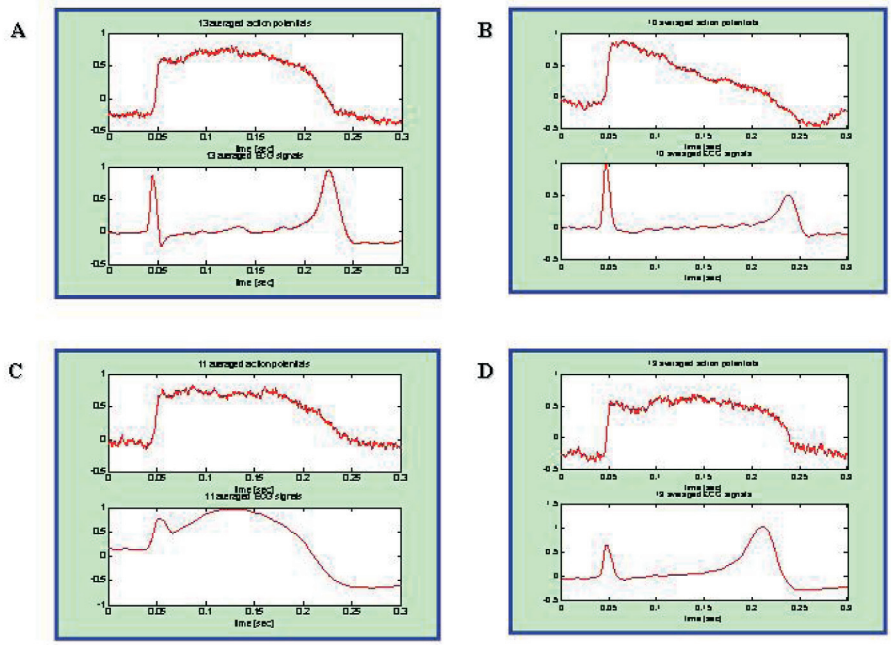

Fig. 3
Summary picture of monophasic action potentials (top) and electrogram from bipolar lead (bottom) in rabbit isolated heart during an experiment. A - control, B – 30 seconds of flow ischemia, C – 10 minutes of flow ischemia, D – 5 minutes of reperfusion. Note the typical changes in MAPs and electrograms. The MAPs and electrograms are summarized from 13 successive records.

In our laboratory, numerous projects are running. Some experiments are focused on detection of early stages of ischemia from electrocardiograms obtained in various experimental models and species. To find tools for uncovering subtle ischemic changes from electrical picture of myocardium it is necessary to record not only summary of electrical changes (electrocardiogram) but also local changes represented as MAPs. However, classical suction or prickle microelectrode always causes a slight damage of myocardium in which it is positioned. Use of voltage-sensitive dye di-4-ANEPPS may be a solution, especially in case when local ischemia changes are studied (e.g. ligation of a branch of the coronary artery). However, spare information in the literature forced us to perform this pilot study in order to obtain at least some data in our experimental model and to decide whether we can rely on the MAPs recorded by optical way in our standardized experimental set-up – isolated heart perfused according to Langendorff.

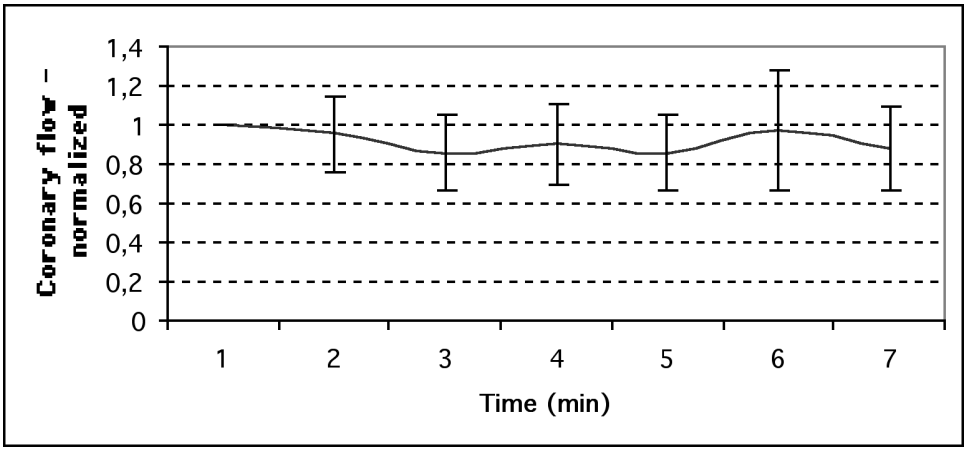

Fig. 4
Normalized coronary flow at the end of control and during three successive periods of 10 minutes ischemia and 10 minutes reperfusion.

There is still one limitation in our study and it is the reason why we stay back with our definite conclusion: not much is known about the direct effects of di-4-ANEPPS on the myocardium. In our previous experiments we observed marked prolongation of RR intervals and various changes of conductivity in AV node as well as changes of ventricular repolarization phase of electrogram (present as impairment of T wave and occasionally ST segment) (*8*). Original recording of electrogram in control period and during staining is pictured in *Fig. 5A* and *5B*.

In conclusion, we consider recording of MAPs by optical probe suitable for the studies of ischemia and reperfusion in rabbit isolated heart. However, certain cautiousness is necessary till the moment when all direct effects of voltage-sensitive dye di-4-ANEPPS will be thoroughly elucidated.

A c k n o w l e d g e m e n t s

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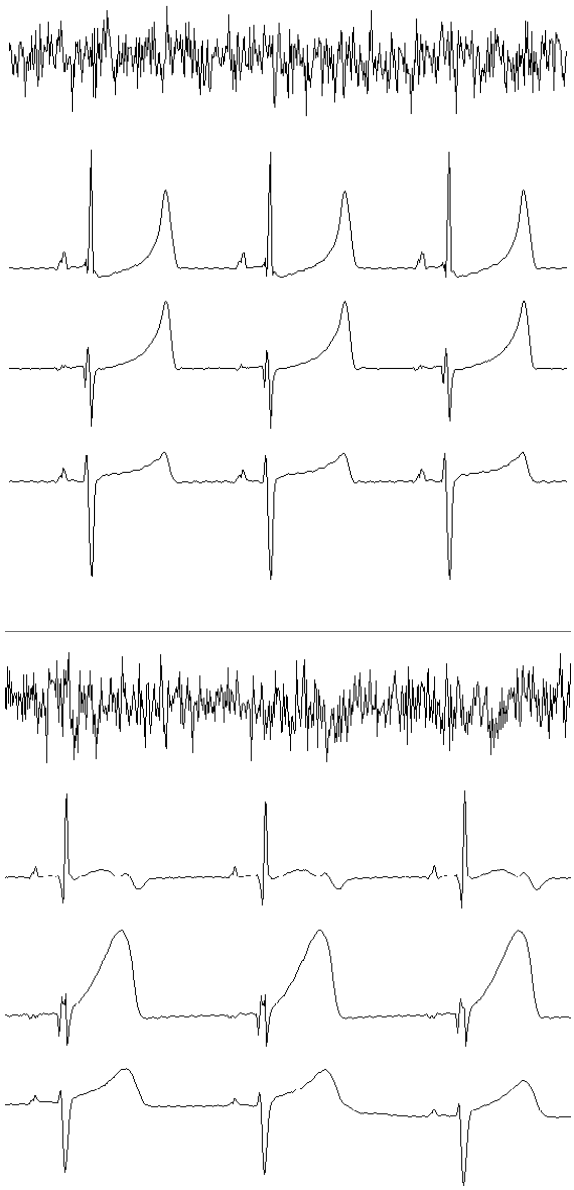

Fig. 5
Original recording of electrogram from 3D bipolar leads in rabbit isolated heart at the end of control (stabilization) period (A) and after 5 minutes of staining with voltage-sensitive dye (B). Note the change of T wave in the first lead and block of conduction between atria and ventricles in the second lead.

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