Isolated Rabbit Hearts – Databases of EGs and MAP Signals

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Abstract

During last decade, the myocardial ischemia was studied in our laboratory in isolated rabbit hearts. The three orthogonal electrograms and optical monophasic action potentials of the left ventricle were continuously recorded according to specific experimental protocol. The original data provided with comments are available in our database and may be useful for other researchers with various areas of interest.

1. Introduction

The database of cardiac signals can be used as a resource to develop and test new approaches for ECG delineation, arrhythmia detection, etc. Database of human ECG (e.g. MIT-BIH database, American Heart Association ECG Database, etc.) are commonly used for these purposes. But there are no databases of animal ECG, which can be useful for basic physiology studies or pharmacological studies [1],[2],[3].

We introduce the database of the signals recorded on isolated rabbit hearts during experiments with repeated ischemia. The isolated heart is a very good model that in particular allows examining myocardial work changes induced by various cardiac diseases, representing serious problem in developed countries. 3D electrograms (EGs) and optical monophasic action potentials (MAPs) recorded in twenty-four isolated hearts during long-term experiments are included in our database and may be useful for studies in various research areas.

2. Animal experiments

All animal experiments were carried out with respect to the recommendations of the European Community Guide for the Care and Use of Laboratory Animals and followed the guidelines for animal treatment approved by local authorities. Data recorded from twenty-four isolated hearts of New Zealand rabbits are included in present signal database.

The animals were introduced into deep anaesthesia by i.m. application of xylazin (2mg/kg) and ketamin (60mg/kg). The chest was opened, the heart with sufficiently long piece of aorta cut-off and placed in a preparation bowl with a cold (5°C) Krebs-Henseleit (K-H) solution of the following composition: NaCl: 118mM, $NaHCO_3$: 24mM, KCl: 4.2mM, $KH2PO_4$: 1.2mM, $MgCl_2$: 1.2mM, $CaCl_2$: 1.2mM, glucose: 5.5mM, and Taurine: 10mM. Aorta was cannulated and the heart retrogradely perfused at the constant perfusion pressure (80 mmHg) with K-H solution. The heart was placed into the test bath, a part of the modified Langendorff setup. The position of the heart in the bath was similar to its physiological orientation in the chest. All experiments were performed at 37°C. The temperature was controlled in cannula and inside the bath.

Experimental protocol included four consecutive phases: control perfusion, loading with the voltage-sensitive dye (VSD), dye washout, and optical MAPs recording (see Fig.1). Duration of control period, loading of the dye and dye washout was approximately 20 minutes each. The phase of MAPs recording (see below) consisted of three consecutive periods of ischemia and reperfusion, 15 minutes each. This experimental protocol allows among others study of heart electrical activity in ischemia preconditioning. During the whole experiment, a function of the heart was controlled by simultaneous touch-free recordings of 3D EGs (see below).

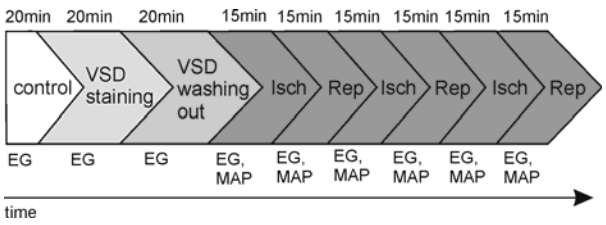

Figure 1. Experimental protocol. VSD-voltage sensitive dye, Isch - ischemia, Rep - reperfusion, EG – three pairs of orthogonal electrograms, MAP - monophasic action potential

3. Data acquisition

The experimental Langendorff setup used in our laboratory allows recording of 3 EGs and MAP from surface on isolated rabbit hearts [2], [4], [5] (see Fig.2). EGs are recorded by three pairs of silver-silver electrodes positioned orthogonally on the inner surface of the bath (see Fig.3). During the experiment, the bath is filled with conductive K-H solution. Thus, EGs are recorded by touch-less method, which does not disturb myocardial tissue.

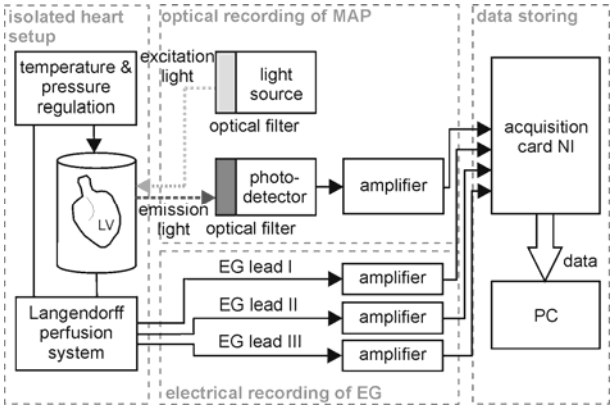

Figure 2. Recording system. LV - left ventricle, EG - electrogram, MAP - monophasic action potential.

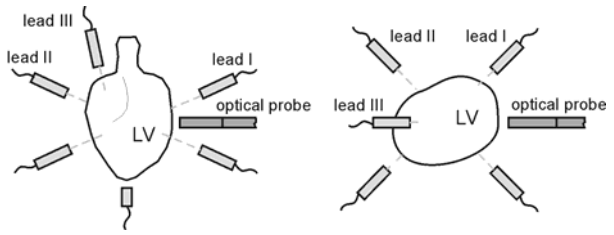

Figure 3. Placement of orthogonal system of electrodes (leads I, II, and III) and optical probe. LV - left ventricle.

MAP recording is based on VSD application and relative value of MAP corresponds to emission light value [6]. MAP is recorded from local area of the left ventricle (LV) surface by a light reflection. The light of special wavelength goes from light source to the heart surface stained with VSD and from the heart surface to a photodetector through a bifurcated fibre cable. The end of cable passes through the bath wall to the heart surface. The single-wavelength measurement of fluorescence emission is used. The emitted light is detected in wavelengths longer than the approximate central frequency of the emission peak. In our laboratory, VSD di-4-ANEPPS is used in MAP recording. Spectral characteristics of this VSD and optical part of used recording system are shown in Fig.4. As can be seen, di-4-ANEPPS has the excitation peak in blue region of spectrum and the excitation one in region between green and red regions. These spectral requirements are fulfilled with the use of excitation and emission filters of appropriate transmission characteristics.

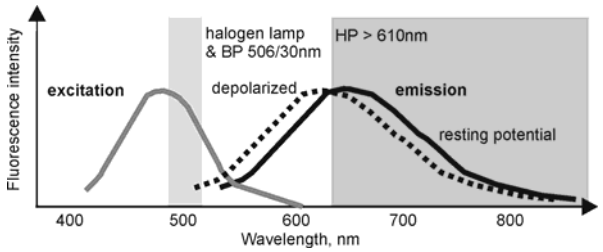

Figure 4. Spectral characteristics of di-4-ANEPPS and acquisition system. BP, HP - band pass and high pass optical filter, respectively

However, emission light detected in this way depends not only on transmembrane potential but also on other factors (e.g. on density of the dye in the area under photodetector, the degree of dye internalization, etc.). Thus, recorded optical signal contains information about the relative changes of potential course, not its absolute value. Recording setup for fluorescent measurement in our experiments was not calibrated due to lack of a proper calibration method. MAP is therefore presented as the relative signal. Another approach is to normalize MAP to voltage range of 100mV [6]. Moreover, MAP signal acquired by the single-wavelength recording setup is often disturbed by motion artefact, which can be suppressed using some special mathematical techniques. No excitation-contraction uncouplers were used in experiments because of possible effect on the course of MAP [7].

Recorded signals are then amplified by bioamplifiers DAM-50 (World Precision Instruments, Inc.), simultaneously digitized by 16-bit AD converters at 2 kHz sampling rate with the LabView compatible data acquisition multifunction card PCI-6250 (National Instruments, USA), and stored on PC.

4. Data

In most cases, EGs and optical signal were simultaneously recorded during the whole experiment focused on repeated ischemia, which contains eight periods (control, loading and washing out of the VSD, three ischemia, and three reperfusion periods). In nine experiments, the incomplete numbers of ischemia-reperfusion periods were acquired. Although all 24 hearts were loaded with VSD, not always the heart was loaded satisfactorily and as a consequence it is not possible to analyse all periods of MAPs recording (which is, for example, useful to study the effects of VSD on heart electrophysiology). From the first ischemia phase to the

end of experiment, excitation of the heart surface was performed in such a way that MAPs appear in recorded optical signal.

Data are stored in binary and also .mat format. The document in .txt format is available to each experiment. This document contains index of beginning of each experimental phase and other important notes which were done during the experiment. Information about date of experiment, animal gender, body mass, specific changes in EG or MAP morphology or EG rhythm, artefact occurrence, amplification value are among the most frequent notes. It may be useful for further analysis of the signals.

The data acquisition setup (especially method of VSD staining and recording system) allows obtaining the high quality signals. Signal-to-noise ratio (SNR) of optical signals is within the range of 17-22 dB depending on duration of VSD staining [4]. In most cases, SNR of EGs is higher than 90 dB. This is high enough for recording of P wave, which can be found in most of EGs. Moreover, sampling frequency of 2 kHz is sufficient for correct detection of R wave. Example of time course of EGs and MAP in control and ischemia is shown in Fig.4.

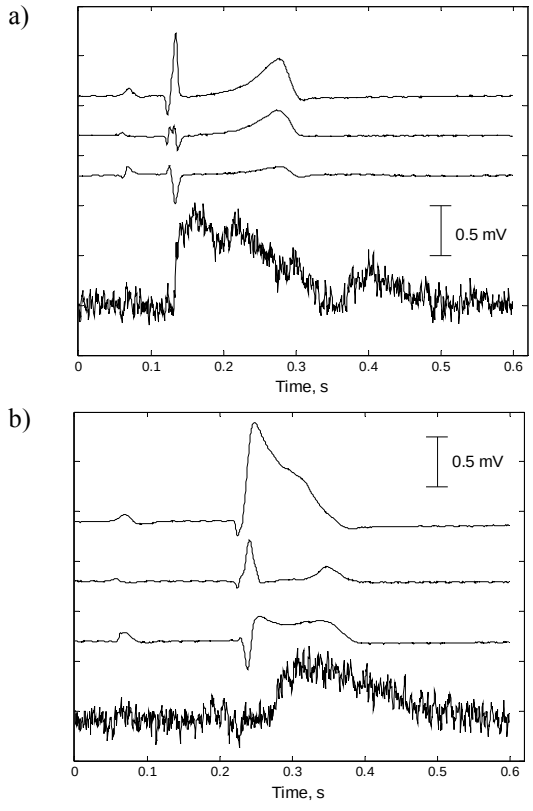

Figure 4. Example of signals recorded in control (a) and ischemia (b): electrograms from lead I, II, and III, and optical action potential (from top to bottom).

As can be seen in Fig.4, there are some changes in EGs and MAP morphology and EGs rhythm caused by perfusion stopping. The downward part of MAP recorded in control phase is disturbed with motion artefact, which is much less pronounced in ischemia (compare Fig.4a and Fig.4b). There are some types of arrhythmias in signals in ischemia. The most frequent of them are shown in Fig.5.

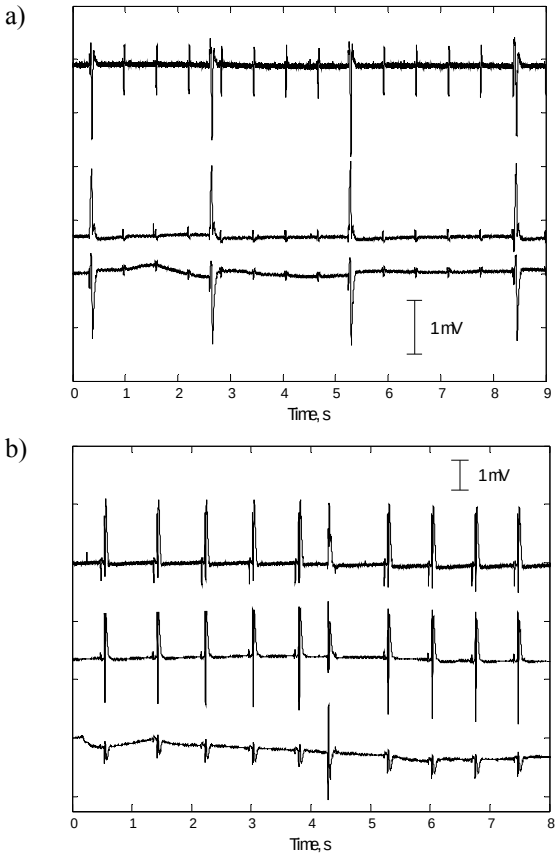

Figure 5. Example of partial AV block (a) and ventricular extrasystoles (b) caused by global ischemia: electrograms from lead I, II, and III (from top to bottom)

5. Possible applications

Fifteen signals were recorded continuously during all phases of experiments and nine were recorded incompletely (without some phases). Whole signals or some of their parts can be used in various research areas, such as:

- influence of di-4-ANEPPS on electrical activity in isolated rabbit heart [8],
- course of global ischemia in isolated heart [2],
- study of various types of arrhythmias,
- evaluation of EG and MAP recorded during experiments with ischemia [2],[9],[10],
- study of relationship between EG and MAP [2],
- comparison of EG and MAP of isolated heart of rabbit and other species [8],

- comparison of EG and MAP of *in vivo* and isolated rabbit heart [11],[12],
- study of preconditioning in isolated rabbit heart [4],
- heart rate variability analysis [11],[12],
- evaluation of vectorcardiogram in control, ischemia, and reperfusion [13],
- testing of approaches for automatic delineation of EG and MAP,
- testing of approach for EG and MAP filtering,
- design and testing of mathematical methods for motion artefact suppression in MAP [14],
- design and testing of automatic classification of EG [15],
- T-wave alternans analysis,
- study of relationship between QT and RR intervals,
- etc.

If the signals are used for evaluation of ischemia or preconditioning, the application of VSD and its possible effects on heart physiology must be taken into account to avoid the incorrect interpretation of the results.

6. Conclusions

More detailed information about data and experiments, and useful links are available on www.ubmi.feec.vutbr.cz/cardio.

Data files can be obtained after sending a request. This database is continually updated by new signals recorded in isolated rabbit hearts according to the present as well as other experimental protocols.

Acknowledgements

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