Electro-Optical Recording System for Myocardial Ischemia Studies in Animal Experiments

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Abstract

Myocardial ischemia is the most common cause of death in the developed countries. The most severe manifestation is sudden death due to electrical instability, terminating in ventricular fibrillation or heart arrest.

This study is based on experiments in which electrograms and monophasic action potentials are recorded from isolated guinea pig hearts perfused according to Langendorff during various phases of acute ischemia and reperfusion. The data are then processed by a wavelet transform in order to obtain complex-valued time-frequency patterns.

1. Introduction

Myocardial ischemia is the most common cause of death in the Western world, and encompasses the range of conditions in which the heart muscle is deprived of oxygen to some extent, and, as a result, becomes damaged (ischemic). In the developed countries, the prominent manifestation of myocardial ischemia is sudden cardiac death. In the majority of cases, sudden death is caused by a severe disturbance of the electrical properties of the cell membranes. Naturally, the study of ischemic heart disease can reveal mechanisms of its genesis and its influence on the electrophysiology of the heart muscle. Results of such studies then contribute to a better understanding of the disease and improve both pre-infarction and post-infarction treatments [1].

We present flexible electro-optical recording system, which allows simultaneous recording of electrograms from orthogonal leads and action potentials from an arbitrary site on heart surface. The system was used to confirm the results of time-frequency analysis of ECG signals for early detection of myocardial ischemia in animal experimental model.

2. Methods

The methods used in the project are based on animal experiments in which electrophysiological data are recorded from isolated guinea pig hearts perfused according to Langendorff during various phases of acute ischemia. The data are then processed by a wavelet transform in order to obtain complex-valued time-frequency patterns.

2.1. Animal experiments

The perfusion apparatus for studying completely isolated mammalian heart introduced by Oscar Langendorff in 1895 [2] has been modified in our laboratory for electrophysiological and pharmacological experiments [3]. The set-up was successfully employed in our current experiments concerning early myocardial ischemic changes.

The study followed the local ethics guidelines for animal research. The animals under deep ether anaesthesia were sacrificed by cervical dislocation. The chest was quickly opened, the heart with sufficiently long piece of aorta immediately cut-off and placed in a preparation bowl with a cold (5°C) Krebs-Henseleit (K-H) solution (1.2 mM Ca^{2+}). The aorta was cannulated and the heart perfused at the constant perfusion pressure (100 mmHg) at the temperature 37°C.

After stabilisation period (15-20 min), the isolated heart was loaded with voltage-sensitive dye (see 2.3) for 20 minutes and washed-out with K-H solution for 15 minutes. Then the preparation was ready for measurements, which were done in three phases: control period (15-20 minutes, all hearts exhibiting any arrhythmias were excluded from experiment), ischemic period (flow ischemia, 20 minutes) and reperfusion period (20 minutes). During all experiment, simultaneous touch-free recordings of electrogram and MAPs were done.

0276–6547/03 $17.00 © 2003 IEEE

Computers in Cardiology 2003;30:573–576.

2.2. Recording set-up

The employed optical recording system is based on application of voltage sensitive dye (VSD) into the examined tissue. VSD's 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 cardiac cells. Fast VSDs (e.g. aminonaphthylethenylpyridinium dyes) are one of the most consistently sensitive probes and can be used in electrophysiological studies in beating hearts. They have a fairly uniform 10% per 100 mV changes in fluorescence intensity in a variety of tissues and cells. Spectral properties of VSD's determine the use of common tungsten or halogen lamps for excitation of dyes and common single photodiode detectors to register changes in emitted fluorescence.

The current optical systems are based on fixed optics. The described system employs a flexible bifurcated fiber cable with seven optical fibers – 1. six illumination fibers positioned in a circle, and 2. a detection fiber positioned in the center of the cable. The fiber optics makes the system flexible so the user is able to scan action potentials from various sites of the preparation with almost no mechanical constraint. The 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 connected to a light source. The "output" (detection) fiber is connected to a light detector that senses the beam of emitted light.

Special fiber cable FCR-7IR200-2-ME (Avantes, the Netherlands) was used in our recording system. Fibers are of 200 μm in diameter and are designed for wavelengths from 350 to 1100 nm. The optical fibers are protected by a silicon inner tube and a flexible chrome plated brass outer tubing. The tubing also gives stress relieve.

A 150W halogen light source Intralux® DC1100 (Volpi AG, Switzerland) was chosen as a source of an excitation light. This cold light source with high intensity light output is designed for fiber optic applications with extremely stable light output (ripple <0.01%). The light source contains a built-in IR filter, which prevents a preparation from heating, and a band-pass filter (560 nm +/- 30 nm), which selects light at excitation maxims of the used dye. The light intensity can be adjusted by a crescent shaped diaphragm and by controlling the lamp voltage. Knobs on front panel and/or through RS232 interface can control the light intensity by original software.

The changes in dynamics of transmembrane potential result in amplitude modulation of the emitted light. This is detected by a photodiode detector with a high-pass (>610 nm) filter. As a detector, the Si photodiode 3 WK 164 87 is proposed (Tesla Blatna, Czech Republic). The output signal of the photodiode detector is preamplified so that the two stage amplifier adjusts the signal to input range of data acquisition card (±1 V). The electrical circuits include also an analogue anti-aliasing filter (low-pass filter fc=2 kHz) and a high-pass filter (f_c=0.05 Hz) to suppress DC offset.

Orthogonal ECG signals were recorded from six silver-silver electrodes positioned on the inner surface of the bath. All signals from the light detector and electrodes were simultaneously digitized by 12-bit AD converters at 4 kHz sampling rate.

The data acquisition card processes the preamplified and filtered signal. The card digitizes the signal with 12 bits dynamic range and at rate of 4000 samples/sec. 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 PCI-6111E (National Instruments, USA). Data acquisition is controlled by subroutines of a software package LabView (National Instruments, USA).

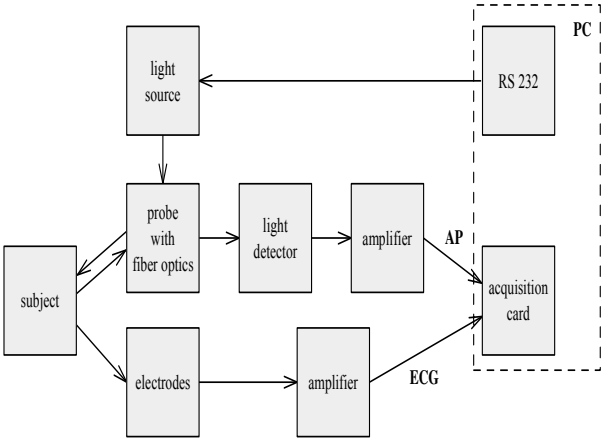

Figure 1. The block diagram of the acquisition system. The excitation light is generated by a light source Intralux DC-1100 with a 150W tungsten-halogen lamp. The light is led by flexible fiber optics to the sample. Fluorescent light is emitted by voltage-sensitive dye present in the sample and led back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

2.3. Voltage-sensitive dye

The aminonaphthylethenylpyridinium (di-4-ANEPPS) produced by Molecular Probes, Inc. was used as a voltage sensitive dye in our experiments. VSD's spectral characteristics are dependent on the type of tissue and solution in which it is applied. The absorption and fluorescence emission maxims of di-4-ANEPPS in methanol are at wavelength of 496 nm and 705 nm,

respectively. Absorption and emission maxims are at shorter wavelengths in membrane environments than in the reference solvent. The difference is typically up to 20 nm for absorption and 80 nm for emission.

2.4. Analysis tools

Wavelets are an efficient tool for analysis of short-time changes in signal morphology [4]. CWT is the preferred type of wavelet transform for signal analysis in opposition to the non-redundant type corresponding to the expansion on orthogonal bases (multiresolution analysis). The reason is that the CWT allows decomposition on an arbitrary scale. Thus, frequency bands of interest can be studied properly at chosen resolution.

The term wavelet analysis represents expansion of a discrete-time or continuous-time signal on wavelet bases. Generally said, the expansion can be provided by any well-known signal-processing tool such as Fourier transform. However, wavelet analysis exploits a simple idea - the signal is expanded on a set of dilated or compressed wavelet functions

$$\psi\left(\frac{t-b}{a}\right)$$

CWT uses shifts b and scales a (dilation and contraction) of a generally complex-valued wavelet $\psi(t)$ instead of its shifts and modulations as for Fourier transform. CWT of signal $f(t)$ is defined as

$$CWT(a,b)=\int_{-\infty}^{\infty}\frac{1}{\sqrt{a}}\psi^{*}\left(\frac{t-b}{a}\right)f(t)dt\,.$$

Complex-valued wavelet transform plays a special role in signal analysis. Complex nature of wavelets provides further improvement in signal detection compared to real-valued wavelet analysis. This is possible due to dual processing through cross-correlation with real and imaginary parts of wavelets. The resulted complex-valued time-frequency spectrum can be further analyzed by detection of significant attributes in its modulus and phase. In this way, not only the waves can be detected but also various shapes and significant points of the waves can be distinguished.

One of the most used complex-valued wavelets in signal processing is a family of complex Morlet wavelets. It is defined by

$${}_{b,c}\psi(t)=\sqrt{\pi f_b}\,\exp(i\pi f_c t)\exp\left(-\frac{t^2}{f_b}\right)$$

where f_b is a bandwidth parameter and f_c is a wavelet center frequency. They are (anti-) symmetric and have infinite support.

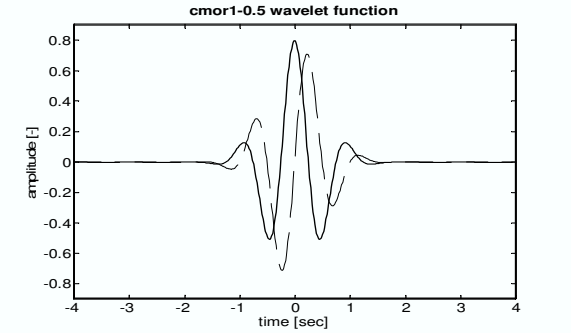

Figure 2. Complex Morlet wavelet No.1-0.5, f_b=1, f_c=0.5. Legend: solid line - real part, dashed line - imaginary part.

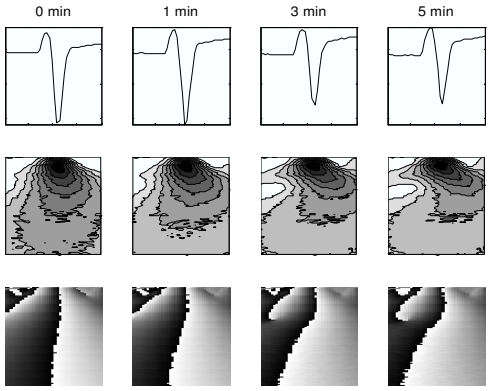

Figure 3. (a) X-lead recordings (80 msec segments, 0.5 mV amplitude window), (b) magnitude time-frequency spectra and (c) phase time-frequency spectra of the above signals using complex Morlet wavelet No.1-0.5. Legend: rows – (a) (b) (c), columns – 0 min (baseline), 1, 3, and 5 minutes after LAD occlusion.

Complex-valued CWT analysis may be further improved by considering its phase. As the second step, the phase of the complex-valued CWT has been studied. It has contained discontinuities along time axis that has revealed as vertically oriented curves. The objects have corresponded to extreme and inflection points of local waves.

The CWT phase spectra have complex structure that is does not allow easy and reliable analysis. However, one can see that the phase contains a number of steps of π (-π) or $\pi/2$ (-$\pi/2$). The steps compose vertical oriented curves in phase spectra images corresponding to time position of significant points of the analyzed signal.

For analysis purposes, the phase can be thresholded to obtain a less complex output image. Thresholded phase is defined as

$$P_{th}(a,b)=\begin{cases}1 & for \quad \left|\arg\left[CWT(a,b)\right]\right|\in\langle th-\varepsilon;th+\varepsilon\rangle\\ 0 & for \quad \left|\arg\left[CWT(a,b)\right]\right|\notin\langle th-\varepsilon;th+\varepsilon\rangle\end{cases}$$

where $th=\{0, \pi\}$ or $\{-\ /2, \pi/2\}$ is threshold, ε is a small real number. The phase thresholding at $\{-\ /2, 0, \pi/2, \pi\}$ results in binary image with vertically oriented curves located at positions of phase steps.

3. Results

7 experiments were included in the study. Simultaneous recording of MAP and electrogram was studied in each. The data were analysed by complex-valued wavelet transform.

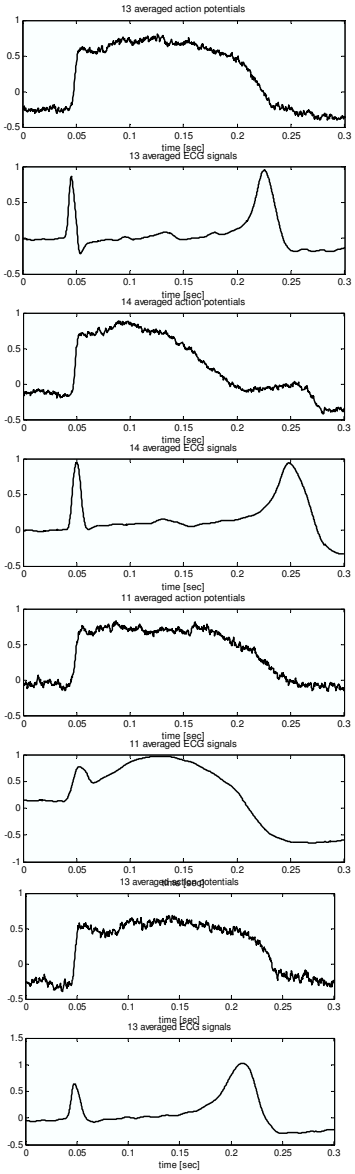

Figure 4. Original recordings of MAP (upper trace) and electrogram (lower trace) during (from top to bottom): control, 2 minutes of ischemia, 10 minutes of ischemia, and 10 minutes of reperfusion.

The ST-segment deviated after 1 minute of LAD occlusion meeting the ST-elevation criteria in 60% of cases. In all of subjects, there was a significantly increased number of phase crosses in phase time-frequency spectra of QRS complexes ($p<0.01$). The typical changes in MAP shape preceded ischemic changes in electrograms (Fig. 4, compare control and 2 minutes of ischemia).

4. Conclusions

The present electro-optical recording system has been verified on isolated Langendorff-perfused hearts. In comparison with standard methods of MAP recoding, its advantage is in the possibility to record without injuring the heart surface, i.e. for a longer period and successively from many sites. The electro-optical recording system has been proved a helpful tool in detection of early ischemic changes.

Acknowledgements

This work has been partly supported by the grant project FRVS MSMT No. 2167/2003/G1, the grant project GACR No. 102/01/1494, and Research Programs of Brno University of Technology No. J22/98:262200011 and J22/01:262200022.

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