Analysis of Voltage-Sensitive Dye Influence on ECG Segment Variability

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Abstract

This paper deals with analysis of changes in ECG signals caused by application of voltage-sensitive dye (VSD), which is necessary part of touch-less recording of electrical activity of the heart by optical way. The dye is injected into perfusate during animal Langendorff experiment.

The recorded ECG signals were divided into P-Q segments, QRS complexes, and ST-T segments of selected consecutive heart cycles in each experimental period. Changes of ECG cycles caused by VSD application were studied during experimental phases. The analysis were performed in time and time-frequency domain and compared.

1. Introduction

Optical mapping of action potentials is a valuable technique, which has been developed in late 1960's [2] as a new high-resolution tool breaking the limits of the traditional microelectrode ECG mapping [5]. At present, the optical mapping is widely used in cardiac electrophysiology research [9], [4], [6]. The optical mapping is also widely used in cardiac electrophysiology animal experiments [2], [5]. The principle of optical mapping is an application of voltage-sensitive dye (VSD) to examined tissue [8] where it binds to a membrane of cardiac cells. The dye undergoes changes in its fluorescence spectra, in response to changes in the surrounding electrical field. Absorption and fluorescence spectra of the dyes are highly dependent on their environment. The dyes are essentially non-fluorescent in water and become quite strongly fluorescent upon binding to membranes. The tissue is illuminated by light with relatively limited narrow spectra. Then, the dye emits fluorescent light of higher wavelength and amplitude proportional to the potential at heart surface. The emitted light can be easily detected and recorded.

The optically recorded signal is then usually used for electrophysiology studies. However, results can be negatively influenced by electrophysiology changes induced by the used VSD.

In this paper, we propose and compare two methods for analysing expected changes in time domain and time-frequency domain.

2. Methods

In this study, hypothesized electrophysiological changes were studied in an animal model. Such a model allows precisely and repeatedly apply voltage sensitive dyes into the heart while its electrical activity can be easily recorded by a number of methods.

2.1. Experimental setup and protocol

Ten guinea pig hearts were included in the study. Each heart was mounted on a Langendorff apparatus, filled with Krebs-Henseleit (K-H) solution (1.25 mM Ca2+) and placed in a bath (37°C) [7]. The hearts were perfused at the constant pressure of 85 mmHg. The hearts were stabilized for at least 15 minutes. After control period, the hearts were perfused with 1mM solution of VSD di-4-ANNEPS diluted in K-H solution (loading period).

ECG signals were continuously recorded for 15 minutes of control parts, than during VSD application till the end of loading period after another 15 minutes. Next experimental periods were washout and acute myocardial ischemia caused by stopping perfusion for 15 minutes.

The ECG signals from orthogonal leads were recorded from Ag-AgCl electrodes positioned on the inner surface of the bath in Langendorff apparatus. The signals were digitized by a 12-bit AD converter at 4 kHz sampling rate using a data acquisition multifunction card PCI-6111E (National Instruments, USA). The digital signal was stored on a hard disk for further off-line processing.

2.2. Analysis

In all segments, heart beats were detected by a common QRS-complex detector and manually segmented into P-Q, QRS, and ST-T intervals.

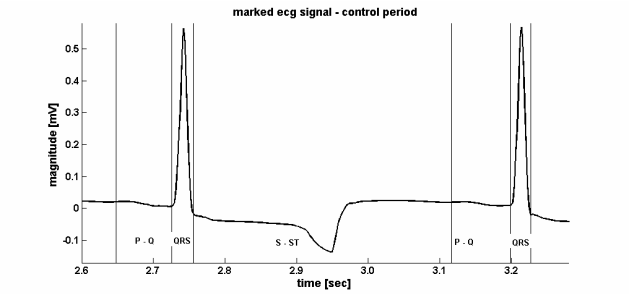

Figure 1. Segmentation of ECG cycles for analysis: P-Q, QRS, and ST-T.

Two methods were used to detect hypothesized electrophysiology changes induced by VSD. The first method is based on analysis of changes in shape of individual segments in time-domain by measuring their distance, and the second method incorporates wavelet transform and dynamic time warping to reveal changes in time-frequency domain also by measuring their distance. Results of both methods are compared to development of R-R intervals during the experiments.

R-R intervals are used to show tendency of duration changes during all experiments parts in this study. Figure 2 demonstrates an example of RR interval measurement on representative recording.

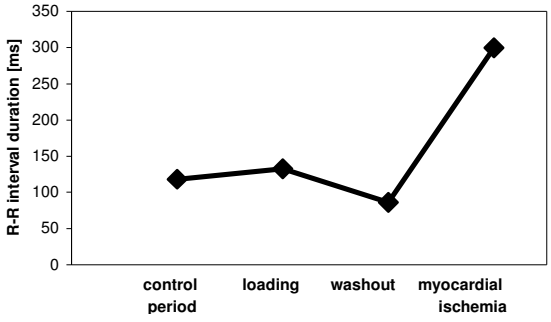

Figure 2. Example of R-R interval duration during an experiment.

2.2.1. Measuring distance in time domain

The first analysis method is based on linear alignment of two selected segments and measuring their distance. A representative control segment is selected from the control period of the experiment. Distance $D_{r,s}$ between two segments represented by vectors (time-sequences) of signal samples is defined in time domain as

$$D_{r,s} = \frac{1}{N}\sum_{i=1}^{N}\left|P_{r,i} - O_{s,i}\right|$$

where pattern P is a representative segment from a control period and O is a segment from other parts of the experiment. Variables r,s symbolize order of cycles, i time samples.

Figures 3 - 5 show variability of distance in time domain in P-Q segments, QRS complexes, and ST-T segments, respectively.

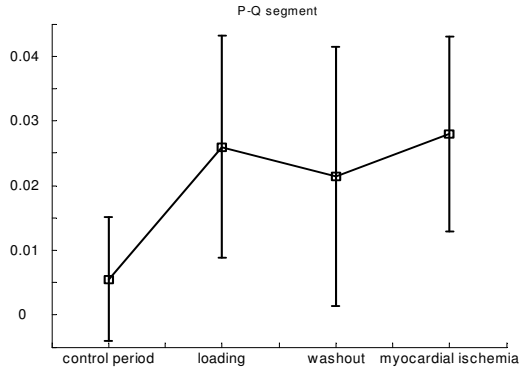

Figure 3. Variability of distance in time domain in P-Q segments.

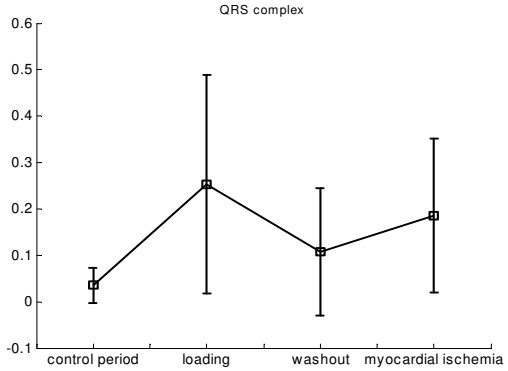

Figure 4. Variability of distance in time domain in QRS complexes.

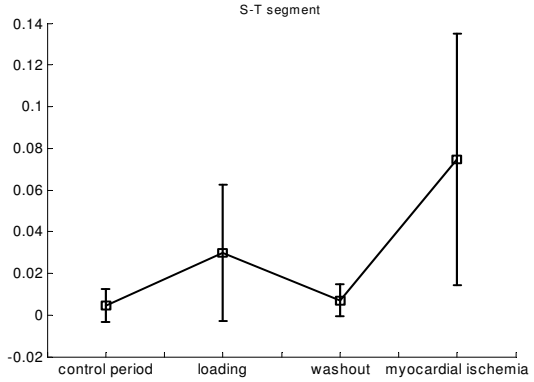

Figure 5. Variability of distance in time domain in ST-T segments.

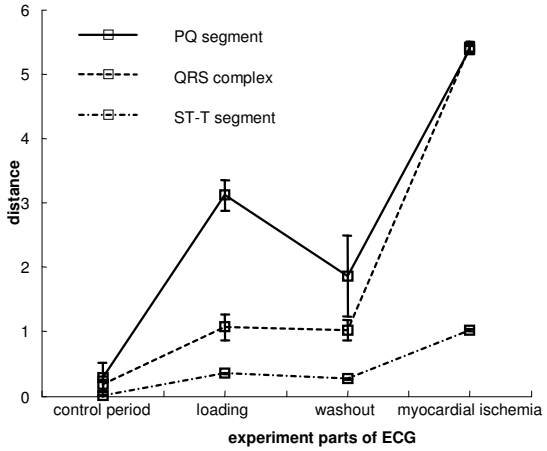

Figure 6. Variability of distance in time-frequency domain. Solid – variability of P-Q segments; dashed – variability of QRS-complexes; dashdotted – variability of ST-T segments.

2.2.2. Measuring distance in time-frequency domain using dynamic time warping

The second method incorporates wavelet transform to reveal short-time frequency limited changes in time-frequency domain using dynamic time warping (DTW) method. DTW finds for each sample in one of compared signals, the correspondent sample in the other signal that is closest to the original sample using predefined metric. Given this correspondence, it is possible to calculate a distance between the signals under comparison.

Continuous wavelet transform (CWT) is defined as correlation of a signal $x(t)$ with wavelets $g^*[(t-t)/l]$, where t is time shift, l is time dilation, and * represents complex conjugate.

$$CWT(\lambda,\tau) = \int_{-\infty}^{\infty} \frac{1}{\sqrt{\lambda}} g * \left(\frac{t-\tau}{\lambda} \right) x(t) dt$$

Morlet wavelet was used for CWT for its relatively smooth shape [1]. The analysis resulted in a sequence of vectors representing frequency components between 0-fs/2 at each time instant. The vectors were used as time sequences for dynamic time warping (DTW) as described below.

Time differences between two time sequences (A and B, of length I and J) are eliminated by warping of time axes. The algorithm with DTW searches for optimal path $m=\psi(n)$ in the plane (n,m), which minimizes a function D. The function D is computed as overall distance between time sequences A and B as

$$D(A,B) = \sum_{n=1}^{I} d[a(n), b(\psi(n))].$$

3. Results

R-R interval responses to VSD application with a delay affected by coronary flow that is individual to each heart. Sharp slow-down of heart rate can be caused by a block of excitation transfer from the sinoatrial node to the atrioventricular (AV) node. Long-term analysis of the R-R intervals reveals further gradual slowing of heart frequency during loading. The heart frequency is partly restored during wash-out after loading period. Time domain and time-frequency domain analysis using measurement of distances revealed large changes in P-Q segment during VSD loading, lower changes in QRS complex and almost no shape changes in S-T segment. P-Q segment and QRS changes were partly restored in wash-out. The detailed beat-by-beat analysis corresponds to results of RR-interval analysis.

4. Discussion and conclusions

The proposed algorithms based on measurement of distances in time domain and time frequency domain using dynamic time warping were applied to ECG recordings from voltage-sensitive dye experiments. Generally, significant changes in all ECG signal segments detected in various phases of the experiment in corresponded in both methods to expected and confirmed changes in RR intervals. In the end of wash-out period, changes were considerably lower for all segments or they

at least showed a decreasing trend. As expected, changes were remarkably different in myocardial ischemia period due to known electrophysiological effect of coronary artery occlusion.

It may be concluded that optimal path of dynamic time warping applied to wavelet transformed ECG signals is sensitive to loading of VSD into examined heart. However, simple measurement of distances in time domain is sensitive as well. They can distinguish particular periods of optical measurement procedure. Further, both methods can be used to analyse various fractions of a heart cycle and thus be exploited for analysis of VSD influence to the conductive system of the heart.

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