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ECG SEGMENT VARIABILITY DURING VOLTAGE-SENSITIVE DYE APPLICATION IN ANIMAL HEART

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Abstract

The optical mapping of action potentials from heart surface has been used extensively as a new possibility of non-invasive recording methods. It employs application of voltage-sensitive dyes (VSDs). Previously, VSDs have been successfully used in myocardial ischemia studies. Our previous Langendorff-perfused heart experiments have suggested shape changes in ECG signals in time and time-frequency domain caused by application of VSD di-4-ANEPPS during optical mapping. These changes went through detailed analysis to reveal impact on particular fractions of a heart cycle. The heart cycle was divided into three parts, corresponding to P-Q segments, QRS complexes, and ST-T segments. Dynamic time warping (DTW) is applied to all marked segments in each experimental period (control, loading, and washout phase). Variability of ECG parts is measured by means of an optimal path resulting from DTW based on minimum distance between the control signal segments (before the application of VSD) and segments recorded in the next two experimental steps.

Key words

Voltage-sensitive dye di-4-ANEPPS, ECG signal, Wavelet analysis, Dynamic Time Warping

INTRODUCTION

Ischemic heart disease (or coronary h. d.), the most common cause of death in industrialised countries (*1*), covers a range of conditions in which blood supply to the heart muscle, and consequently the heart functions, become limited. As a rule, it is caused by a narrowing of a branch of the coronary artery (stenosis) by the atherosclerotic process.

The diagnostic processes of ischemic heart disease consist of invasive and non-invasive methods. The development of non-invasive methods has been required by the tendency to avoid the untoward side effects during instrumentation, risk of iatropathogenic damage (mainly by infection) and, last but not least, the economic aspects of cardiovascular diagnostics. Further, the results could help to develop a new non-invasive method needed in cardiology diagnostics.

Ischemic heart disease is instrumentally diagnosed by coronary angiography, which represents the reference method. Echocardiographic data offer valuable information on regional or global impairment of cardiac function as do the radionuclide perfusion methods based on thallium 201 or technetium 99 uptake. For more than a half of a century, the electrocardiographic method has been the most widely used diagnostic test for the detection and evaluation of cardiac ischemia (*2*). The attenuated or stopped coronary perfusion causes a potential difference between the ischemic and normal regions during the ST segment and, eventually, also at rest. While ST depression is the common manifestation of transitory, exercise-induced cardiac ischemia, ST elevation is related to severe transmural ischemia, acute myocardial infarction. Myocardial ischemia also affects other features than the ST segment (*3*) including an increase of QRS amplitude, a subtle prolongation of QRS duration, and shifts of QRS axis (*4*). These changes reflect abnormal conduction due to irregular membrane depolarisation. There is ample evidence that cardiac ischemia may change the QRS spectrum as the expression of fragmentation of ventricular depolarisation.

Recent findings show that acute myocardial ischemia in early stages can be detected using the analysis of intra-QRS changes. These changes reveal local ischemia-induced propagation changes earlier than do traditional ECG-based indices (*5*), (*6*). Recent findings specify the changes as low-amplitude short-time events within a window of ventricular depolarisation. The actual time and frequency localisation of the changes depend on the heart status.

Myocardial ischemia studies using optical mapping consist of four experimental phases: control period, dye loading, washout, myocardial ischemia. The use of VSDs may negatively affect the electrophysiology of the examined heart. Therefore, dynamic changes in electrocardiograms during the particular phases of the experiment must be studied.

A number of methods based on the deterministic or stochastic theory can be applied. One of the possibilities is analysis of time-frequency spectra of the signals. Time-frequency analysis reveals changes in signals with the optimum time- and frequency resolution. Thus, it can help to detect low-level and short-time changes at the same time. However, the time-frequency spectrum is a complex parameter and its further analysis is complicated. Regarding variability in the length of the particular ECG signal segments during the experiment, dynamic time warping (DTW) applied to parts of the time-frequency spectra seems to be one of the suitable mathematical tools.

MATERIALS AND METHODS

The methods used in the project are based on animal experiments when electrograms are recorded from Langendorff-perfused rabbit hearts during various phases of application of a voltage-sensitive dye. The recorded ECG signals were divided into three parts corresponding to P-Q segments, QRS complexes, and ST-T segments. All marked segments were processed by the wavelet transform in

each experimental period (control, loading, and washout phase). The acquired time-frequency patterns were used for calculation of the variability of ECG parts. The variability of two different sequences was measured by means of an optimal path resulting from DTW based on minimum distance between the sequences. In the experiments, variability was calculated between the control signal segments (before the application of VSD) and segments recorded in the next two experimental steps.

Experimental setup and protocol

All experiments were approved by the Ethical Committee of Masaryk University in Brno. Four rabbits were included in this study. Each animal was introduced into deep anaesthesia by xylazine and ketamine. The chest was opened, the heart with a sufficiently long piece of the 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; NaHCO3, 24mM; KCl, 4.2mM; KH2PO4, 1.2mM; MgCl2, 1.2mM; CaCl2, 1.2mM; glucose, 5.5mM, and taurine, 10mM. The aorta was cannulated and the heart perfused at a constant perfusion pressure (80 mmHg) with K-H solution. All experiments were performed at the temperature of 37 °C. During the control period (20–30 minutes), all hearts exhibiting any reperfusion arrhythmias were excluded from the experiment. Afterwards, the heart was loaded with a voltage-sensitive dye di-4-ANEPPS diluted in K-H solution (22–27 minutes – depending on actual coronary flow). Then the period of washout with K-H solution followed (of the same length as the loading period). The preparation was then ready for measurements of monophasic action potentials. During all experiments, simultaneous touch-free recordings of electrograms were performed.

The employed optical recording system is based on application of a voltage sensitive dye di-4-ANEPPS (Molecular Probes, USA) into the examined tissue (*7*). The dye undergoes changes in its fluorescence spectra, in response to changes in the surrounding electrical field.

The ECG signals from orthogonal leads were recorded from Ag-AgCl electrodes positioned on the inner surface of the bath. The signals were digitised 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.

Data processing

The data recorded were downsampled from 4000 Hz to 500 Hz. Then all the data were divided for P-Q segments, QRS complexes, and ST-T segments of selected consecutive heart cycles in each experimental period, see *Fig. 1*. Then, the wavelet transform and dynamic time warping were applied as described below.

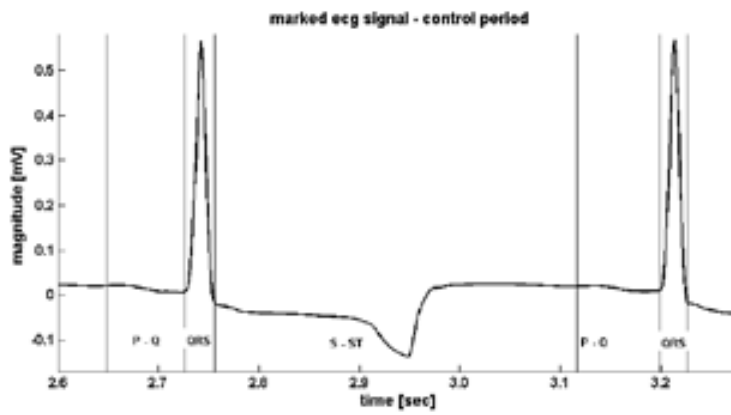

Fig. 1.
Segments for analysis: P-Q segments, QRS complexes, and ST-T segments of selected consecutive heart cycles

Wavelet analysis

A common mathematical tool for time-frequency analysis is the short-time Fourier transform (STFT). STFT is a Fourier transform applied to a windowed signal while the window is shifted in time. A constant width of the window causes low time resolution in various frequency bands. To overcome this problem, the wavelet transform (WT) can be used. WT uses a basis of functions of two parameters: time shift and time dilation. To decompose the analysed signal, the projection to each dilated basis function (wavelet) must be computed. WT is then defined as the correlation of signal x(t) with wavelets g*[(t-τ)/λ], where τ is time shift, λ is time dilation, and * represents complex conjugate:

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

For the time-frequency analysis, Morlet wavelet has been used for its relatively smooth shape (*8*). The analysis resulted in a sequence of vectors representing frequency components between 0.01–250 Hz at each time instant. The vectors were used as time sequences for DTW as described below.

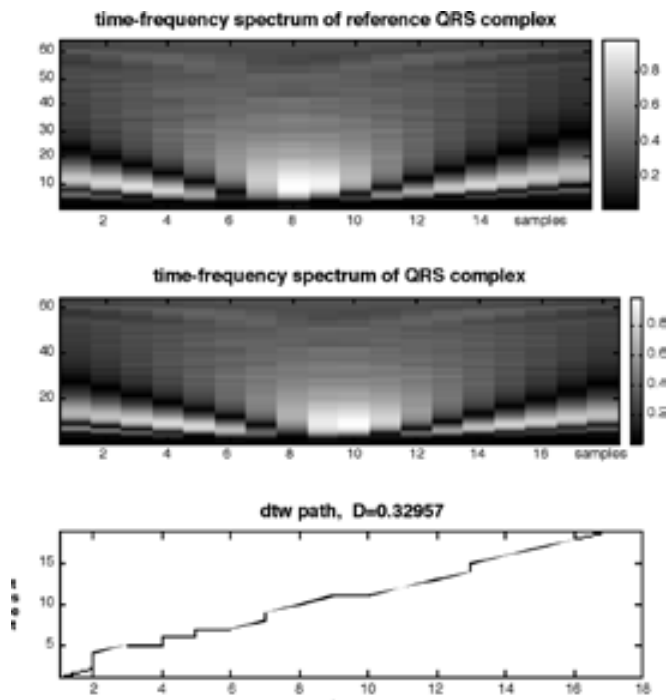

Fig. 2.

a) Time-frequency spectrum of the reference QRS complex, recorded before loading VSD;
b) Time-frequency spectrum of the QRS complex compared with that of reference;
c) Progress of DTW optimal path – x-axis corresponds to the length of reference sequences of vectors, taken from time-frequency spectrum of the control period of ECG, y-axis corresponds to the length of test sequences of vectors, taken from time-frequency spectrum of ECG

Dynamic time warping in ECG signal processing

Dynamic time warping is a method which calculates the distance between two time sequences (signals) and searches for a single optimal sequence. A component of the first signal has its corresponding counterpart in the second signal but may be distorted in time (dilated, compressed, shifted, etc.). This also applies in the case of signals with different lengths. DTW works with non-linear time normalisation. Moreover, the normalisation can be limited by specific parameters to prohibit large fluctuation in a time domain model. The time differences between two time sequences are eliminated by warping of time axes. This problem is formulated as a maximum likelihood path searching optimisation process (*9*), (*10*).

In the algorithm proposed, sequences of the frequency spectra are used as time sequences. These frequency spectra are extracted from the time-frequency spectrum which was calculated by wavelet transform for each ECG signal (see *Fig. 2a*) and *Fig. 2b*) (*11*).

Consider two time sequences A and B, of lengths I and J respectively, where:

$$A=[a(1),a(2),...,a(n),..., A(I)], B=[b(1), b(2),..., b(m),..., b(J)] \quad (2)$$

The algorithm with DTW searches for an optimal path m=ψ(n) in the plane (n,m), which minimises function D. The function D is computed as the overall distance between time sequences A and B as

$$D(A,B)=\sum_{n-1}^{I} d[a(n),b(\psi(n))] \quad (3)$$

where d[a(n),b(ψ(m))] is the local distance between the n-th element of A and the m-th element of B.

The optimal path m=ψ(n) expresses the relation between m and n by a simple function. Suppose a general time variable k, both time variables m and n can be expressed as a function with the parameter k as, n=i(k), m=j(k) for k=1,...,K, where K is the length of the general time axis for comparison of sequences A and B, especially the reference sequence and the test sequence.

In this case, dynamic time warping (DTW) is applied to the time-frequency spectrum of P-Q segments, QRS complexes, and ST-T segments of selected consecutive heart cycles in each experiment period, namely control period, loading of VSD, washout, and ischemia. An optimal path resulting from DTW based on the minimum distance between the control segments (before the application of VSD) and segments recorded in the next two experiment steps is computed. The length of discussed segments was significantly different, namely the length of the ST-T segment ranged around 0.6 sec and that of the P-Q segment in the same heart cycle around 0.05 sec. To decrease the effect of the length, the final distance between two vector sequences was divided by the length of the DTW optimal path

$$D=D(A,B)/K \quad (4)$$

An example of the DTW optimal path is shown in *Fig. 2 c*).

RESULTS

The proposed algorithm based on dynamic time warping was tested on ECG signals regarding application of voltage-sensitive dyes. Generally, significant changes in the optimal path computed according to Eq. 3 were observed during the VSD loading. At the end of the washout period, changes in the optimal path were considerably lower for all segments, or at least showed a decreasing trend; see *Fig. 3*.

It may be concluded that the optimal path of dynamic time warping applied to ECG signals is sensitive to the loading of VSD into the examined heart. It can

distinguish particular periods of optical mapping procedure. Further, DTW can be used to analyse various fractions of a heart cycle and thus be exploited for an analysis of the VSD influence on the conductive system of the heart.

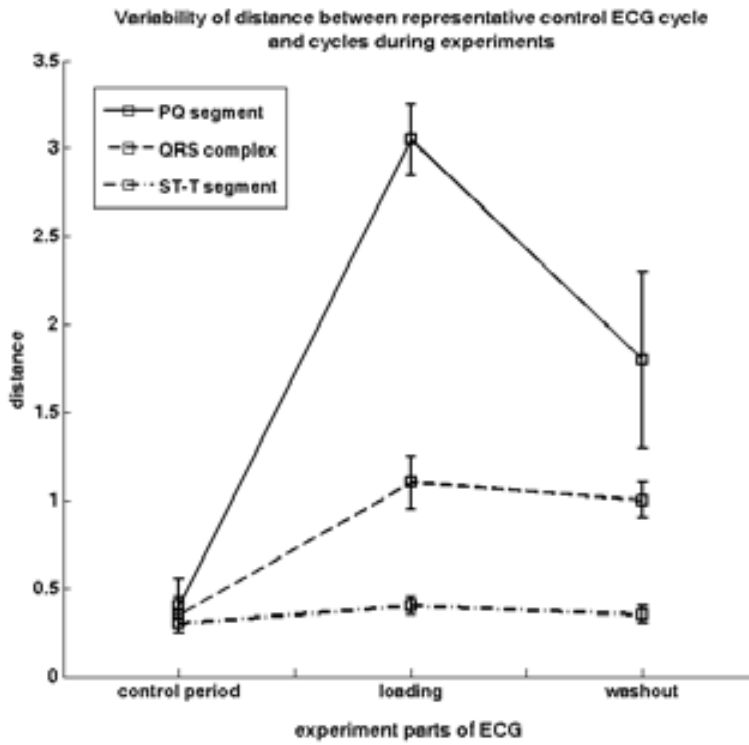

Fig.3
Variability of distance between a representative control ECG cycle and cycles during all experiments. Solid – variability of P-Q segments; dashed – variability of QRS-complexes; dashdotted – variability of ST-T segments

DISCUSSION

Changes in the optimal path were observed during VSD loading. The changes exceeded few times the length of the path in all experiments. At the end of the washout period, changes in the optimal path were considerably lower. It may be concluded that the optimal path of dynamic time warping applied to ECG signals is sensitive to particular periods of optical mapping procedure. Further, DTW can be used to analyse various fractions of the heart cycle and thus be exploited for an analysis of the VSD influence on the conductive system of the heart.

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VARIABILITA SEGMENTŮ EKG SIGNÁLU BĚHEM APLIKACE NAPĚŤOVĚ CITLIVÉHO BARVIVA VE ZVÍŘECÍM SRDCI

Souhrn

Napěťově citlivá barviva (VSD) jsou využívána pro optické snímání akčních potenciálů z povrchu zvířecích srdcí. VSD byla úspěšně použita ve studiích ischemické choroby srdeční. Experimenty se zvířecími srdci perfundovanými podle Langendorffa však naznačovaly vznik tvarových změn EKG signálu v časové i časově-frekvenční doméně způsobené aplikací VSD di-4-ANEPPS během optického měření. Je nutné provádět detailní analýzu signálů k potvrzení individuálních změn v jednotlivých částech EKG cyklu. Byla aplikována metoda borcení časové osy (DTW) na úseky P-Q, QRS a ST-T vybraných posloupností srdečních cyklů v každém kroku experimentu. Byla vypočítána optimální cesta jako výsledek DTW založený na minimální vzdálenosti mezi kontrolními segmenty signálů (před aplikací VSD) a segmenty zaznamenanými v dalších dvou krocích experimentu.

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