High-resolution electrical mapping of depolarization and repolarization alternans in an ischemic dog model

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Gordon D, Kadish AH, Koolish D, Taneja T, Ulphani J, Goldberger JJ, Ng J. High-resolution electrical mapping of depolarization and repolarization alternans in an ischemic dog model. Am J Physiol Heart Circ Physiol 298: H352–H359, 2010. First published November 13, 2009; doi:10.1152/ajpheart.00914.2009.—Cardiac electrical alternans have been associated with spontaneous ventricular arrhythmias during myocardial ischemia. The study aims were to use a new algorithm to measure depolarization and repolarization alternans from epicardial electrograms in an ischemia model and to evaluate which features are predictive of ventricular fibrillation (VF). The left anterior descending coronary artery was occluded in 21 dogs, of which 6 developed spontaneous VF. Four seconds of unipolar epicardial electrograms was recorded before and 5 min after occlusion from an 8 × 14-electrode plaque placed on the anterior left ventricle. Alternans amplitudes were estimated with a triangular wave-fitting algorithm and for each unipolar electrogram for various measurements of the QRS and ST-T wave amplitude. The root mean square error was computed for each fit. Receiver-operator characteristic curves were used to determine whether prevalence of alternans having estimated alternans amplitude-to-error ratio (AE) above a given threshold could distinguish the dogs who had and did not have spontaneous VF. The prevalence of alternans after ischemia was highly predictive of VF when measured both during depolarization (sensitivity of 83% and specificity of 87%) and during repolarization (sensitivity of 100% and specificity of 73%). The optimal alternans AE ranged from 1 to 4. There were no differences in the level of discordance or alternans specificity of 73%). The optimal alternans A/E ranged from 1 to 4.

METHODS

Experimental preparation. Studies approved by the Northwestern University animal facility were conducted on 21 adult mongrel dogs weighing 25–35 kg. The animals were anesthetized with 30 mg/kg pentobarbital, intubated, and mechanically ventilated. Anesthesia was maintained with 1–1.5% halothane. Via a median sternotomy, a 112-uni-polar silver electrode recording plaque consisting of an 8 × 14 array with a 2.5-mm interelectrode distance (Bard, Billerica, MA) was sutured to the left ventricle with its long axis parallel to the left anterior descending coronary artery (LAD). The LAD was exposed ~1–2 cm from its origin proximal to the first major diagonal branch and was then completely occluded by ligation. In two dogs, the first major diagonal was also ligated. Of the 21 dogs, 6 developed spontaneous VF after occlusion.

Signal acquisition and analysis. The 112 unipolar electrogram signals and the standard surface ECG limb leads and arterial blood pressure were acquired at a sampling rate of 1,000 samples/s with 8-bit resolution and stored continuously in a digitized form on videotape with a cardiac mapping system (Map Tech, Maastricht, The Netherlands). The stored signals were later analyzed off-line with custom-made software written in the Matlab programming language (Mathwork, Natick, MA). The signals were band-pass filtered (0.5–500 Hz). Four seconds of data from the first 2 min before occlusion of the LAD and 4 s of data 5 min after occlusion were analyzed. Beats were automatically detected from the epicardial electrograms with a combination of slope detection and template matching, and heart rates were computed. The onset of the QRS and the offset of the T wave may not be clearly delimited in the unipolar electrogram, particularly at high heart rates. Because the onset of the QRS and the isoelectric line are generally more clearly defined in the surface ECG, the surface ECG signals were used as a reference to manually determine QRS onset, J point, and T wave offset.

ST height. ST height was measured for each of the unipolar electrograms before and after occlusion. ST height was defined as the amplitude at the J point expressed as a percentage of the peak-to-peak QRS amplitude. The mean ST heights during ischemia for the dogs

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ELECTRICAL ALTERNANS (5, 9), which consists of the beat-to-beat oscillation in the amplitude of a particular electrocardiographic segment, may be a marker of electrical instability (16, 17). Microvolt-level alternans of the T wave has been shown to be associated with ventricular fibrillation (VF) in animal models (13). It has also been correlated with the inducibility of ventricular tachycardia at electrophysiological testing (6, 15), and it has been approved as a technique for risk stratification of patients with structural heart disease (7, 11, 18). Unlike measurements of alternans in the nonischemic state, alternans is a visible macroscopic phenomenon during ischemia. Local T-wave alternans from unipolar electrograms have been seen in animal models of acute ischemia (3, 4, 13, 17). Here we implemented a method to analyze temporal fluctuations in ECG morphology over short time segments (4-s time window), using a short-segment time-domain algorithm. This algorithm is robust in its detection of alternans and also takes into account the impact of noise on the determination of the alternans signal. This technique could be useful in the setting of very short data streams of 6–12 beats in which neither spectral analysis of morphology (15) nor the modified moving average (12) can be implemented. Most experimental and clinical studies have focused on T-wave or repolarization alternans. However, the presence of QRS alternans has not been as carefully studied. The goal of the present study was to use the algorithm to measure depolarization and repolarization alternans in a canine model of ischemia and to evaluate which alternans features are predictive of VF.
that developed spontaneous VF after occlusion were compared with those that did not develop spontaneous VF after occlusion.

**Quantifying alternans.** The QRS complex and ST-T wave segments of the 112 unipolar electrograms were delimited. The window for the QRS complex was the QRS onset to the J point. The window for the ST-T wave segment was the J point to the end of the T wave. The mean peak-to-peak QRS amplitude in a 4-s window was computed. All subsequent measurements were scaled as a percentage of the mean peak-to-peak QRS amplitude. The following four measurements (see Fig. 1) were made for each beat in the 4-s window and for each of the 112 unipolar electrograms: the peak-to-peak QRS amplitude (QRSpeak), the mean QRS absolute amplitude (QRSmean), the peak-to-ST-T wave amplitude (STTpeak), and the mean ST-T wave absolute amplitude (STTmean). The sequences of QRSpeak, QRSmean, STTpeak, and STTmean taken before coronary occlusion and another sequence after coronary occlusion constitute the raw data base.

If the sequence of beat-to-beat numbers alternates in the 4-s window as the sequence ABABAB…AB, there is by definition alternans. We call this perfect alternans. A plot of perfect alternans behavior would be a regular triangular wave function. In practice, perfect alternans is rarely observed, and the data sequence resembles ABA’B’A''…B'' in which the primed values are not identical but are “approximately” identical to A and B (see Fig. 1). When there is no alternans, the values do not fit a regular triangular wave function but vary slightly because of noise and other biological factors (see Fig. 2, example 2).

Estimation of the amount of alternans present in each of the sequences was performed with the steps illustrated in Fig. 2. First, the sequence is linearly detrended with respect to the beat numbers. The odd values are then multiplied by −1. The absolute value of the mean of this new sequence is then half the estimated alternans amplitude since it represents the deviation in the amplitude from the detrended baseline. To assess the goodness of fit of the estimated alternans, the root mean square error of the reconstructed triangular wave pattern was compared with the original detrended sequence. The ratio of the estimated alternans amplitude and the root mean square error provides a confidence measure of whether alternans is present in the sequence.

**Alternans prevalence.** Because variations in biological signals may be due to alternans, other time-varying signals, and noise, it is important to relate the degree of observed alternans to the magnitude of these alternative sources of variation, hereafter termed collectively as noise. To study the effects of noise, we evaluated the ratio of the magnitude of the estimated alternans amplitude versus the estimated error and defined significant alternans as present when this ratio exceeded a defined threshold. This threshold was allowed to vary from 1.0 to 10.0 and incremented with a step size of 0.5. For each of the four types of measurements of the unipolar electrograms (QRSpeak, QRSmean, STTpeak, and STTmean), we quantified the percentage of the 112 electrodes that had ratios of the magnitude of the estimated alternans amplitude versus the estimated error greater than the threshold value. We refer to this percentage as the percent sites. We expected that a smaller alternans amplitude-to-error ratio (A/E) would result in a higher prevalence of alternans, measured in percent sites, since smaller ratios are easier to obtain.

**Optimal A/E in predicting VF.** To find the optimal A/E for which the alternans values have physiological significance, we constructed receiver-operator characteristic (ROC) curves for each of the ratios to distinguish the dogs that had spontaneous VF versus the dogs that did not have spontaneous VF, using percent sites as the predictive variable. The ratio for which the area under the ROC curve (AUC) was the greatest was considered optimal. We performed this analysis for the four types of data obtained after occlusion. Because a small amount of alternans was possibly present at baseline, this analysis was repeated for the percent sites of the ischemia alternans after subtracting the

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Fig. 1. Illustrations for the measurements of peak-to-peak QRS amplitude (QRSpeak), mean absolute QRS amplitude (QRSmean), peak-to-peak ST-T amplitude (STTpeak), and mean absolute ST-T amplitude (STTmean).

Fig. 2. Examples for the steps to estimate the alternans signal, amplitude, and error. **Example 1** has obvious alternans with an estimated alternans amplitude of 0.236 and alternans amplitude-to-error ratio (A/E) of 5.76. **Example 2** does not have obvious alternans and thus has an estimated alternans amplitude of only 0.03 and a ratio of 1.3.
percent sites of the baseline alternans. All subsequent analyses were performed with the optimal A/E for each of the four variables.

**Discordant alternans.** It is possible for alternans from one electrode of a plaque to be completely out of phase with another electrode from the same plaque, i.e., at the time when one electrode is at the peak phase of alternans the other is in the trough phase (see Fig. 7). This occurrence, called discordant alternans, is thought to be more arrhythmogenic than concordant alternans. For each dog during ischemia, the percent discordance was quantified as the percentage of the electrodes that were out of phase of the prevalent phase. Thus the percentage will have a possible range of 0% (no discordance) to 50% (complete discordance). The optimal A/E were used in this analysis. Percent discords of QRSpeak, QRSmean, STTpeak, and STTmean were tested to see whether they were predictors of VF.

**Alternans amplitude.** To examine the significance of the amplitudes of the alternans from the unipolar recordings, the mean and maximum alternans amplitude (absolute value) during ischemia for the electrodes with the optimum A/E were analyzed. As above, maximum alternans and mean alternans of QRSpeak, QRSmean, STTpeak, and STTmean were tested to evaluate whether any were predictors of VF.

**Statistical analysis.** All data are shown as means ± SE. Heart rate and ST height differences between baseline and ischemia were analyzed with a paired *t*-test. The effects of VF on heart rate and ST height were analyzed with an unpaired *t*-test for baseline and ischemia measurements. Differences in alternans measurements that were not normally distributed were tested with the Mann-Whitney U-test for unpaired analysis and with the Wilcoxon signed rank test for paired analysis. A *P* < 0.05 was considered statistically significant.

**RESULTS**

**Heart rate and ST height changes with ischemia.** The average heart rate for the 21 dogs did not change significantly during coronary occlusion-induced ischemia [baseline 144.2 ± 5.8 to 148.9 ± 5.8 beats per minute (bpm) during coronary occlusion-induced ischemia, *P* = not significant (NS)]. There was also no difference in heart rate between the 6 dogs that developed VF after coronary occlusion and the 15 dogs that did not develop VF (148.4 ± 8.5 vs. 149.0 ± 7.6 bpm, *P* = NS). As expected, the mean ST height expressed as a percentage of QRS amplitude increased significantly from baseline to ischemia (from 4.4 ± 0.8% to 19.1 ± 2.5%, *P* < 0.0001). An example of this is shown in Fig. 3. However, mean ST height was not different between dogs who developed VF and dogs who did not develop VF (19.1 ± 4.5% vs. 19.2 ± 3.1%, *P* = NS).

**Alternans changes with ischemia.** Figure 3 shows an example of how the electrograms change with ischemia. Note the elevated ST segment and the alternans that is prominent in the ST segment. The average percent sites of the number of signals that have alternans A/E greater than values from 1 to 10 (with step size of 0.5), before and during ischemia for QRSpeak, QRSmean, STTpeak, and STTmean.

**Fig. 4.** The average percent sites, the percentage of signals that have alternans A/E greater than values from 1 to 10 (with step size of 0.5), before and during ischemia for QRSpeak, QRSmean, STTpeak, and STTmean.

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**Fig. 3.** Example of electrograms at baseline and during ischemia. Note the prominent T-wave alternans during ischemia.
determined by the maximum AUC of QRSpeak, QRSmean, STTpeak, and STTmean were 4, 3.5, 1, and 1, respectively. These maximum AUCs ranged from 0.81 to 0.89. The ROC curves for these values and their respective AUCs are shown at center. The difference in percent sites using the optimal A/E for the dogs that developed VF from those that did not develop VF are shown on right.

This analysis was repeated for the percent sites during ischemia relative to the baseline percent sites (Fig. 6 has the same format as Fig. 5). The optimal A/E of QRSpeak, QRSmean, STTpeak, and STTmean were 2, 3.5, 3.5, and 3.5, respectively. These maximum AUCs ranged from 0.85 to 0.88. The percent

Table 1. Results from optimal ROC curves for QRSpeak, QRSmean, STTpeak, and STTmean values during ischemia

<table>
<thead>
<tr>
<th>Alternans Measure</th>
<th>Optimal A/E</th>
<th>AUC</th>
<th>Percent Sites Cutoff, %</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRSpeak</td>
<td>3.5</td>
<td>0.81</td>
<td>0.9</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>QRSmean</td>
<td>4</td>
<td>0.82</td>
<td>6.3</td>
<td>83</td>
<td>87</td>
</tr>
<tr>
<td>STTpeak</td>
<td>1</td>
<td>0.89</td>
<td>71.9</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>STTmean</td>
<td>1</td>
<td>0.85</td>
<td>72.3</td>
<td>83</td>
<td>73</td>
</tr>
</tbody>
</table>

ROC, receiver-operator characteristic; QRSpeak, peak-to-peak QRS amplitude; QRSmean, mean QRS absolute amplitude; STTpeak, peak-to-peak ST-T wave amplitude; STTmean, mean ST-T wave absolute amplitude; A/E, alternans amplitude-to-error ratio; AUC, area under the curve.
sites using the optimal A/E were significantly greater in all four measures for the dogs that developed VF than in those for the dogs that did not develop VF. The sensitivities, specificities, and percent cutoff values for the optimal ROC curves are shown in Table 2.

Discordant alternans. Figure 7 shows an example of an alternans map for STTpeak of one dog showing discordant alternans during ischemia. Each box represents the relative spatial location of each electrode on the 14 × 8 electrode array. A box was colored either dark gray for positive-phase alternans or light gray for negative-phase alternans if the A/E for the alternans at that electrode was >3. At baseline only 1 electrode (percent count of 0.9%) had a ratio >3, while during ischemia 88 electrodes (percent count of 78.6%) had ratios >3. The electrograms at sites A and B of the alternans map are shown in Fig. 7, bottom. Note that for electrogram A the alternans pattern is small-large-small, whereas for electrogram B the pattern is large-small-large.

During ischemia, discordant alternans were found in 7.9 ± 4.6% of the electrodes with significant alternans (greater than the optimal A/E) for QRSpeak, 14.5 ± 4.6% for QRSmean, 27.3 ± 2.9% for STTpeak, and 25.8 ± 2.9% for STTmean. The alternans map in Fig. 7 shows alternans with 41% discordance during ischemia. The percent discordance was not significantly different in the dogs that developed VF from that in the dogs that did not develop VF.

Alternans amplitude. During ischemia, the maximum amplitude of the significant alternans was 6.3 ± 1.6% for QRSpeak, 3.8 ± 0.6% for QRSmean, 18.7 ± 3.5% for STTpeak, and 11.0 ± 2.3% for STTmean. The mean amplitude of the significant alternans was 3.2 ± 0.6% for QRSpeak, 2.3 ± 0.5% for QRSmean, 3.7 ± 0.7% for STTpeak, and 2.0 ± 0.4% for...
STTmean. Neither the maximum nor mean amplitudes of the significant alternans showed any significant difference between the 6 dogs that developed VF and the 15 dogs that did not develop VF.

DISCUSSION

Ischemia results in alternans in both the QRS complex (depolarization) and the ST segment (repolarization). Repolarization alternans was more prevalent than depolarization alternans. Discordant alternans was observed in both depolarization and repolarization. The prevalence of depolarization or repolarization alternans was highly predictive of VF. However, there were no differences in the level of discordance or alternans amplitude between dogs who developed VF and dogs who did not. This suggests that the prevalence of alternans in the ventricles may be the key risk factor for developing VF during myocardial ischemia when short-term recordings are used.

Depolarization alternans. Depolarization alternans was shown to be as predictive of VF as repolarization alternans, both when adjusting and not adjusting for baseline level of alternans. Depolarization alternans was recognized along with repolarization alternans as a contributor to electrical instability in the work in the mid to late 1980s of Smith et al. (17) in canine experiments and in human patients at the time of electrophysiological study. Later, Rosenbaum et al. (15) in their seminal paper linking alternans of the ST segment and T wave with increased risk of ventricular arrhythmias deemphasized the importance of QRS alternans by showing that T-wave alternans was much more prominent than QRS alternans. However, they pointed out that in 10 patients the magnitude of ST-segment and T-wave alternans remained constant over a range of rates (from 95 through 150 bpm) while the magnitude of QRS alternans increased with rate. The rate dependence of depolarization alternans in a guinea pig Langendorff-perfused heart model was also noted by Pastore et al. (14). Their study implicated repolarization dispersion due to alternans for the development of ventricular arrhythmia; however, they did not comment on the potential role of depolarization alternans.

It is possible that QRS alternans may be linked to repolarization alternans through conduction velocity restitution. Because repolarization alternans will lead to alternans in action potential duration, alternans in action potential duration will in turn lead to alternans of the diastolic interval, assuming a steady heart rate. Because conduction velocity has been shown to be a function of diastolic interval (conduction velocity restitution) (2), the changes in conduction velocity will lead to changes in the QRS. A hypothetical situation in which repolarization and depolarization alternans may not be linked is the occurrence of postrepolarization refractoriness. Postrepolarization refractoriness has been shown to occur as a result of ischemia. Alternans of the postrepolarization refractory period may result in detectable alternans in the QRS but not the ST segment.

Method of analysis of alternans. Identification of alternans in short time segments may be useful in real-time monitoring or archived data. In the present study the average number of beats in the 4-s window was 10. Because of this it is not feasible to use conventional spectral methods (15), nor was it feasible to use the modified moving average time-domain method (12), both of which require many more beats. Thus we developed the simple short time segment time-domain algorithm illustrated in Fig. 2 for macroalternans. In contrast, the previously published spectral and time-domain methods were primarily designed for microvolt alternans. Not only does our method quantify the amplitude of depolarization and repolarization alternans, but it also deals explicitly with background noise and variability that is not 2:1 in nature by having a statistical measure that quantifies the “goodness” of the constant triangular waveform fit. It was also computationally efficient in dealing with 4,704 short time segments in this

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Table 2. Results from optimal ROC curves for QRSpeak, QRSmean, STTpeak, and STTmean values during ischemia relative to baseline values

<table>
<thead>
<tr>
<th>Alternans Measure</th>
<th>A/E</th>
<th>AUC</th>
<th>Percent Sites</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRSpeak</td>
<td>2</td>
<td>0.87</td>
<td>5.4</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>QRSmean</td>
<td>3.5</td>
<td>0.87</td>
<td>6.3</td>
<td>83</td>
<td>87</td>
</tr>
<tr>
<td>STTpeak</td>
<td>3.5</td>
<td>0.85</td>
<td>18.8</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>STTmean</td>
<td>3.5</td>
<td>0.88</td>
<td>15.6</td>
<td>100</td>
<td>73</td>
</tr>
</tbody>
</table>

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Fig. 7. An example of an alternans map for STTpeak of 1 dog during ischemia with discordant alternans. Each box represents the relative spatial location of each electrode on the 14 × 8-electrode array. A box was colored either dark gray for positive-phase alternans or light gray for negative-phase alternans if the A/E for the alternans at that electrode was >3. The electrogroma at sites A and B are shown at bottom. Note that for electrogram A the alternans pattern is small big small, whereas for electrogram B the pattern is big small big.
We reported QRS alternans based on two methods of identifying the phenomenon. One is the alternation in the QRS peak, and the other is the alternation in the QRS mean. There are limitations regarding the use of QRS mean. First, the J point may be difficult to estimate during ischemia. Second, a repolarization component may be included in this measurement, particularly near the time of the J point. Despite these limitations, the results of QRSmean followed the same trend as those of QRSpeak.

It is important to emphasize as have Walker and Rosenbaum (19) that the mechanism of alternans is probably different in the ischemic heart model than the alternans found in nonischemic myocardium. Ischemia causes alternans in the action potential upstroke, repolarization, action potential amplitude, and/or magnitude of hyperpolarization (1). Thus the risk prediction benefit of the technique may be limited to the time period shortly after acute myocardial infarction. It is not known whether alternans would still be present after the infarction has healed.

Conclusions. A simple method of analyzing short time segments of data for repolarization and depolarization alternans is presented. We found that prevalence of repolarization alternans compared with amplitude of alternans is presented. We found that prevalence of repolarization and depolarization alternans was the key factor in determining which dogs developed VF from short-term recordings during ischemia. This methodology provides a practical way to measure alternans and/or time dependence of arrhythmia risk with very short-term recordings. This may be useful to evaluate because alternans has been shown to be a predictor of ventricular arrhythmias in a number of studies.

GRANTS
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DISCLOSURES
No conflicts of interest are declared by the author(s).

REFERENCES


