Relation between activation sequence fluctuation and arrhythmogenicity in sodium-channel blockades

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Watanabe, Tetsu, Michiyasu Yamaki, Isao Kubota, Hitetada Tachibana, and Tetonobu Tomoike. Relation between activation sequence fluctuation and arrhythmogenicity in sodium-channel blockades. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H971–H977, 1999.—To examine the correlation between activation sequence fluctuation and arrhythmogenicity, we investigated temporal changes in the activation sequence by measuring activation times [negative first derivative of voltage over time (–dV/dt) in ORS] from the entire heart in 18 dogs. The heart was paced by constant atrial stimulation. The character of the activation sequence fluctuation was established by a principal component analysis, in which the first principal component was defined as a stable component of the sequence and the second or third component as a fluctuated component. Steady state contained stable component of the sequence and the second or third fluctuation was established by a principal component analysis.

METHODS

Instrumentation. Eighteen adult mongrel dogs (wt 12–33 kg) were anesthetized with pentobarbital sodium (30 mg/kg iv) and received supplemental doses as needed. Dogs were ventilated by a respirator with room air supplemented with oxygen (3–5 l/min). The thorax was opened in the fifth intercostal space, the pericardium was opened, and a pericardial cradle was made to support the heart at an appropriate position. The sinus node was crushed, and the right atrium was paced at a cycle length of 400 ms using a model SEN-7203 stimulator (Nihon Koden, Tokyo, Japan). After an intravenous bolus administration of heparin (10,000 IU), a 24-gauge plastic cannula was inserted into the left anterior descending artery (LAD) at the distal site of the second diagonal branch. The cannula was kept open by continuous infusion of saline at 1 ml/min (14). A sock-shaped electrode array was placed on the ventricular surface for simultaneous recording of electrograms from 60 epicardial sites. Each unipolar electrode consisted of fine silver wire (0.2-mm diameter) sutured to the sock. The electrode array was of 6 rows (1–6) and 10 columns (A–J) (Fig. 1). All recording electrodes were referenced to the Wilson’s central terminal, and multichannel electrograms were digitized every millisecond using a multiplexed data processing system (CD-G015, Chunichi Denshi, Nagoya, Japan) as described in a previous study (16). The thoracic cavity was covered with plastic wrap to prevent cooling and dehydration. Body temperature was maintained at 37–38°C. An arterial line was inserted into the right femoral artery to continuously monitor mean arterial pressure. Electrocardiogram lead II and blood pressure were simultaneously monitored throughout the study on a model 2G66 recorder (NEC San-ei, Tokyo, Japan).

Experimental protocol. Flecainide (low dose: 10 µg·kg⁻¹·min⁻¹, high dose: 100 µg·kg⁻¹·min⁻¹; n = 6), lidocaine (low dose: 0.12 mg·kg⁻¹·min⁻¹, high dose: 0.6 mg·kg⁻¹·min⁻¹; n = 6), or disopyramide (low dose: 20 µg·kg⁻¹·min⁻¹, high dose: 200 µg·kg⁻¹·min⁻¹; n = 6), was intracoronarily infused using an infusion pump (model SP-100, J MS, Hiroshima, Japan). The lower doses of flecainide, disopyramide, and lidocaine corresponded to 1% and the higher doses to 5–10% of the intravenous doses in the previous study (14). After the baseline measurements, a low-dose protocol of 10-min duration was first performed, and a high-dose protocol of 10 min duration followed. Epicardial electrograms were recorded every 5 min after the start of the low-dose infusion. During the high-dose infusion, when spontaneous ventricular tachyarrhythmias were recognized, electrograms were immediately recorded.

In the present study, we investigated whether 1) ventricular activation sequences fluctuate in an intact heart, 2) sodium-channel blockades enhance activation sequence fluctuation, and 3) enhanced activation sequence fluctuation causes proarrhythmia.

T WAVE ALTERNANS is an important indicator of life-threatening arrhythmias in patients with ischemic heart disease (1, 8). We previously reported (15, 27) that the magnitude of S-T alternans increased and discordance of S-T alternans appeared during myocardial ischemia, which resulted in ventricular arrhythmias. These studies mainly focused on electrical alternans in the repolarization phase. However, electrical instability in the depolarization phase, especially in ischemic heart disease (1, 8). We previously reported (15, 27)

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Study of principal components. Multichannel epicardial electrograms were later processed on a SUN 4/2 microcomputer (SUN Microsystems, Mountain View, CA). The cross-correlation function was used to reject ectopic beats and artifacts (cross-correlation coefficients below a threshold value: 0.95) (11). The epicardial activation of each electrogram was defined as the time at the minimum derivative of the QRS signal (25), and the recovery time was defined as the time at the maximum derivative of the T wave (10, 20). QRS deflection area (sum of all positive and negative potentials from QRS onset to end of T deflections) was calculated (2, 12, 16). The earliest activation among the entire cardiac surface electrogram was assigned to time 0, and activation time (AT) was determined as the duration between time 0 and each activation. An isochronal map was constructed (16).

The data sets of AT or QRS deflection area were analyzed by principal component analysis (19). This analysis permitted us to evaluate the quantitative changes in multivariable data with time series (7, 19). We used this technique for estimating temporal changes in AT or QRS deflection area from multiple leads. A detailed description of the mathematical procedures of the principal component analysis is given in the APPENDIX. The orthogonal vector (eigenvector; $l_{ki}$) was derived after the extraction of the principal components. The principal component score ($z$) was calculated for every cardiac cycle. The $z$ score, which represents total AT or QRS deflection area change, was used to quantify the fluctuation in AT or QRS deflection area. The percentage of the $k$th principal component score ($k$th %PC) is defined as the $k$th $z$ as a percentage of the sum of the $z_k$ score ($k = 1, 2, ..., 60$). Factor loading, which was defined as the correlation coefficient between the derived principal components ($z$) and original data set of $X_i$, was calculated for each $i$ and used as a parameter indicating the spatial contribution of the principal components on the cardiac surface.

Statistical analysis. Quantitative data are reported as means ± SD. Statistical analysis was performed with ANOVA.
A confidence level of 95% was considered statistically significant.

RESULTS

Effects of sodium-channel blockades on activation sequences. Eighteen dogs were included in the analysis. Six were given flecainide, six lidocaine, and six disopyramide. Figure 2 illustrates cardiac surface distribution of AT before (control) and after flecainide administration (low and high dose) in a representative experiment. A slight delay in AT appeared on the perfused area during low-dose infusion of flecainide, and the area with delayed AT expanded after high-dose infusion. The electrogram configuration showed a widened QRS complex and an increased R wave on the perfused area.

A high dose of flecainide evoked beat-by-beat alternans in AT on the perfused area, and then ventricular fibrillation (VF) ensued in all dogs (n = 6). On the other hand, disopyramide and lidocaine evoked no VF and did not induce alternans in AT. Figure 3 shows representative maps of AT and recovery times of three consecutive beats just before VF. Beat-by-beat alternans in AT (activation sequence alternans) was evident on the perfused area; nevertheless, obvious changes in recovery time were not recognized. In this dog, activation sequence alternans appeared to be independent of recovery sequence.

Hemodynamic data, AT, and incidence of VF are summarized in Table 1. Sodium-channel blockade did not affect the systemic pressure, except for high doses of lidocaine.

Activation sequence fluctuation. Figure 4 illustrates factor loading maps of AT in the same experiment represented in Fig. 2. Flecainide caused diminution in factor loading of the first principal component on the

Table 1. Effects of sodium channel blockade on hemodynamic data and activation times

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<tr>
<th></th>
<th>Flecainide</th>
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<th>Lidocaine</th>
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<td>High dose</td>
<td>Control</td>
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<td>High dose</td>
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<td>BP, mmHg</td>
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<td>86 ± 14</td>
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<td>45 ± 20*</td>
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<tr>
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<td>18 ± 3</td>
<td>18 ± 5</td>
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<td>VF</td>
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Values for blood pressure (BP), activation time on center of perfused area (ATp), and activation time on nonperfused area (ATn) are means ± SD; ventricular fibrillation (VF) values are no. of dogs in which VF occurred. *P < 0.05 vs. control.
perfused area, in contrast to the increase in the second principal component. This indicates that activation sequence fluctuated mainly on the perfused area. Even at a control state, %PC of the first three components fluctuated in a beat-by-beat manner (Fig. 5). This fluctuation was suddenly augmented during high-dose flecainide; the decrease in the first %PC and the increase in the second %PC are seen. VF occurred after 10-min administration of high-dose flecainide in this dog. VF occurred only when flecainide was infused (Table 1).

%PC, which reflects the level of each principal component, was calculated during the infusion of each sodium-channel blockade in all experiments. Figure 6 shows the first three %PC of AT. At a control state, the contribution of the first %PC on activation sequence was 97.8%. Steady state contained 2.2% of fluctuated components. Activation sequence fluctuation should exist even at a control state. Although lidocaine and disopyramide did not change each %PC, a high dose of flecainide significantly decreased the first %PC and increased the second %PC. Flecainide uniquely augmented the amplitude of activation sequence fluctuation, and VF followed after this augmentation, lasting for several minutes in all dogs exposed to flecainide (n = 6). Specific change in the third %PC was not recognized during the infusion of each sodium-channel blockade.

We measured QRST deflection area in the dogs exposed to flecainide and evaluated the quantitative changes in repolarization using the principal component analysis (Fig. 7). The QRST deflection area is independent of changes in activation sequence and represents local recovery properties (2). Figure 7 shows that the contribution of the first %PC was relatively small at a control state. This observation suggested that the steady state contained a relatively large quantity of variable components on the QRST deflection area. However, flecainide did not modify the ratio of each component.

Fig. 5. Changes in the percentage of each premature ventricular contraction (%PC) of first (A), second (B), and third (C) components on AT during flecainide administration. PVC, premature ventricular contraction; VT, ventricular tachycardia. Sudden decrease in the first %PC and increase in second %PC were seen during exposure to high-dose flecainide. PVC beats were excluded from %PC calculation. VT occurred 10 min after high-dose (VT/VF).
DISCUSSION

The Cardiac Arrhythmia Suppression Trial (CAST) study focused on the possible proarrhythmia of sodium-channel blockades (3). Recently, genetic disturbance of the sodium channel was found in patients with the Brugada form of idiopathic VF (4). Increased attention has been given to the relation between sodium-channel suppression and arrhythmia.

The present study demonstrated that 1) ventricular activation sequence is fluctuated even in an intact heart in a beat-by-beat manner (activation sequence alternans); 2) flecainide uniquely enhances the amplitude of activation sequence alternans; and 3) an increase in the amplitude of activation sequence alternans ends in VF.

Application of principal component analysis on AT. In this study, we used principal component analysis as a tool for evaluating activation sequence. The principal component analysis (7, 19) condensed the information from the activation sequence of the whole heart into the three orthogonal principal components without loss of information (cumulative contribution of the first 3 principal components was 99.9%). We could quantify the changes in activation sequence by the derived principal component. Factor loading, defined as a correlation coefficient between the derived principal components and original AT, is another parameter indicating the character of the principal component. This parameter represents the spatial contribution of each principal component on the cardiac surface. In this study, the factor loading of the first principal component decreased on the perfused area, whereas that of the second principal component increased on that area. The contribution of the third principal component was relatively low and was assumed to be a nonspecific component. This suggested that the first two principal components were worthwhile to analyze.

Activation sequence fluctuation and arrhythmogenicity. Recently, detection of T wave alternans has been applied to clinical use for assessing the vulnerability of ventricular arrhythmias (1, 8). Electrical alternans in the repolarization phase has been recognized as an important factor for predicting life-threatening arrhythmias (15, 21, 22). In the depolarization phase, alternans in R wave amplitude on the electrocardiogram was reported as a useful parameter for estimating arrhythmogenicity (24). El-Sherif et al. (5a, 6) also observed that temporal dispersion of AT increased in an ischemic myocardium, and spontaneous reentrant arrhythmias easily ensued. These findings suggest that

Fig. 6. Changes in each %PC (first (A), second (B), and third (C)) on activation sequence by sodium-channel blockades. Significant decrease in first %PC and increase in second %PC were recognized during high-dose flecainide infusion. Lidocaine and disopyramide did not alter any %PC on AT. * P < 0.05 vs. control.

Fig. 7. Quantitative changes in QRST deflection areas due to flecainide. Contribution of first %PC was ~50% at a control state. Flecainide did not affect any component %PC on QRST deflection area.
Alternation of depolarization is another important factor of arrhythmogenicity. In the present study, the contribution of the first PC on activation sequence was 97.8% at control (Fig. 5). This indicated that 2.2% variability was present in the activation sequence of an intact heart. Beat-by-beat oscillations in the first and second %PC (Fig. 4) also suggested the presence of activation sequence alternans. It is commonly accepted that sodium channel current fluctuates under control conditions by a basic ion channel study. Simulation studies also suggested that open and closed conformation states of ion channels during depolarization are chaotically determined (17, 18). Thus activation sequence fluctuation should exist even at a control state. In the present study, the activation sequence fluctuation increased only when a high dose of flecainide was applied. Furthermore, VF occurred in the same protocol. In this group exposed to flecainide, we analyzed QRS/T deflection area, as well as AT, to evaluate the quantitative changes in repolarization. The QRS deflection area represents local recovery properties, and its changes are independent of changes in depolarization (2). The results indicated that flecainide did not modify the contribution of each principal component of QRS/T deflection area. This indicated that repolarization alternans was not determinant of flecainide-induced arrhythmia. Flecainide-induced arrhythmia was a simple model for examining the relation between activation alternans and arrhythmia. This finding may not be simply extended to other arrhythmogenic circumstances such as ischemia. Because ischemia influences both activation and recovery properties, electrical alternans in ischemia is supposed to be more complicated. (23) Therefore, further examination is needed to solve the mechanisms of arrhythmia in these circumstances.

A study with single sodium channels reported that sodium-channel blockades decrease the open probability of the sodium channel and suppress the sodium current (9). This possibly enhances the activation sequence fluctuation that may exist at a control state. It is noteworthy that only flecainide increased the alternans and was arrhythmogenic. The reason why flecainide, and not disopyramide or lidocaine, induced alternans was not identified. Flecainide is classified as a slow kinetic drug and is known to have strong sodium-channel blockades (28) may explain the unique action of flecainide on activation alternans.

In conclusion, flecainide infusion induced local activation sequence alternans and caused VF. This result suggests that activation sequence alternans may play an important role in arrhythmogenicity as a new substrate of arrhythmia.

**APPENDIX**

The calculation made here is substantially the same method as that of Lux et al. (19). It is applied as follows. The 60 activation times (AT) or QRST deflection areas in a single beat are formed into a 60-dimensional vector in beat i

\[ X_i = (X_{i1}, X_{i2}, \ldots, X_{i60}) \]

where \( \mathbf{X}_{ij} \) is the AT or the QRST deflection area on lead j. Covariance matrices that yield eigenvectors \( \mathbf{V}_j \) for each beat were calculated

\[ \mathbf{V}_j = \sum_{i=1}^{60} (\mathbf{X}_{ij} - \bar{\mathbf{X}}_j)(\mathbf{X}_{ij} - \bar{\mathbf{X}}_j)/(n-1) \]

\( Z_k \) may be represented by a linear sum of basis vectors

\[ Z_k = \sum_{i=1}^{60} \lambda_{ik} X_i \quad (k = 1, 2, \ldots, 60) \]

where \( \lambda_{ik} \) is a set of orthogonal basis vectors.

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