Mechanism of ST Elevation and Ventricular Arrhythmias in an Experimental Brugada Syndrome Model

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Background—Although phase 2 reentry is said to be responsible for initiation of ventricular tachycardia (VT) in Brugada syndrome, information about the activation sequence during VT is limited.

Methods and Results—We developed an experimental Brugada syndrome model using a canine isolated right ventricular preparation cross-circulated with arterial blood of a supporter dog and examined the VT mechanism. Two plaque electrodes (35×30 mm) containing 96 bipolar electrodes were attached to the endocardium and epicardium. Saddleback and coved types of ST elevation in transmural ECG were induced by pilsicainide, a pure sodium channel blocker, and pinacidil, a K<sub>A<sub>ATP</sub></sub> channel opener. Eighteen polymorphic VT episodes were recorded in 9 of the 12 preparations associated with ST elevation. Fourteen episodes spontaneously developed in 5 preparations after an extrasystole during basic drive pacing. Analysis of local recovery times revealed increased dispersion especially in epicardium, and the extrasystole originated from a site with a short recovery time, suggesting that phase 2 reentry was its mechanism. The other 4 VTs in 4 preparations were induced by premature stimulation. Analysis of the activation sequences during VT revealed reentry between epicardium and endocardium or reentry around an arc of a functional block confined to epicardium or endocardium with bystander activation of the other.

Conclusions—Electrical heterogeneity in the recovery phase was induced in this experimental Brugada syndrome model, which can be a substrate for the development of phase 2 reentry and the subsequent reentry around an arc of the functional block, resulting in sustained VT. (Circulation. 2004;109:125-131.)

Key Words: electrocardiography ■ mapping ■ reentry

Brugada syndrome is characterized by peculiar ST elevation in the right precordial leads and the development of life-threatening ventricular arrhythmias. A previous experimental study on canine right ventricular (RV) wedge preparation demonstrated that ST elevation can be induced by administration of pinacidil and flecainide and suggested a transmural voltage gradient created by loss of the action potential dome in epicardial myocytes as a mechanism for ST elevation. As an initiatory mechanism for ventricular tachycardia (VT) and ventricular fibrillation (VF) in this syndrome, phase 2 reentry attributable to an electrical propagation of the epicardial action potential dome from existing to abolished sites has been proposed. However, information about the activation sequences during VT/VF is still limited.

We developed an experimental Brugada syndrome model using a canine isolated RV preparation cross-circulated with arterial blood. Not only saddleback and coved types of ST elevation but ventricular arrhythmias were induced by administration of pilsicainide, a sodium channel blocker, and pinacidil, a K<sub>A<sub>ATP</sub></sub> channel opener. By analyzing local transmural ECG, local electrograms, local recovery times, and activation sequences of endocardium and epicardium, we examined the mechanisms for ST elevation and ventricular arrhythmias induced in this preparation.

Methods

Animals
Experiments were performed using HBD dogs of either sex (weighing 11 to 18 kg), which were bred for laboratory use (Kitayama Labes, Yoshiki Farm, Gifu, Japan). In one experiment, 2 dogs were used; 1 served as a supporter dog and the other as the experimental dog. Twelve experiments were performed using 24 dogs. The experiments were performed according to the Guidelines for Animal Experimentation of Hirosaki University.

Drugs
Pilsicainide was provided by Suntory Limited, Osaka, Japan, and pinacidil was purchased from Sigma. Pilsicainide was dissolved in saline, and pinacidil stock solution was made in DMSO and diluted in saline before use. The final concentration of DMSO was 0.03%, a concentration that had been found not to alter the effect of pinacidil. Both drugs were infused continuously into the cross-circulation system. The doses were adjusted to obtain the concentrations of 5 to
30 μmol/L in the arterial blood when administered into the cross-circulation system.

Isolation of RV Preparation
After general anesthesia with intravenous sodium pentobarbital (30 mg/kg) and calcium heparin administration (500 U/kg), the experimental dogs (n=12) were euthanized by releasing blood from the right carotid artery and the chest was opened via the right thoracotomy. The heart was quickly removed and plunged into Tyrode’s solution kept at 4°C and equilibrated with 95% O₂ and 5% CO₂. The RV free wall including the portion of the RV outflow tract and the right coronary artery was dissected and cannulated for perfusion with arterial blood from the supporter dog (Figure 1).

Preparation of Arterial Blood Cross-Circulation System
Supporter dogs (n=12) of either sex, weighing 14 to 21 kg, were anesthetized with intravenous sodium pentobarbital (30 mg/kg as a bolus injection followed by constant infusion at 5 mg/kg per h) and ventilated with a respirator. Heparin calcium (500 U/kg) was administered intravenously (followed by an additional dose of 200 U/kg every hour). ECG and arterial pressure were continuously monitored throughout the experiment. Arterial blood gasses were analyzed at appropriate intervals and were maintained within their physiological ranges by adjusting the ventilation rate and supplementing oxygen.

The details of the cross-circulation system have been described previously. Briefly, arterial blood was conducted from the carotid artery of the supporter dog into the right coronary artery of the RV preparation with a Harvard peristaltic pump. Constant perfusion pressure at 100 mm Hg was obtained with a Starling pneumatic resistance placed parallel to the perfusion system, and the flow rate was continuously monitored with an electromagnetic flowmeter.

The RV preparation was placed on stainless steel mesh set in a double-wall glass jacket, which was warmed to 37°C by circulating water from a water bath. Venous blood from the RV preparation and the excess blood passing through the pneumatic resistance were collected in a blood reservoir and returned to the jugular vein of the supporter dog. Drugs were administered to the cross-circulation system using a syringe pump. The RV preparation was paced continuously from the center of the endocardial aspect at a cycle length (CL) of 600 ms.

Recordings of Electrograms and Transmural ECG
Two plaque electrodes (30×35 mm) made from soft silicon plates, each containing 48 bipolar electrodes (interelectrode distance, 1.5 mm) in 6 rows and 8 columns, were attached to the endocardial and epicardial aspects of the RV preparation and used for mapping activation sequences (Figure 1). Special care was taken not to institute any mechanical forces against the preparation. The plaque electrode also contained a bipolar electrode in the center of the plaque for electrical stimulation and 4 electrodes in the center of every quarter of the plaque for recording 4 transmural ECGs while using 2 opposite electrodes in the endocardial and epicardial plaque electrodes. All bipolar electrograms were filtered with the use of a bandpass between 50 Hz and 1 kHz. For analyzing the recovery phase of local sites (n=3), bipolar electrograms were filtered with a bandpass between 0.16 Hz and 1 kHz to retain the low frequency component. These electrograms were simultaneously recorded with a mapping system (HPM-7100, Fukuda Denshi) along with 4 transmural ECGs. All data were stored on floppy disks for later analysis.

In this study, ST elevation was defined when ST segment was elevated by ≥0.2 mV compared with the baseline level. According to the configuration of the elevation, ST elevations were divided into saddleback and coved types. When both types were observed in 1 preparation, it was represented as a coved type.

Study Protocol
Approximately 1 hour was allowed for the preparation to be stabilized electrically. After control recording during basic drive pacing, to induce ST segment changes, incremental doses of pinacidil (5 to 10 μmol/L, in the initial 3 preparations and 5 to 30 μmol/L in the other 9) were infused into the cross-circulation system for 2 minutes for each dose. After a 20-minute period for washout of the pinacidil, 5 μmol/L of pilsicainide was continuously infused into the cross-circulation system and incremental doses of pinacidil were again infused. After a 20-minute period for washout of both drugs, the administration of pilsicainide and pinacidil was repeated in the same way except that the dose of pilsicainide was increased up to 30 μmol/L. When VT/VF was induced by drug administration, the dose of pilsicainide was not additionally increased. At each dose of pinacidil, transmural ECG and bipolar electrograms were recorded during pacing at CLs of 1000, 800, and 600 ms to observe CL-dependent ST segment changes. When VT/VF did not occur during basic drive pacing, a single premature stimulus during basic drive pacing at 600 ms was delivered to the preparation. When VT/VF occurred, the administration of drugs was terminated, which resulted in the interruption of VT/VF, usually within 90 seconds.

Assessment of Local Recovery Time
The interval from the stimulus artifact to the end of the T wave of each bipolar electrogram recorded with a bandpass between 0.16 Hz and 1 kHz was measured during basic drive pacing at 600 ms and was defined as local recovery time. This time interval was used as an index of local repolarization in the preparation.

Statistical Analysis
All data are shown as mean±SE. The Student t test was used for comparison of 2 variables. A P value <0.05 was considered to be statistically significant.

Results
Mean blood pressure and heart rate of the supporter dogs (n=12) were 124±4 mm Hg and 129±6 bpm, respectively, at the initiation of the study and 113±7 mm Hg and 145±8 bpm, respectively, at the termination of the study (both P=NS).

ST Elevation Induced in RV Preparation
Figure 2 shows 2 types of ST elevation induced by pilsicainide and pinacidil. When pinacidil up to 10 μmol/L was administered in the absence of pilsicainide, no ST change was induced. When pinacidil was administered in the presence of 5 μmol/L of pilsicainide, saddleback-type ST elevation was induced. When pinacidil was administered in the presence of...
8 μmol/L of pilsicainide, coved-type ST elevation was induced. The degree of ST elevation became greater as the dose of pinacidil was increased. In the other preparations, saddleback-type ST elevation was induced after larger doses of pinacidil were administered or pinacidil was administered in the presence of pilsicainide. Coved-type ST elevation was induced by pinacidil in the presence of pilsicainide at doses of 10±2 μmol/L.

**Ventricular Arrhythmias Induced in RV Preparation**

At baseline, no ventricular arrhythmia occurred spontaneously or was induced by premature stimulation. When ST elevation was induced, 18 episodes of polymorphic VT occurred in 9 of the 12 preparations. Fourteen occurred spontaneously during basic drive pacing, and the other 4 were induced by a single premature stimulus. Nine occurred associated with saddleback-type ST elevation, and the other 9 occurred with coved-type ST elevation. All VT was sustained until the drugs were withdrawn. VT occurring spontaneously was reproducible except for 1 preparation when the same amounts of the drugs were administered. VT induced by premature stimulation was not reproducible. All 3 preparations in which any VT did not occur were isolated from female dogs, whereas the others were from male (n=7) and female dogs (n=2).

**Activation Maps During Spontaneous VT**

Of the 14 spontaneously occurring VT episodes observed in 5 preparations, 3 episodes in 1 preparation were likely to be attributable to reentry between the epicardium and endocardium (an example is shown in Figure 3), 10 episodes in 3 preparations attributable to reentry confined to the epicardium (an example is in Figure 4), and the remaining 1 episode in the other preparation attributable to reentry confined to the endocardium. In the preparation shown in Figure 3, saddleback-type ST elevation was induced by 5 μmol/L pilsicainide and 10 μmol/L pinacidil, and polymorphic VT occurred during pacing at CL of 1000 ms. After the first paced beat, in which the wavefront spread radially from the stimulation site, a premature beat (the first VT beat) sponta-

Figure 2. Two types of ST elevation induced by pinacidil and pilsicainide in 1 preparation. Each panel shows transmural ECG, and the pacing cycle length was 1000 ms. Pinacidil was administered in the absence (A) and presence (B and C) of pilsicainide. The recording site was in the fraction close to the RV outflow tract.

Figure 3. Activation maps of spontaneously occurring VT. A, Anatomic orientation (TVA indicates tricuspid valve annulus; RVOT, RV outflow tract). B, Transmural ECG at the beginning of VT after pilsicainide (5 μmol/L) and pinacidil (10 μmol/L). The recording site is indicated by a star in A. C, Endocardial (ENDO) and epicardial (EPI) activation maps of the basic drive beat (S1) and the first 4 VT beats (V1 through V4). Time 0 indicates stimulation artifact of the basic paced beat preceding VT.
neously occurred from the corner of the epicardium in the middle of the repolarization phase. No bridging activation from the previous paced beat to the first VT beat was noted. The wavefront spread from the right upper corner to the left in the epicardium, and then the wavefront spread from the center to the periphery in the endocardium. Almost the same activation sequences were noted in the second VT beat, suggesting reentry between the epicardium and endocardium.

In the preparation shown in Figure 4, coved-type ST elevation was induced by 20 μmol/L pilsicainide and 10 μmol/L pinacidil. The first VT beat originated from the epicardial site. No bridging activation from the previous paced beat to the first VT beat was noted. Then an arc of block developed and clockwise reentry in the epicardium followed. In the endocardium, the wavefront spread radially from the site that was at the opposite of the earliest activation site in the epicardium.

**Figure 4.** Relation of the origin of the first beat of spontaneously occurring VT to local recovery times. A, Coved-type ST elevation and VT after pilsicainide (20 μmol/L) and pinacidil (10 μmol/L). B, Activation sequence of the first VT beat originating from site a in the nadir of the local recovery times in the epicardium (D). An anatomic orientation is indicated in Figure 3A. C, Bipolar electrograms recorded at sites indicated in B. The ordinate in D indicates local recovery time.

**Activation Maps During Induced VT**

All 4 VT episodes induced by a premature stimulus, which was delivered from the center of the endocardium, in 4 preparations were likely to be attributable to reentry in the...
endocardium. In the preparation shown in Figure 5, coved-type ST elevation was induced by 10 μmol/L pilsicainide and 20 μmol/L pinacidil and polymorphic VT was induced by a premature stimulus (coupling interval, 170 ms). During basic drive stimulation, the wavefront spread radially from the center of the endocardium. After the premature stimulation, the wavefront initially spread radially but an arc of block developed in the left of the stimulation site (Figure 5B). The wavefront took a circuitous route from site d to site h around the arc of the block and reciprocated to site a, producing the first VT beat. Double potentials were recorded at site i, indicating the presence of functional block (Figure 5C). Thus, reentry presumably localized to the endocardium was induced by premature stimulation. Figure 5D shows 3D representation of the activation pattern between endocardium and epicardium. It is noted that the activation in the endocardium showed a counterclockwise rotation, whereas that in the epicardium showed a clockwise rotation. Because the activation in the endocardium always preceded that in the epicardium, the rotating epicardial pattern in the epicardium was considered to be a bystander for reentrant VT.

**Figure 5.** Activation maps of VT induced by a premature stimulus to the endocardium (ENDO). A, Induction of VT by a premature stimulus with a coupling interval of 170 ms after pilsicainide (10 μmol/L) and pinacidil (20 μmol/L). B, Activation sequences in ENDO and epicardium (EPI) during the initial phase of VT. An anatomic orientation is indicated in Figure 3A. C, Bipolar electrograms at sites a through i indicated in B. D, 3D representation of the activation patterns in ENDO and EPI.

Electrical Heterogeneity and Its Relation to Arrhythmias
Mean values of the average of all local recovery times measured in the endocardium and epicardium of each preparation (n=3) were 246±6 and 235±6 ms, respectively, at baseline and 272±1 and 276±1 ms, respectively, after...
administration of pilsicainide and pinacidil that caused ST elevations. Mean values of dispersions of local recovery times in the endocardium and epicardium of each preparation were $23 \pm 2$ and $22 \pm 3$ ms, respectively, at baseline and were increased to $35 \pm 1$ and $45 \pm 3$ ms, respectively, after drug administration (both $P<0.05$).

An example of the relations of local recovery times to the local transmural ECGs is shown in Figure 6. At baseline, local recovery times at every recording site were almost the same in the endocardium and epicardium. Administration of pilsicainide (20 $\mu$mol/L) and pinacidil (10 $\mu$mol/L) increased dispersion of local recovery times in both the endocardium and epicardium. Bipolar electrograms recorded at the sites indicated by the ellipses (a, a’, b, b’, c, and c’) and local transmural ECGs recorded at these sites (a-a’, b-b’, and c-c’) are shown in Figures 5C and 5D, respectively. Coved-type ST elevation in the local transmural ECG was recorded in the area where local recovery time in the epicardium was longer than that in the endocardium (b-b’), whereas saddleback-type ST elevation was seen in the area where local recovery time in the epicardium was shorter than that in the endocardium (c-c’). Thus, when heterogeneity in the recovery phase was increased, different patterns of local transmural ECG were recorded in adjacent sites.

An example of the relationship between the heterogeneity of local recovery times and the origin of the first VT beat is shown in Figure 4D. The first VT beat originated from the site where local recovery time was markedly shortened.

**Discussion**

**Major Findings**

We developed an experimental Brugada syndrome model in which saddleback and coved types of ST elevation were induced by pilsicainide and pinacidil and VT occurred spontaneously or was induced by premature stimulation. The analysis of local recovery times revealed heterogeneous recovery, especially in the epicardium, and the first VT beat originated from a site with a short local recovery time. During VT, reentry around an arc of functional block confined to endocardium or epicardium or reentry between the epicardium and endocardium was demonstrated.

**Mechanism of ST Elevation**

ST elevation in Brugada syndrome is caused by a transmural voltage gradient during repolarization phase in the RV. Transmural voltage gradient is induced by a relative decrease of inward current or an increase of outward current through RV wall. In this study, we used pilsicainide to decrease inward current through sodium channels and pinacidil to increase outward current through ATP-sensitive K channels. The results showed that saddleback-type ST elevation was induced by relatively large doses of pinacidil and coved-type ST elevation was induced by administration of both pilsicainide and pinacidil. Antzelevitch et al described that accentuation of the notch of the action potential of the RV epicardial myocyte as accompanied by exaggeration of the transmural voltage gradient and, thus, exaggeration of J wave and the appearance of saddleback-type ST elevation. Addi-
tional accentuation of the notch may be accompanied by prolongation of the epicardial action potential such that the direction of repolarization across the RV wall and the transmural voltage gradients are reversed, thus leading to the development of coved-type ST elevation and an inversion of the T wave.

By analyzing the local recovery times, we showed that after pilsicainide and pinacidil, when the recovery time in the epicardium was shorter than that in the opposite endocardial site, saddleback-type ST elevation was induced in local transmural ECG. When the recovery time in the epicardium became longer than that in the endocardium, coved-type ST elevation was induced. Prolonged local recovery time in the epicardium relative to that in the opposite endocardial site may be related to accentuation of the notch of the epicardial action potential presumably induced by pilsicainide. Thus, the present findings are consistent with those of Antzelevitch and colleagues and could represent an in vivo mechanism for different types of ST elevation in Brugada syndrome. This study additionally showed that both types of ST elevation in the local transmural ECG were simultaneously observed in 1 preparation. This indicates the heterogeneity of recovery times, providing a substrate for the development of reentrant arrhythmias.

Reentry as a Mechanism for VT/VF in This Model

Phase-2 reentry in the epicardium has been suggested to be responsible for the initiation of ventricular arrhythmias in Brugada syndrome. In the present study, after administration of pilsicainide and pinacidil, VT occurred spontaneously in 5 of the 12 preparations. The analysis of the relation of the origin of the first VT beat to local recovery times revealed that the first VT beat originated from the epicardial site, where local recovery time was shortened compared with those in the neighboring area. Heterogeneity of recovery times seems to promote the development of phase-2 reentry in the epicardium, as described previously.

There has been no systematic study on the mechanism for repetitive excitation during VT in Brugada syndrome. By analyzing the activation sequences during spontaneously occurring VT or VT induced by a premature stimulus delivered from the endocardium, we demonstrated that reentry localized to either the endocardium or epicardium with passive activation of the other (in most preparations) or reentry between the endocardium and epicardium (in 1 preparation) is the most likely mechanism for VT. Thus, reentry in Figure 4 (spontaneously occurring VT) was confined to the epicardium, whereas that in Figure 5 (VT induced by premature stimulation) was confined to the endocardium. For these 2 VT runs, the endocardium in the former and epicardium in the latter were activated passively. During spontaneously occurring VT in Figure 3, reciprocation from the epicardium to the endocardium and then to the initial epicardial site was observed. These activation sequences during VT strongly suggest the presence of a transient block between the endocardium and epicardium, ie, in the M cell area. Using a transmural optical imaging technique in the canine model of long-QT syndrome, Akar et al demonstrated functional expression of M cells in intact myocardium and a central role for M cells in the development of reentrant tachycardia. In recent study using a canine RV wedge preparation perfused with Tyrode’s solution, Di Diego et al reported 1 case in which a marked difference in the cycle length of VT between the endocardium and epicardium was observed. Additional studies on the role of M cells in the development of not only phase 2 reentry but also subsequent repetitive excitations in Brugada syndrome are indicated.

It is worthy of note that VT was not induced in 3 preparations, all of which were isolated from female dogs. Di Diego et al reported that typical electrophysiological changes were hardly induced in the ventricular wedge model obtained from female dogs. Gender might actually be a determinant factor in the development of VT in Brugada syndrome and needs additional investigation. In this study, local recovery time was measured by analyzing bipolar electrograms filtered between 0.16 Hz and 1 kHz. Such bipolar recording system was reported to underestimate the repolarization time compared with unipolar system. The use of a unipolar recording system could demonstrate a greater degree of heterogeneity.

References

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_Circulation_. 2004;109:125-131; originally published online December 8, 2003; doi: 10.1161/01.CIR.0000105762.94855.46
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/1/125

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