High-Density Substrate Mapping in Brugada Syndrome
Combined Role of Conduction and Repolarization Heterogeneities
in Arrhythmogenesis

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Background—Two principal mechanisms are thought to be responsible for Brugada syndrome (BS): (1) right ventricular (RV) conduction delay and (2) RV subepicardial action potential shortening. This in vivo high-density mapping study evaluated the conduction and repolarization properties of the RV in BS subjects.

Methods and Results—A noncontact mapping array was positioned in the RV of 18 BS patients and 20 controls. Using a standard S1-S2 protocol, restitution curves of local activation time and activation recovery interval were constructed to determine local maximal restitution slopes. Significant regional conduction delays in the anterolateral free wall of the RV outflow tract of BS patients were identified. The mean increase in delay was 3-fold greater in this region than in control (P=0<0.001). Local activation gradient was also maximally reduced in this area: 0.33±0.1 (mean±SD) mm/ms in BS patients versus 0.51±0.15 mm/ms in controls (P<0.0005). The uniformity of wavefront propagation as measured by the square of the correlation coefficient, r2, was greater in BS patients versus controls (0.94±0.04 versus 0.89±0.09 [mean±SD]; P<0.05). The odds ratio of BS hearts having any RV segment with maximal restitution slope >1 was 3.86 versus controls. Five episodes of provoked ventricular tachycardia arose from wave breaks originating from RV outflow tract slow-conduction zones in 5 BS patients.

Conclusions—Marked regional endocardial conduction delay and heterogeneities in repolarization exist in BS. Wave break in areas of maximal conduction delay appears to be critical in the initiation and maintenance of ventricular tachycardia. These data indicate that further studies of mapping BS to identify slow-conduction zones should be considered to determine their role in spontaneous ventricular arrhythmias. (Circulation. 2009;120:106-117.)

Key Words: arrhythmia ■ Brugada syndrome ■ conduction ■ mapping

The pathophysiological basis of Brugada syndrome (BS) remains contentious. Characterized by a triad of right bundle-branch block, ST elevation in the right precordial leads, and lethal ventricular arrhythmia, BS remains one of the important causes of sudden cardiac death in the young, accounting for 20% of all cases.1 Currently, ventricular tachycardia/ventricular fibrillation (VT/VF) is thought to arise in BS patients either as a result of conduction block in the right ventricle (RV), the “depolarization hypothesis,”2,3 or from subepicardial attenuation of the action potential dome in the right ventricular outflow tract (RVOT) and phase 2 reentry in the midmyocardium of the RV free wall, the “repolarization hypothesis.”4,5 Data supporting the latter have been derived from the canine RV wedge preparation with the use of pharmacological manipulation of the ion channel currents, simulating the critical changes in this condition.6,7

To date, only single catheter recordings in the human RV have provided evidence to support the repolarization hypothesis, demonstrating differences in activation-recovery interval (ARI) between RV endocardium and epicardium.8,9 There are no published high-density in vivo mapping studies of the RV in BS patients. A single Langendorff study of a perfused BS patient’s ex vivo heart demonstrated conduction delay localized to the RVOT and no evidence of steep restitution slopes in this area.10 Furthermore, no significant transmural differences in repolarization time were observed, even at long cycle lengths. One limitation to this study was the fact that the heart had been examined ex vivo and therefore was subject to possible myocardial ischemia that could modify the behavior of the substrate. We therefore sought to investigate the in vivo electrophysiological properties of the entire RV endocardium using noncontact endocardial mapping in patients diagnosed
with BS. The principal objectives were to determine whether localized endocardial conduction delay in the RVOT is a characteristic feature of BS and to identify whether significant endocardial repolarization gradients exist in the RV compared with controls. The study evaluated regional differences in repolarization time with the use of a standard S1-S2 restitution protocol. The degree of regional11 and local conduction delay was measured with 2 techniques, including activation gradient (AG) quantification.12

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Methods

Mapping was performed in 38 patients (BS group, n=18; control group, n=20). The control group consisted of 12 patients with unifocal RVOT ectopics and 8 with supraventricular tachycardia. They had normal resting and signal-averaged ECGs, a negative ajmaline challenge test, structurally normal hearts on echocardiography, and no family history of sudden cardiac death. Informed consent was obtained after local research ethics committee approval. The noncontact mapping study and pacing protocol were performed before ablation in the control patients or VT stimulation studies in the BS group. The technique for noncontact mapping in the ventricle has been described in detail elsewhere.13 The multielectrode array (Ensite, St Jude Medical) was deployed via the left femoral vein in the RVOT (see Methods in the online-only Data Supplement for detailed protocol).

Genetic Testing

Blood samples (10 mL) were obtained from participating BS subjects. Genomic DNA was isolated from peripheral blood leukocytes with the use of a commercial kit (Genta System, Puregene). The exons of SCN5A were amplified and analyzed by direct sequencing. Polymerase chain reaction products were purified with Exosap (USB) and were directly sequenced from both directions with the ABI PRISM BigDye Terminator Cycle Sequencing Reaction and the ABI PRISM 3130XL Automatic DNA Sequencer.

Study Protocol

After 3 minutes of steady state RV apical pacing at 400 ms, an extrastimulus (S2) was introduced after a drivetrain of 10 beats according to a standard S1-S2 restitution protocol (see Methods in the online-only Data Supplement) and was repeated during pacing from the RVOT. All patients were studied in the absence of ajmaline administration. A VT stimulation protocol was also performed (see Methods in the online-only Data Supplement).

The time from the pacing artifact to electrogram peak negative dV/dt was used as the local activation time. ARI was defined as the interval between activation time and repolarization time and measured as described previously.14,15 The slope of the ARI restitution curve was calculated by the least mean squares method.16 The RV was divided into 16 anatomic segments,15 and the restitution slopes were studied in the segments from the RVOT, RV body, and apex.

Endocardial Regional Delay

Mean increase of delay (MID) was calculated by dividing the integrated increase of delay (area under the curve) by the interval between baseline cycle length and the refractory period.11,12 The degree of delay was measured from 4 segments in the RVOT and the RV body and 4 segments at the apex. Activation times in the left ventricular outflow tract (LVOT) were recorded from a steerable quadrapolar catheter positioned retrogradely through the aortic valve and applied to the septal surface of the LVOT.

Endocardial Local Activation Delay

Areas of local activation delay during sinus rhythm were determined to estimate the location of the diastolic pathway with the use of AG quantification. During sinus rhythm without pacing, activation times determined from noncontact unipolar recordings at 64 locations defined by Cartesian coordinates were used to construct a 3-dimensional activation map.12 This is described in detail in the online-only Data Supplement. In summary, AG was determined by computing the linear regression of activation times from 4 to 6 recording sites that were spatially aligned in the direction of the propagating wavefront and in proximity to one another along the endocardial surface in sinus rhythm. Where slow conduction occurred on the map (ie, greatest local activation delay), the 3-dimensional position, AG magnitude, and square of the correlation coefficient (r2) was calculated, which is a measure of uniformity of wavefront propagation along the endocardial surface, were calculated.

In 5 patients with VT/VF induced during the electrophysiology study, the reentrant VT circuit activation wavefront was mapped; an arrow was drawn directly through the midline of the diastolic pathway on the computerized mapping grid, and the difference between the estimated and actual isthmus was determined as follows. Five equally spaced digital points were placed along each arrow on the computerized mapping grid. The absolute distance between corresponding points along each arrow ([point 1, arrow 1 to point 1, arrow 2], [point 2, arrow 1 to point 2, arrow 2], . . .) was calculated and averaged for the 5 distances.

Statistical Analysis

The mean±SD of computed values of AG and r2 at the region of greatest local activation delay was determined for BS patients versus control patients. The unpaired t test was used to assess the statistical significance of the difference between BS and controls for each of these parameters (SigmaStat version 3.11, 2004). The MID and electrogram data were modeled with the use of mixed effects linear regression (software, MLwIN version 2.01), and statistical significance was inferred from the model. The ARI data were compared with the use of curve fitting. MLwIN was used to fit a regression model to the ARI restitution curve data, and statistical significance was inferred from the model fit. A logistic regression model (STATA) was fitted to evaluate the odds of a segment of control/BS hearts having maximal restitution slopes (Smax) >1. As multiple segments were analyzed in each heart, clustering was used to adjust for error.

To power the study to 80% with a significance of 0.05, STATA 8.2 software was employed. From pilot studies, mean MID was 8.3 (SD 2.5) in control hearts, and mean MID was 14.7 (SD 5.9) in BS hearts. Seventeen patients in each group were calculated as sufficient to detect at least a 50% regional difference in MID. In a normal heart, the mean AG was 0.5 mm/ms (SD 0.15). To identify a ≥40% difference in AG between BS and control groups, n=10 was required. Finally, with the mean maximal slope of the normal heart being 0.5 (SD 0.2), n=10 was required to detect an increase in mean Smax of ≥40%.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Patient Characteristics and Results of SCN5A Mutation Screening

Demographics of the BS study subjects are shown in the Table. The 20 control subjects had a mean age of 48 years (9 men, 11 women). Eighteen BS subjects were studied; 28% had spontaneous resting type 1 ECG changes, and the remainder had a type 1 response to ajmaline provocation. Four had a previous VT/VF arrest, and 5 had a positive VT simulation study at which sustained VT/VF was induced.

We screened by direct sequencing the exons and exon-intron boundaries of SCN5A of the BS patients. Nucleotide sequencing in individuals revealed a missense mutation (G to
A substitution at nucleotide 5038) in 1 BS subject (Table, patient 8), causing the substitution of alanine to threonine (designated A1680T). Mutation A1680T had been described previously as being responsible for sudden arrhythmic death syndrome.17

Control Group

The conduction and repolarization data were initially compared in the RVOT ectopic and supraventricular tachycardia patients. No significant differences in MID (RVOT [mean±SEM], 6.0±0.8 versus 6.8±0.8 ms; body, 8.5±2.2 versus 9.9±2.4 ms; apex, 8.5±2.2 versus 8.4±3.2 ms), AG (mean, 0.52±0.16 versus 0.49±0.14 mm/ms), or proportion of Smax;>1 were identified between these patients’ RVs. They were therefore grouped together and treated as a single control group of patients for the rest of the analysis.

Isochronal Mapping to Identify the Site of Maximal Delay

Figure 1 illustrates the isochronal maps recorded during RV apical pacing at 400 ms obtained from a control patient and at 400-, 600-, and 800-ms coupling intervals in a BS patient. There are clear differences in depolarization time in the RV between the 2 patients. Overall activation of the RV took almost twice as long to propagate across the chamber compared with the control. The isochrones are homogeneously separated in most of the BS RV, but isochronal crowding was evident in the anterolateral RVOT, representing a line of conduction delay of ≥40 ms over this site (Figure 1B). The mean endocardial activation time in patients with type 1 resting ECG during sinus rhythm was 125±10 ms, coinciding with the peak of J-point elevation in the type 1 patients (Figure 1C). The lines of conduction delay in BS patients were functional. There was evidence of conduction slowing in this basal area at 800-ms pacing cycle length without evidence of double potentials (Figure 1B). This contrasts with the control group, in which conduction was more rapid and there were no consistent lines of conduction delay in the anterolateral RVOT.

Quantification of Local and Regional Conduction Delay

As the graphs in Figure 2A and 2B demonstrate, significant local differences in the RVOT activation time occurred in BS patients. Statistically significant regional conduction delay was found between the RVOT and apex of BS and control subjects (Figure 2B). Within the BS group, significant delays were also identified between the RVOT and apex, which was not evident in the control RV (Figure 2B). The LVOT activation curves also had a normal appearance without the delayed upstroke of the BS RVOT activation curve (data not shown).

With the use of an alternative well-validated method to quantify the extent of regional conduction delay and inhomogeneity,12,18 significant reductions in AG in the RVOT of BS patients versus controls were identified (Figure 3A). AG was reduced by 35%. At the slow-conducting region, the mean AG as determined by linear regression was 0.33±0.01 mm/ms in BS patients versus 0.51±0.15 mm/ms in control patients (P<0.0005). Significantly higher correlation coefficients were measured at the site of maximal delay in BS, a measure of local stability in conduction. The uniformity of wavefront propagation as measured by the square of the correlation coefficient, r², was 0.94±0.04 for BS patients versus 0.89±0.09 for control patients (P<0.05) (Figure 3B). With the use of a linear discrimination function of AG=0.425 mm/ms

Table. BS Subjects: Demographics

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ICD indicates implantable cardioverter-defibrillator.

125±10 ms, 0.49±0.14 mm/ms, or proportion of Smax;>1 were identified between these patients’ RVs. They were therefore grouped together and treated as a single control group of patients for the rest of the analysis.
Figure 1. Isochronal maps of RV activation during pacing from RV apex (RVA) at 400-ms cycle length with color coding of each time frame represented on the left. The segment represents a period of 200 ms recorded immediately after the pacing artifact. A, Control. Activation of the RV is completed in 60 ms with evidence of heterogeneity in the breakthrough of activation in the posterior RV body vs the anterior wall. B, In this BS patient who had a prior cardiac arrest, homogeneous depolarization of the RV occurs, taking 120 ms for the RV to depolarize. The mid area of the RV activates over a similar time period compared with control. However, maximal delays occurred in the higher basal region. The area of maximal delay indicated in the anterolateral RVOT had evidence of double potentials (separated by 42 ms) along this site of functional delay (evenly spaced points 1 to 5, along the line) at short coupling intervals of 400 and 600 ms. These double potentials were absent at the longer coupling interval of 800 ms. C, Example of J-point elevation in BS subject 11 with a resting type 1 ECG. Endocardial activation persisted to the peak of the J point at 140 ms after the onset of the QRS complex but not beyond it. PV indicates pulmonary valve location.
for classification, the method of AG determination has a sensitivity of 89% and a specificity of 86% to predict BS in this study population (Figure 3C). No significant differences in AG and $r^2$ were identified between the spontaneous and ajmaline-induced patients.

**Fractionation of Electrograms**

Significant prolongation of the sinus rhythm electrograms was identified in the RVOT versus apex of BS subjects, which was not apparent in the controls (Figure 4A and 4B). These electrograms were also significantly more prolonged (Figure 4B) and complex in the BS RVOT segments versus controls (3.1±0.5 versus 2.5±0.5 polyphasic components; $P<0.05$). The electrograms in the BS RVOT segments were more polyphasic than RV body and apex (3.1 versus 1.8 [RV body] and 1.7 [RV apex]; $P<0.01$), which did not occur in controls.

**Endocardial ARI Restitution Curves**

The mean ARIs of all the segmental restitution curves at 400-ms baseline cycle length in the BS RV and control RV during RV apical pacing were compared. Overall, there were significant differences in ARI in the midportion of the restitution curve between BS and control hearts (Figure 5A and 5B). The mean ARI in the midrange of diastolic intervals was 15 ms longer in control hearts versus BS ($P<0.05$; confidence interval, 4-24 ms) at 400 ms, and this difference persisted over the 210- to 600-ms diastolic interval range.

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**Figure 1.** Continued.

**Figure 2.** A, Conduction curves derived from 5 sites: RVOT, RV body (>2), RV apex, and LVOT of a BS subject. There is a delayed upstroke in RVOT conduction curve, resulting in an increased area under the curve. AT indicates activation time; CI, coupling interval. B, MID for BS and control subjects (mean±SEM). *$P<0.05$ vs BS RVOT.
When the ARI restitution curves in all the RVOT and apical segments were compared in the BS and control patients, there was a significant dispersion of repolarization between these segments versus controls (Figure 5C). Analysis of all the curves by model fitting and testing for interaction confirmed significant differences in the ARI at apex and base in BS at both long and short diastolic intervals, which did not occur in controls. Segmental ARIs were longer at apex versus RVOT in controls only at long diastolic intervals \( (P<0.05) \). There was a crossover of ARIs, resulting in significantly more prolonged ARIs at long diastolic intervals in the RVOT versus apex in BS, which reversed at short diastolic intervals, such that at negative diastolic intervals the apical ARI became longer than the basal in BS hearts \( (P<0.05) \). This suggests that there is a large interval-dependent dispersion of ARI in BS patients.

The maximal slopes of ARI restitution curves were compared between control and BS subjects (examples are shown in Figure 5D). The odds of a BS heart having any segment with a maximum ARI slope \( S_{\text{max}} \) of 1 was 3.86 (95% confidence interval, 1.48 to 10.07) times higher than that in the control heart \( (P<0.05) \). There was a trend in the proportion of RVOT and apical segments with \( S_{\text{max}} \) being significantly greater in BS versus controls, but this did not reach statistical significance. No significant differences in proportion of \( S_{\text{max}} \) segments with \( S_{\text{max}} \) greater than 1 between BS and control subjects were observed.
were identified between the spontaneous and ajmaline-induced BS patients in the 3 regions.

The mean of the greatest segmental $S_{\text{max}}$ from the RVOT of BS was twice that of control RVOT segments (0.84±0.3 versus 0.42±0.2; $P=0.002$, unpaired $t$ test). The apical-basal differences in maximal local restitution slopes were preserved with pacing from the RVOT (RVOT, $S_{\text{max}}$ 0.93±0.3 versus apex, 0.51±0.2; $P<0.01$, paired $t$ test) in BS hearts.

Initiation of VT and VF

In 5 BS patients, VT was induced that degenerated into VF during the VT stimulation protocol. (An example of the initiation is shown in detail in Figure 6.) A consistent feature seen in all 5 cases that provoked VF was progressive slowing of conduction, followed by formation of an arc of functional conduction block on the anterior RVOT endocardium. Each episode of VF initiation consistently demonstrated the site of earliest wave break, and fractionation arose from an anterior RVOT site. These key areas that initiated VF correlated with the same regions of altered myocardial electrophysiology, identified during sinus rhythm and steady state remote RV pacing, characterized by altered dispersion of ARI and slow conduction in BS patients. The restitution slope between the lines of conduction block in Figure 6 was <1, suggesting that conduction delay accounted for wave break, ultimately leading to polymorphic VT and VF. Lines of functional block evolved on a beat-to-beat basis, but the region of slow conduction that these lines bordered was limited to the anterior wall of the RVOT. Furthermore, the same data were analyzed with the custom-designed AG quantification technique developed by Ciaccio et al. The site of wave break occurred in areas of local conduction delay, as shown for 2 BS subjects 17 and 18 in Figure 7. This slow-conduction zone formed at the region of greatest local activation delay as determined by sinus rhythm AG quantification. Wave break tended to coincide with slow-fast conduction boundaries at the edges of this region. From the set of 5 patients in which the isthmus location estimated by sinus rhythm analysis was compared with the location of the diastolic pathway as in Figure 7, the mean difference in location was 8.7±1.6 mm.

Discussion

This is the first study to describe the electrophysiological substrate responsible for the changes seen in BS in vivo, with the use of high-resolution noncontact endocardial mapping.
Figure 6. Initiation of VT (which subsequently degenerated into VF) after development of an arc of functional block during a VT stimulation study (BS subject 18). Orthogonal isochronal maps on each panel show activation from the entire RV. A sequence of isochronal maps with reference time scale on the left of each map corresponds to 5 sampled time windows. The Ensite computer-generated isochrones correspond to the peak negative $dV/dt_{max}$ time point of each electrogram. The time scale of the isochrones is shown on the left of each map in 20-ms increments. The position of the Ensite array and mapping catheter can also be seen within the RV. Sampled areas of reconstructed electrograms are shown in the lower panel. The position of each electrogram is also marked on the isochronal maps (electrograms: 1 to 10). Two reference ECG electrodes are also included at the top of the figure. A shows the first ectopic beat that occurs after a fusion beat seen on the second pacing artifact (at 600-ms coupling interval). A time window of 150 ms is taken to create the map. Earliest activity arises from the midanterior RV wall. No lines of block are seen from the reconstructed electrograms or isochronal maps. In B, a further ectopic arising from a position similar to the first beat occurs (time window, 150 ms); limited lines of conduction block starting to develop above and below the earliest points of activation on the anterior wall (>50 ms activation difference between 2 adjacent areas) can be seen on the isochronal activation, and slowing of conduction is suggested by the more prolonged electrograms. C, With a time window of 120 ms, the next sequence of RV activity arises just after the next pacing artifact. This arises from a superior and more septal point on the anterior RV wall. A region of conduction block with crowding of isochrones and temporal differences in activation of immediate adjacent areas of 80 to 90 ms is present. Electrograms at positions 6 to 8 demonstrate a single early negative unipolar activation, which contrasts with the double potentials seen in the reconstructed electrograms (electrograms 3 to 5) within the adjacent area forming a border of conduction block. D shows the next time window of 110 ms after C. Continued activity with the earliest region (now in white) follows from the latest regions in C. Activity revolves around lines of conduction block and propagates into the previously protected area. There is a delay of 100 ms between the latest activation of the beat on electrogram 3 in C and the earliest electrogram 6 in this panel. This sequence of activation may be consistent with reentry. However, there is a delay of 100 ms between the latest activation of the beat on electrogram 3 in C and the earliest electrogram 6 in this panel. This could be explained by delay created by turning of the wavefront around the line of conduction block in this area. The earliest electrograms progress from electrogram 6 to 8 with far field deflections seen on 3 to 5, which lie on a border of conduction block. E shows the immediate time window spanning 110 ms after D. The earliest region is now white, replacing the latest area on D (blue and purple). Activation during this beat follows the same path of activity shown in D, proceeding around the region of conduction block in the RVOT with evidence of double potentials in electrograms 3 to 5 and sharp negative deflection in electrograms 6 to 8 at the site of latest activation.
and confirmed by AG quantification. Analysis of conduction curves from the RVOT, RV body, and apex revealed significant conduction delay in the RVOT compared with the RV body and apex. Isochronal maps confirmed significant reductions in AG and formation of lines of conduction delay in the RVOT of BS patients versus controls. There was evidence of steep restitution gradients in the RVOT of these patients, with a greater proportion of RV segments in BS patients having slopes $>1$ compared with controls. The same region of delayed conduction gave rise to wavefront fragmentation that led to initiation of polymorphic VT, then VF, similar to the mechanisms reported in human ischemic cardiomyopathy by Chow et al.19

Our observations of delayed conduction in the RVOT are consistent with the depolarization hypothesis. Similar observations were made by Coronel et al10 in an explanted BS heart, in which delayed endocardial conduction was most prominent in the RVOT. A computational model of these conduction data reproduced the classic ECG signs of BS. A separate model investigating activation delay based on BS ECG analyses demonstrated that slowing the AG to 0.2 m/s in the anterior RV could reproduce the BS ECG changes and result in VF initiation from that area that approximates the AG observed in BS patients.20

Clinical evidence for conduction delay in BS comes from echocardiographic, signal-averaged ECG, and body surface

**Figure 7.** Relation of VT diastolic pathway to sinus rhythm activation in 2 other examples of VT. A, BS subject 17. The vector projected along the area of slow and most uniform conduction, determined from sinus rhythm analysis, approximately coincides with the actual direction of propagation through the diastolic pathway during VT. The mean distance between estimated (blue arrow) and actual vectors (white arrow) was 10.1 mm. The AG along the estimated vector during sinus rhythm was 0.398 mm/ms. The pathway had an $r^2$ value of 0.99 (i.e., very uniform conduction along the estimated vector). B, Illustration of another VT diastolic pathway in BS subject 18 estimated from the septal paced rhythm activation analysis. The actual pathway (white arrow) is 0.8 to 1 cm (figures shown) away from the estimated diastolic pathway in the anterolateral region of the RV. RVA indicates RV apex; PV, pulmonary valve.
mapping data. The latter 2 have been shown to be independent predictors of VT/VF. Nagase et al showed that delayed potentials from the epicardium coincided with late potentials from signal-averaged ECG. However, to date, there has been no in vivo determination of the precise location and extent of delayed conduction in the RV endocardium of the human BS heart. With the use of 2 methods to measure conduction delay, our study demonstrates that endocardial activation delay is most pronounced in the RVOT. This regional inhomogeneity of conduction, especially in the RVOT, reinforces the concept that conduction delay contributes to the signature ECG changes of BS and is proarrhythmic. AG is 35% lower in BS patients. In BS patients, this conduction delay increases the likelihood of reentry across boundaries of slow-fast conduction, where there is the potential for impedance mismatch and functional unidirectional block.

The lower uniformity of activation in the controls and greater variability from patient to patient as measured by the square of the correlation coefficient ($r^2$) (0.89±0.09 in controls versus 0.94±0.04 for BS; $P<0.05$) may be due to the fact that normal endocardial surface conduction arises from deeper tissue via the Purkinje network, and thus more endocardial breakthrough occurs. The greater uniformity of depolarization in BS may be due to the fact that surface conduction is limited to the endocardium and occurs less by breakthrough from mid and epicardial tissue. The lower AG observed in specific areas of the RVOT in BS may suggest a structural basis for the creation of the substrate from which conduction abnormalities arise, as has been described in infant-related ventricular arrhythmias. Fibrosis has been documented in clinical BS cases and SCN5A knockout murine models of the condition. Such a process would serve to uncouple myocardial layers perhaps through disease of the Purkinje network, which has been demonstrated in another disorder arising through mutations in the SCN5A sodium channel gene: Lev-Lenègre disease.

SCN5A mutations currently occur in only 20% of BS cases (6% in this study), and the consistent identification of RVOT conduction delay in all the patients from this series suggests that the features of BS arise from a number of factors leading to conduction block as opposed to a single sodium channelopathy. As Coronel et al suggested recently, the fundamental problem in BS may be secondary to conduction delay arising from genetic, structural, or pharmacological causes. The right precordial ST elevation characteristic of the condition is a final phenotypic expression of this conduction abnormality, and the regional ECG abnormalities would correlate to the location of the conduction abnormality in the RV. Although endocardial activation did not continue into the coved ST segment in this study, RV epicardial activation may continue beyond the J point, or endocardial activation delay itself may cause prolongation of repolarization, contributing to persistent ST elevation. Indeed, BS may represent a myocardial disorder in which specific ion channelopathies such as SCN5A mutations interact with structural abnormalities to promote arrhythmia, although these mutations may not be as proarrhythmic or clinically significant in normal myocardium.

Repolarization Abnormalities

This study demonstrated that steep restitution slopes $>1$ exist in a greater proportion of segments in BS hearts versus controls. Contrary to the report of Hayashi et al, slopes $>1$ were identified in all BS patients independent of inducible or spontaneous VF. This can be explained by the fact that Hayashi et al mapped only 2 sites and therefore could not examine the heterogeneity in ARI restitution in the entire RV. The greater percentage of slopes $>1$ in the BS RV versus controls implicates a global repolarization abnormality in the RV endocardium, which may enhance the vulnerability to arrhythmia.

The finding of slopes $>1$ in normal RV myocardium has been described previously by 2 independent groups with contact and noncontact recordings and suggests that the identification of slopes $>1$ alone does not necessarily indicate a high-risk substrate. The greater proportion of steep restitution slopes in the BS subjects and apical-basal dispersion of repolarization over a range of coupling intervals in the RVOT would increase the probability of wave break of local reentrant wavefronts and the degeneration of stable VT into VF as proposed by the action potential duration restitution hypothesis. The combination of conduction delay and heterogeneities in ARI restitution slopes would create the ideal substrate for local wave break to initiate VT and the degeneration of VT into VF. It is interesting to note that a crossover of regional ARI occurs in BS and not controls at longer coupling intervals, suggesting persistence of action potential duration dispersion at both rapid and slow heart rates. Therefore, at rapid heart rates, regional action potential duration dispersion facilitates conduction block to create the conditions to promote reentry and VT. Steep restitution slopes would then serve to destabilize the VT and promote degeneration into VF.

Data from the canine RV wedge preparation implicate phase 2 reentry as a result of epicardial dispersion of repolarization without significant shortening in endocardial action potential duration at closely coupled extrasystoles as the trigger for ventricular arrhythmia. This is consistent with heterogeneous Ito channel distribution with higher membrane densities in the epicardial tissue than in the endocardium. Nagase et al have recently demonstrated prolonged epicardial ARIs in BS after administration of pilsicainide. However, type 1 ECG changes and epicardial prolongation in ARI also occurred in SCN5A mutation-negative subjects, implying other mechanisms of transmural repolarization differences. Such dispersion in repolarization could be maximized if local fibrosis or another mechanism of myocyte uncoupling were to promote the differences in repolarization gradients.

In a recent canine optical mapping study, Aiba et al showed marked dispersion of repolarization in the RVOT epicardium compared with the endocardium with maximum repolarization gradients at the initiation of VF. Furthermore, conduction velocity restitution was progressively depressed from polymorphic VT to VF, suggesting that conduction delay is an important determinant of wave break and VF initiation/maintenance in this model.
We conclude that although epicardial repolarization gradients might explain phase 2 reentry and the initiation of VT in BS, marked regional endocardial conduction delay in combination with regional differences in repolarization kinetics are key elements of the arrhythmogenic substrate in this condition and could be critical in the initiation and maintenance of VT. The degeneration of VT to VF in humans is due to wave break at areas of steep ARI restitution slopes. The development of wave break would be consistent with high impedance mismatch, similar to the situation in infarct border zones. This may result from fibrosis in the tissue in this condition, for which there is evidence from other studies. Biopsy data from these sites of maximal conduction slowing and wave break would enhance our understanding of the substrate in these specific sites but are not currently available from these clinical cases.

**Study Limitations**

This study employed noncontact mapping to investigate endocardial conduction and repolarization kinetics. Epicardial data were not collected because of ethical limitations in mapping RV epicardium via the conus branch so that the roles of epicardial conduction block and action potential duration changes were not examined. However, epicardial RV sites may also be clinically important. This study investigated VT initiation after programmed stimulation, which may differ from spontaneous VT/VF in this syndrome. However, practical considerations prevented mapping of spontaneous VF, which did not occur during the mapping studies in these patients.

**Clinical Implications**

Risk stratification in BS remains a significant challenge. The inducibility of VT during an electrophysiological study is a potential determinant of sudden death. The identification of regional RVOT conduction delay and block in this condition suggests that conduction delay is an important feature of BS. The recent observation that fragmented QRS complexes are a predictor of prognosis in BS supports the contention that these conduction abnormalities are an important contributor to the arrhythmogenic substrate in this disease. Why this form of channelopathy has such a localized predilection affecting the RVOT rather than a more widespread ventricular manifestation remains unexplained.

Having identified slow-conduction zones in the BS RVOT, future studies should investigate whether they play a role in spontaneous arrhythmogenesis. This information could inform potential targeted therapies if a local endocardial or epicardial abnormality is confirmed to play a role in spontaneous VT/VF.

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**Disclosures**

Dr Lambiase reports having served on the St Jude Medical Advisory Board. The other authors report no conflicts of interest.

**References**


CHLONICAL PERSPECTIVE

Characterized by coved J-point elevation in leads V1 through V3, and ventricular arrhythmia in apparently structurally normal hearts, the risk stratification and treatment of Brugada syndrome remain areas of active research. There is some debate in the literature in regard to whether proarrhythmia is secondary to localized right ventricular conduction abnormalities or transmural differences in repolarization in the anterior right ventricular wall. A single Langendorff study in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. Circulation. 2003;8:30–36.

Peters NS, Coromilas J, Seves NJ, Wit AL. Disturbed connexin43 gap junction distribution correlates with the location of reentrant circuits in the coved J-point elevation in leads V1 through V3 and ventricular arrhythmia in apparently structurally normal hearts, the risk stratification and treatment of Brugada syndrome remain areas of active research. There is some debate in the literature in regard to whether proarrhythmia is secondary to localized right ventricular conduction abnormalities or transmural differences in repolarization in the anterior right ventricular wall. A single Langendorff study in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. Circulation. 2003;8:30–36.


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Methods
The multi-electrode array (MEA) (*Ensite*, St Jude Medical) was deployed via the left femoral vein in the RVOT. Right ventricular geometry was created by dragging a 5F mapping catheter along the endocardial surface of the RV. The RV geometry was created in the usual manner taking care to ensure that the array was positioned at a site within 4cm of the RV apex, body and RVOT surfaces to obtain accurate NC unipolar electrogram data from these sites. Detailed geometry creation was assessed by ensuring dense surface point acquisition and editing during collection to eliminate inaccurate interpolated surface projections with sparse geometric points in any given area. RV pacing was established using a quadrapolar catheter placed in the RV apex and RVOT.

Study Protocol
After 3 minutes of steady state RV apical pacing at 400msec, an extrastimulus (S2) was introduced after a drivetrain of 10 beats according to a standard S1-S2 restitution protocol. The coupling interval of S1-S2 was increased in stepwise fashion in increments of 50msec until a sinus beat occurred and then decremented by 50ms every cycle to the baseline coupling interval, by 20 ms every cycle to 300ms and by 5ms every cycle from 300ms until the ventricular effective refractory period (ERP) was encountered. This was repeated during pacing from the right ventricular outflow tract. All patients were studied in the absence of ajmaline administration.

The VT stimulation protocol consisted of a 9 beat drivetrain at 600, 500 and 400 msec baseline cycle length respectively followed by S2 until ventricular refractoriness (VERP) or 200msec coupling interval was reached. This was repeated for each baseline drivetrain adding
S3 to S2 until ventricular refractoriness or 200msec coupling interval. Finally S4 was added keeping the preceding beat (S3) 10msec above VERP or at 200msec. This protocol was performed with pacing in the RV apex and RVOT.

The non-contact mapping data were collected with a recording bandwidth of 0-300Hz in all cases and measurements made with a filter band width of 0.1-25Hz as previously described\textsuperscript{14}. Investigators (PL, AA, EJC, SJ) were blinded to the measurements of other investigators. The array was maintained in the same position throughout the performance of the restitution curves and VT stimulation study. In 2 cases the array was repositioned to ensure acquisition of accurate apical data when the distance was greater than 4cm from the array.

The time from the pacing artefact to electrogram peak negative dV/dt was used as the local activation time (AT). Activation-recovery interval (ARI) was defined as the interval between activation time (AT) and repolarization time (RT) and measured as previously described\textsuperscript{14, 15}. In summary, the RT was measured at the dV/dt\textsubscript{max} for the negative T-wave, the dV/dt\textsubscript{min} of the positive T-wave, and at the mean time between dV/dt\textsubscript{max} and dV/dt\textsubscript{min} for the biphasic T-wave. T waves with an interrupted descending or ascending phase, resulting in double-peak derivatives at these sites were measured at the mean time between two peak derivatives. The unipolar electrograms with flat T waves and ST-segment elevation without discernable T-wave upstroke were excluded from measurement. Diastolic interval was measured from the end of repolarization (RT) from the preceding beat to the AT of the following beat. The slope of the restitution curve was calculated using the least mean squares method\textsuperscript{16}. The RV was divided into 16 anatomical segments\textsuperscript{15} and the restitution slopes studied in the segments from the RVOT, RV body and apex.
Measurement of Endocardial Local Activation Delay

Areas of local activation delay during sinus rhythm were determined to estimate the location of the diastolic pathway using a method termed activation gradient quantification. During sinus rhythm without pacing, activation times determined from NC unipolar recordings at 64 locations defined by Cartesian coordinates were used to construct a three-dimensional activation map. Conduction velocity was determined by computing the linear regression of activation times from 4-6 recording sites that were spatially aligned in the direction of the propagating wavefront and in proximity to one another along the endocardial surface in sinus rhythm. From regression analysis, the slope of the regression line in milliseconds and the regression coefficient is obtained. The conduction velocity of the propagating wavefront was calculated from the mean distance between adjacent recording sites used for regression divided by the slope of the regression line. The regression coefficient is an estimate of the uniformity of wavefront propagation along the endocardial surface at any measured region. This regression analysis was performed at all regions of the activation map. Where slow conduction occurred on the map (i.e., greatest local activation delay), the three-dimensional position, conduction velocity magnitude (CV), and regression coefficient ($r^2$) were calculated.

The vector along which CV was least with $r^2$ value >0.9 was considered to be the best estimate of the direction of propagation along the diastolic pathway during ventricular tachycardia re-entry. The CV and $r^2$ value was tabulated at these points for all patients. In 5 patients with VT/VF induced during the EP study, the re-entrant VT circuit activation wavefront was mapped; an arrow was drawn directly through the midline of the diastolic pathway on the computerized mapping grid, and the difference between the estimated and actual isthmus was determined as follows. Five
equally spaced digital points were placed along each arrow on the computerized mapping grid. The absolute distance between corresponding points along each arrow: (point 1, arrow 1 - point 1, arrow 2), (point 2, arrow 1 - point 2, arrow 2) ... was calculated, and averaged for the five distances.