

PRE-CLINICAL RESEARCH

Abnormal Repolarization as the Basis for Late Potentials and Fractionated Electrograms Recorded From Epicardium in Experimental Models of Brugada Syndrome



Tamás Szél, MD, PhD,^{*†} Charles Antzelevitch, PhD^{*}
Utica, New York; and Szeged, Hungary

- Objectives** The aim of this study was to test the hypothesis that late potentials and fractionated electrogram activity are due to delayed depolarization within the anterior aspects of right ventricular (RV) epicardium in experimental models of Brugada syndrome (BrS).
- Background** Clinical reports have demonstrated late potentials on signal-averaged electrocardiography (ECG) recorded in patients with BrS. Recent studies report the appearance of late potentials and fractionated activity on bipolar electrograms recorded in the epicardium of the RV outflow tract in patients with BrS.
- Methods** Action potential and bipolar electrograms were recorded at epicardial and endocardial sites of coronary-perfused canine RV wedge preparations, together with a pseudo-ECG. The transient outward potassium current agonist NS5806 (5 μM) and the Ca^{2+} -channel blocker verapamil (2 μM) were used to pharmacologically mimic the BrS genetic defect.
- Results** Fractionated electrical activity was observed in RV epicardium, but not in endocardium, as a consequence of heterogeneities in the appearance of the second upstroke of the epicardial action potential, and discrete high-frequency spikes developed as a result of concealed phase 2 re-entry. In no case did we observe primary conduction delay as the cause of the BrS ECG phenotype or of late potential or fractionated electrogram activity. Quinidine (10 μM) and the phosphodiesterase-3 inhibitors cilostazol (10 μM) and milrinone (2.5 μM) restored electrical homogeneity, thus abolishing all late potentials and fractionated electrical activity.
- Conclusions** These data point to an alternative pathophysiological basis for late potentials and fractionated electrical activity recorded in the right ventricle in the setting of BrS. We demonstrate an association of such activity with abnormal repolarization and not with abnormal depolarization or structural abnormalities. (J Am Coll Cardiol 2014;63: 2037–45) © 2014 by the American College of Cardiology Foundation

Brugada syndrome (BrS) is a disease that causes vulnerability to ventricular tachycardia (VT) and sudden cardiac death in young adults with structurally normal hearts. The electrocardiographic (ECG) pattern of BrS is characterized by the appearance of prominent J waves, often appearing as ST-segment elevation in the right pre-cordial leads (1–3), which are often concealed, but can be unmasked by vagal stimulation and potent sodium-channel blockers (3,4). BrS

has been linked to mutations causing decreased inward currents such as peak sodium-channel current (I_{Na}) or L-type calcium-channel current (I_{Ca}) or increased outward currents, especially the transient outward potassium current (I_{to}) (5,6). The net outward shift of current during phase 1 of the epicardial action potential (AP) leads to accentuation of the spike-and-dome morphology, most prominently in the epicardium of the right ventricular outflow tract (RVOT) (3). Loss of the dome (phase 2 of the AP) and consequent development of dispersion of repolarization within epicardium and between epicardium and endocardium create the substrate for phase 2 re-entry and polymorphic VT (7).

Previous clinical reports have demonstrated the presence of late potentials on the signal-averaged ECG (SAECG) in patients with BrS (8). These investigators also recorded

From the ^{*}Masonic Medical Research Laboratory, Utica, New York; and the [†]Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary. This study was supported by grant HL47678 from the National Heart, Lung and Blood Institute (Dr. Antzelevitch); New York State Stem Cell Science grant C026424 (Dr. Antzelevitch); and the Masons of New York, Florida, Massachusetts, Connecticut, Maryland, Delaware, New Hampshire, and Wisconsin. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Abbreviations and Acronyms

AP	= action potential
BrS	= Brugada syndrome
ECG	= electrocardiography
EG	= electrogram
I_{Ca}	= L-type calcium-channel current
I_{Na}	= sodium-channel current
I_{to}	= transient outward potassium current
PDE	= phosphodiesterase
RV	= right ventricular
RVOT	= right ventricular outflow tract
SAECG	= signal-averaged electrocardiography
VF	= ventricular fibrillation
VT	= ventricular tachycardia

delayed potentials using a unipolar electrogram (EG) lead introduced into the conus branch through the right coronary artery. This activity was hypothesized to be due to a “myocardial abnormality” in the epicardium, but not the endocardium, of the RVOT. Other investigators documenting late potentials on SAECG in patients with BrS have attributed this activity to structural abnormalities and delayed conduction within the RV (9,10). Using bipolar EGs, Nademanee et al. (11) demonstrated late potentials and fractionated activity in RVOT epicardium in patients with BrS and hypothesized that these represent regions of delayed conduction or depolarization defects.

A recent publication by Sacher et al. (12) demonstrated such activity in patients with BrS challenged with potent sodium-channel blockers, and the investigators likewise hypothesized that these are due to depolarization defects.

The present study provides a test of this hypothesis using experimental models that closely recapitulate the ECG and arrhythmic manifestations of BrS. The principal focuses of the study were to elucidate the cellular mechanisms involved and to test the hypothesis that late potentials and fractionated EG activity recorded from the epicardial surface of experimental models of BrS are associated with the development of abnormal repolarization rather than abnormal depolarization.

Methods

Wedge preparations. All experiments were carried out in compliance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (publication no. NIH 85-23, revised 1996) and approved by the Institutional Animal Care and Use Committee. Detailed methods for isolation and recording of transmembrane activity from coronary-perfused canine RV wedge preparations have been reported previously (13,14). Briefly, adult mongrel dogs (20 to 35 kg) of either sex were used. Transmural wedge preparations were dissected (1.9 × 0.9 × 0.9 cm to 3.2 × 1.6 × 1.3 cm) from the RV free wall in the dogs. The preparations were cannulated via the marginal branch of the right coronary artery and perfused with cardioplegic solution (Tyrode’s containing 12 mmol/l KCl). Nonperfused tissue was carefully removed using a razor blade. The preparations were then placed in a tissue bath and perfused with oxygenated Tyrode’s solution (mM: NaCl 129, KCl 4, NaH₂PO₄ 0.9, NaHCO₃ 20, CaCl₂ 1.8,

MgSO₄ 0.5, and glucose 5.5; pH: 7.4). The perfusate was delivered using a roller pump (Cole-Parmer, Niles, Illinois) at a constant flow rate of 8 to 10 ml/min and warmed to 37 ± 0.5°C.

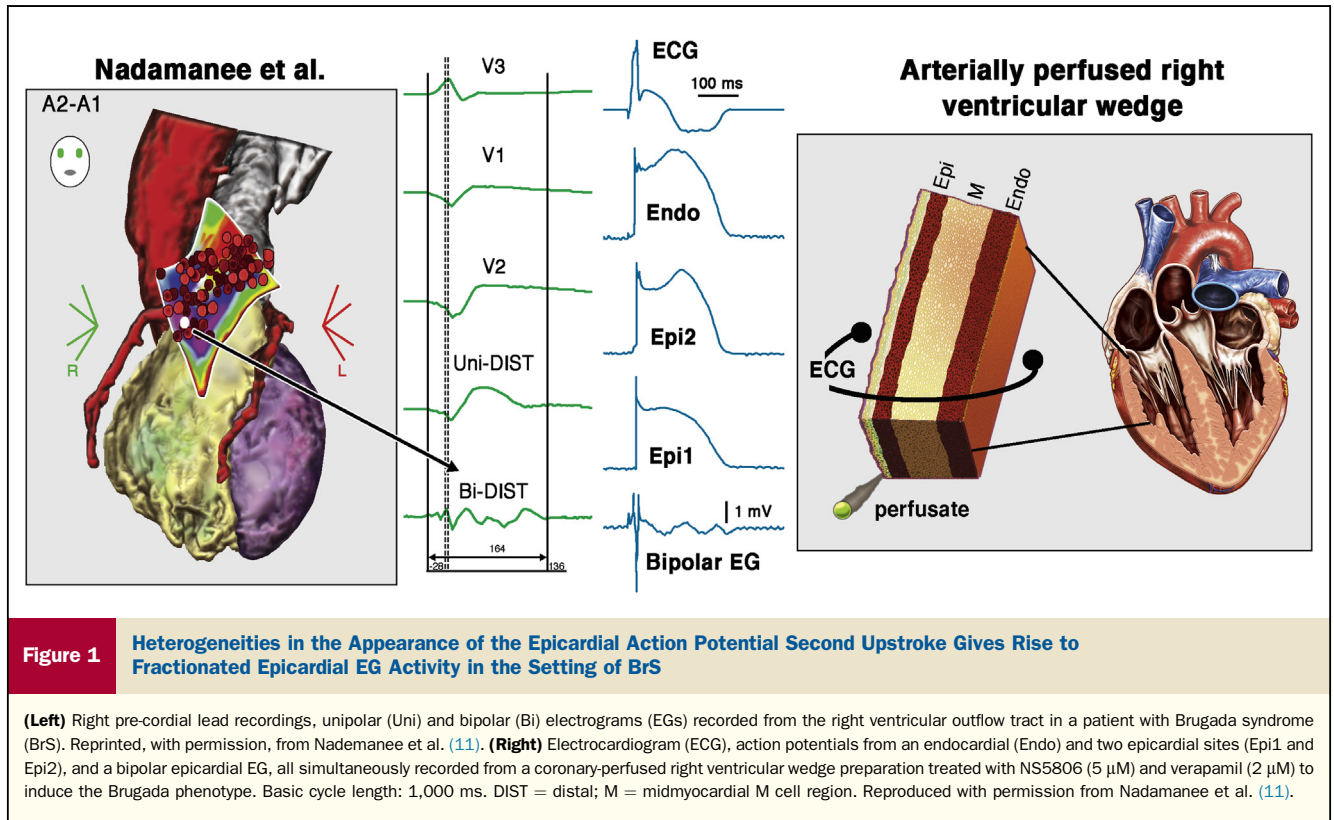
The preparations were equilibrated in the tissue bath until electrically stable, usually 1 h, while stimulated at a basic cycle length of 1,000 ms using bipolar silver electrodes insulated except at the tips, applied to the endocardial surface. A transmural ECG was recorded using two electrodes consisting of AgCl half-cells placed in the tissue bath, 1.0 to 1.5 cm from the epicardial and endocardial surfaces of the preparation, along the same axis as the transmembrane recordings (the epicardial electrode was connected to the positive input of the ECG amplifier).

Transmembrane APs were simultaneously recorded in two epicardial sites (Epi 1 [distal] and Epi 2 [proximal]; distance from Epi 1 to Epi 2: approximately 5 to 10 mm) and in one endocardial site, with the use of floating microelectrodes (DC resistance: 10 to 20 MΩ) filled with 2.7-mol/l KCl, each connected to a high-input impedance amplifier. Impalements were obtained from the epicardial and endocardial surfaces of the preparation at positions approximating the transmural axis of the ECG recording. Two unipolar EGs were placed in the epicardium or endocardium. Virtual bipolar EGs were derived as the difference of two unipolar EGs.

Spike 2 software (Cambridge Electronic Design Ltd., Cambridge, United Kingdom) was used to record and analyze the ECG, EGs, and the AP. NS5806, verapamil, quinidine, cilostazol, and milrinone were dissolved in dimethyl sulfoxide (10-mM stock).

Results

Using coronary-perfused canine RV wedge preparations, we induced the Brugada phenotype by the addition of NS5806 (5 μM) (an I_{to} activator) and verapamil (2 μM) (a Ca²⁺-channel blocker) to the coronary perfusate. NS5806 has previously been shown to increase I_{to} in isolated canine cardiomyocytes, resulting in augmentation of the notched appearance of the RV AP, most notably in the epicardium (15,16). NS5806 (5 μM) and verapamil (2 μM) accentuated the AP notch in RV epicardium, leading to the development of a prominent J point and ST-segment elevation, characteristic of the Brugada phenotype. Late potentials and fractionated activity were often observed in the bipolar EGs comparable to those recorded in the clinical cases reported by Nademanee et al. (11) (Fig. 1). The accentuation of the AP notch in the epicardium, but not the endocardium, generates a transmural voltage gradient involved in the accentuated J waves in the ECG. The fractionated EG activity was observed to have resulted from the heterogeneity in the appearance of phase 2 of the AP. In no case did we observe major delays in phase 0 depolarization or any type of conduction problem of the primary depolarization wave.



With longer exposure to the provocative agents, we observed loss of the AP dome at some epicardial sites but not others. This further accentuated the transmural voltage

gradient, thus contributing to the manifestation of J waves, often appearing as ST-segment elevation. The dispersion of repolarization generated as a result of abbreviation of the

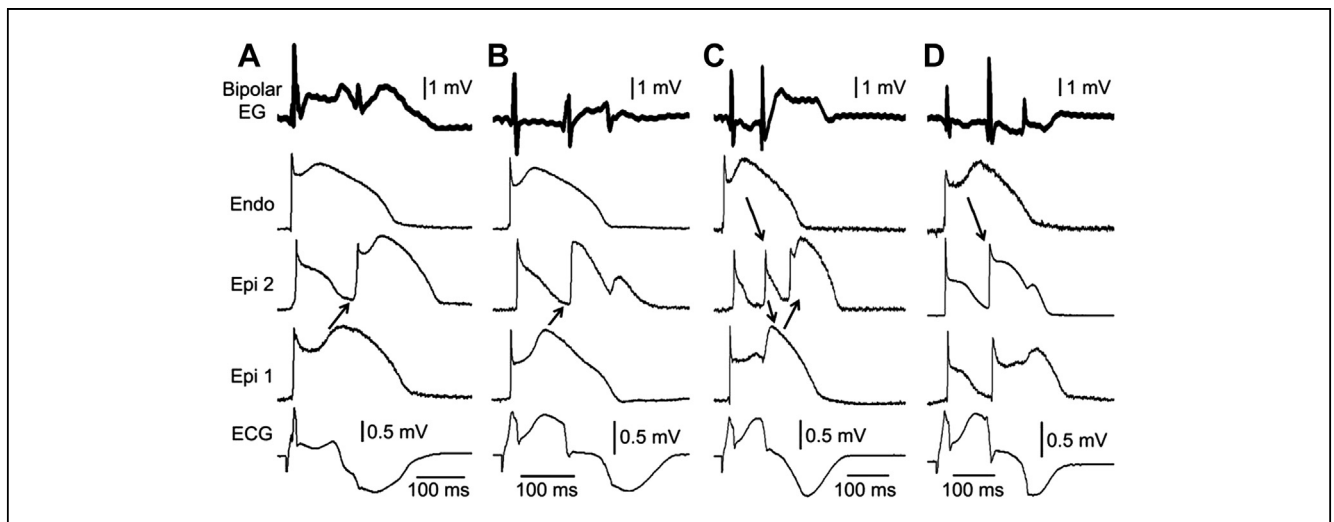
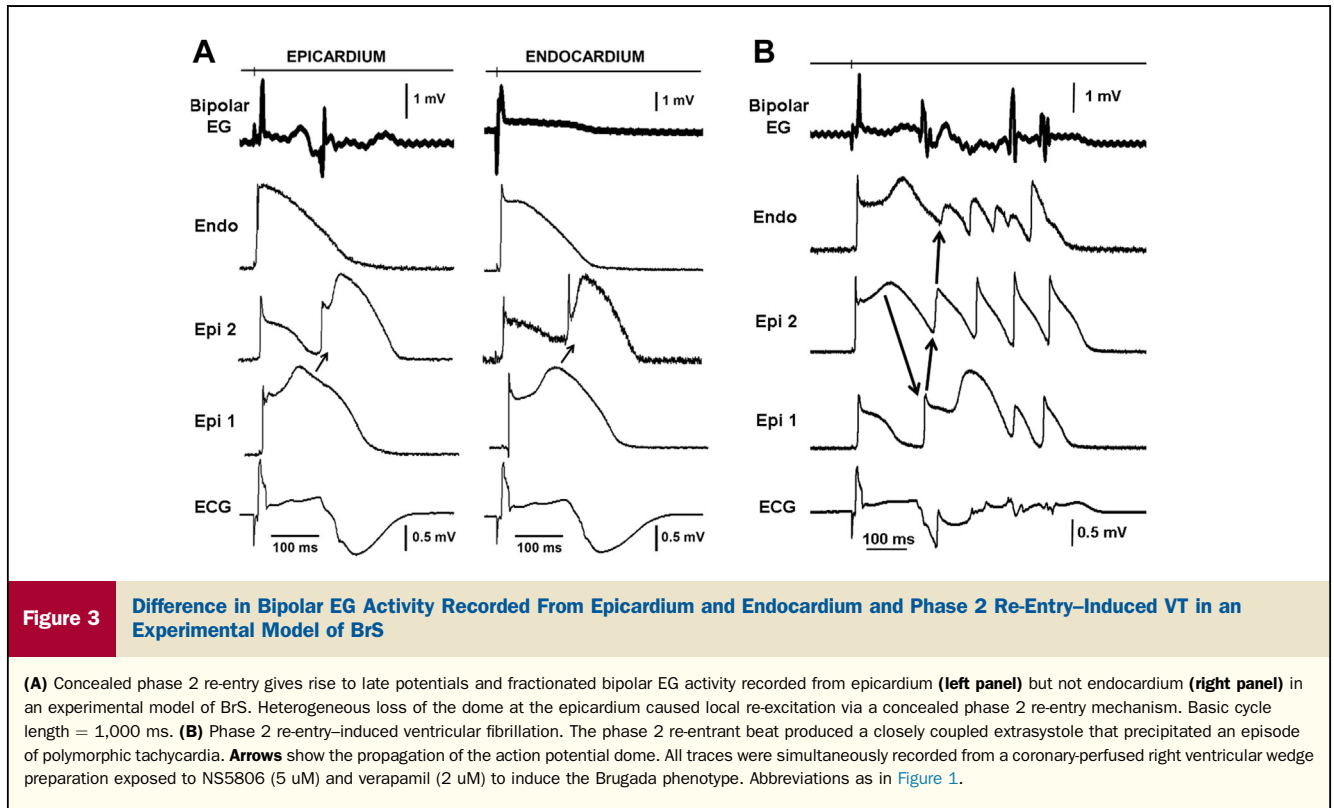


Figure 2 Concealed Phase 2 Re-Entry as the Basis for Late Potential and Fractionated Bipolar EG Activity in an Experimental Model of BrS

The panels from top to bottom show a bipolar EG, action potentials recorded from an endocardial and two epicardial sites, and an ECG, all simultaneously recorded from a coronary-perfused right ventricular wedge preparation exposed to NS5806 (5 μ M) and verapamil (2 μ M) to induce the Brugada phenotype. Each panel (A–D) shows results from a different preparation. Heterogeneous loss of the dome at the epicardium caused local re-excitation via a concealed phase 2 re-entry mechanism, leading to the development of late potentials and fractionated bipolar epicardial EG activity. No major delays in conduction of the primary beat were observed. Basic cycle length = 1,000 ms. Arrows show the propagation of the AP (action potential) dome. Each panel shows results from a different preparation. Abbreviations as in Figure 1.



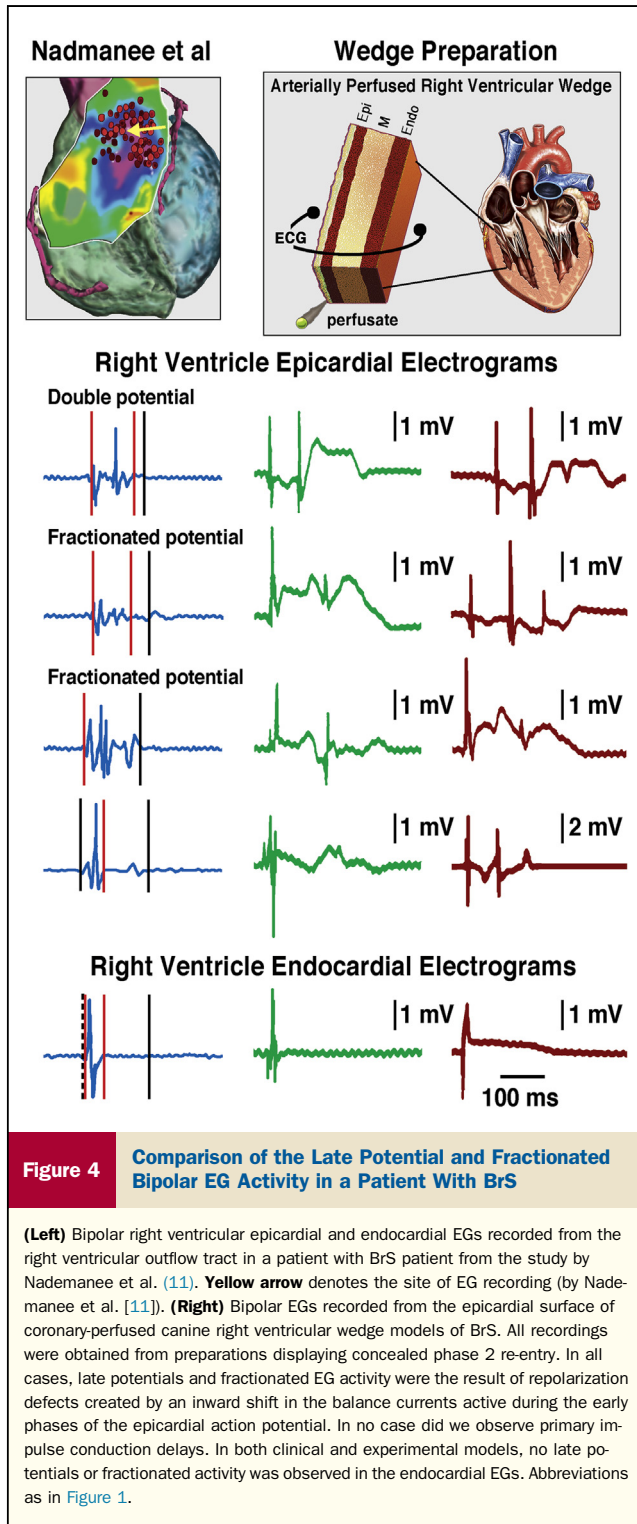
epicardial AP at some sites but not others, or in the endocardium, created a window vulnerable to the development of re-entrant arrhythmias. Propagation of the dome from regions at which it was maintained to regions at which it was lost caused local re-excitation via a phase 2 re-entrant mechanism, leading to the development of very closely coupled re-excitation of epicardium. More often than not, because of its early appearance, the phase 2 re-entrant beat was unable to propagate out of the epicardial focus in which it was generated, thus resulting in “concealed phase 2 re-entry,” which was not manifested as an extrasystole in the ECG. This local activity, however, accentuated the inverted T-wave and caused late potentials and fractionated activity on EGs recorded in epicardium but not endocardium, very similar to those observed in the clinical cases reported by Nademanee et al. (11) (Figs. 2 to 4). Concealed phase 2 re-entry associated with the appearance of late potential activity was observed in seven of seven wedge preparations. The mean \pm SEM coupling interval of concealed phase 2 re-entrant beat and the delay in the appearance of the associated high-frequency late potentials in the bipolar EG were 117.5 ± 8.2 ms (range: 81 to 138 ms) and 121.1 ± 8.9 ms, respectively (n = 7).

When the phase 2 re-entrant beat was able to exit the epicardial focus in which it was generated, it produced a closely coupled extrasystole capable of initiating VT/ventricular fibrillation (VF), as illustrated in Figure 3B. When the temperature of the coronary perfusate was reduced to

30°C, verapamil (3 μ M) alone was able to induce concealed phase 2 re-entry and delayed potentials (Fig. 5).

Figure 5 illustrates an example of 2:1 alternans of concealed phase 2 re-entry, giving rise to marked T-wave alternans and 2:1 alternation in the appearance of the epicardial EG late potential. In the presence of NS5806 (5 μ M) and verapamil (2 μ M), the introduction of a stimulated premature beat (S₂) restored homogeneity of the transmural APs and abolished the late potential and fractionated epicardial EG activity (Fig. 6).

Quinidine and cilostazol have been shown to suppress arrhythmogenesis associated with BrS. Quinidine and cilostazol have been reported to eliminate SAECG late potentials in patients with BrS (17,18). In another series of experiments, we tested the hypothesis that the ameliorative effects of these 2 agents are due to the effect of these agents to reverse the repolarization defect, thus suppressing concealed phase 2 re-entry and late potential activity. We tested the effects of quinidine and two phosphodiesterase (PDE)-3 inhibitors, cilostazol and milrinone. As illustrated in Figures 7 and 8, quinidine (10 μ M), cilostazol (10 μ M), and milrinone (2.5 μ M) all promptly restored the AP dome throughout epicardium, thus dramatically reducing epicardial and transmural dispersion of repolarization, normalizing the ECG, abolishing concealed phase 2 re-entry, and eliminating all late potential and fractionated epicardial EG activity.



Discussion

It is well-established that mutations leading to a decrease in inward currents (I_{Na} or I_{Ca}) or to an increase in outward currents (I_{to} and I_{K-ATP}) are capable of causing BrS in humans (5,19–22). We used NS5806 (I_{to} agonist) and

verapamil (I_{Ca} antagonist) to pharmacologically model the BrS genotypes involved in a loss of function of I_{Ca} (BrS types 3, 4, and 9) (23) and a gain of function of I_{to} (BrS types 5, 6, and 10) (5,6,24,25) so as to induce the Brugada phenotype in coronary-perfused canine RV wedge preparations. The NS5806-induced increase in I_{to} caused an outward shift in the balance of current in the early phase of the epicardial AP, leading to a moderate increase in notch in epicardium but little change in endocardium. The greater accentuation of the AP notch in epicardium than in endocardium gives rise to an increase in transmural voltage gradient, causing an increase in the magnitude of the J wave (Figs. 7 and 8). The addition of verapamil to the coronary perfusate caused a further outward shift in the balance of current, leading to further accentuation of these parameters. Longer exposure caused all-or-none repolarization at the end of the epicardial AP phase 1, leading to loss or delay of the AP dome at some epicardial sites. In some preparations, the increased repolarization forces caused variable delay in the appearance of the second epicardial AP upstroke, leading to the appearance of low-voltage fractionated activity in the bipolar EG recording, as previously demonstrated in clinical recordings reported by Nademanee et al. (11) (Fig. 1).

Loss of the dome and marked abbreviation of AP duration at some epicardial sites but not others caused a prominent increase in epicardial and transmural dispersion of repolarization, thus creating the substrate for the development of phase 2 re-entry and VT (Figs. 2 and 3). Conduction of the AP dome, from sites at which it was maintained to sites at which it was lost, caused local re-excitation via a phase 2 re-entry mechanism. In the majority of cases, phase 2 re-entry was concealed because the extra beat was unable to exit the focus in which it was generated. However, the concealed phase 2 re-entrant beat was able to produce high-frequency late potentials and fractionated bipolar EGs in epicardium but not in endocardium (Figs. 2 to 4), comparable to clinical observations reported by Nademanee et al. (11) and Sacher et al. (12).

The hypothesis proposed by Nademanee et al. (11) and Sacher et al. (12) that late potentials and fractionated activity recorded in the RVOT of patients with BrS are due to depolarization defects is not supported by our results, which provide an alternative explanation for the appearance of such activity, based on the development of primary repolarization defects. Our observations suggest that without the benefit of AP recordings, it is difficult to discern between repolarization and depolarization defects. It is noteworthy that recordings of monophasic APs in the epicardium and endocardium of the RVOT in patients with BrS have demonstrated AP activity very similar to that recorded from the canine coronary wedge preparations, with no major transmural conduction delay (26,27).

One way to distinguish between the two mechanisms in the clinic might be to examine the effect of rate or atrial

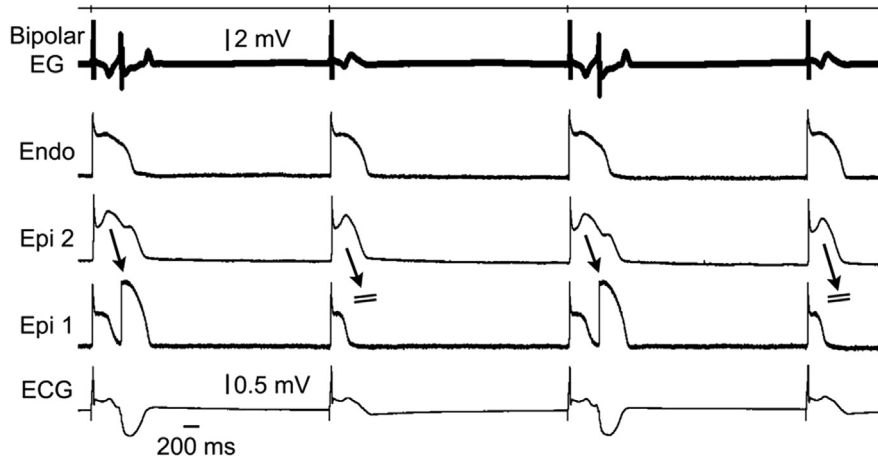


Figure 5 Alternans of “Concealed” Phase 2 Re-Entry Gives Rise to Alternans of Late Potential and T Wave

Traces as in Figure 2, all simultaneously recorded from a coronary-perfused right ventricular wedge preparation exposed to verapamil (3 μ M) and hypothermia (30°C) to induce the Brugada phenotype. **Arrows** indicate successful conduction of the Epi2 AP dome in alternate beats to generate concealed phase 2 re-entry. Abbreviations as in Figure 1.

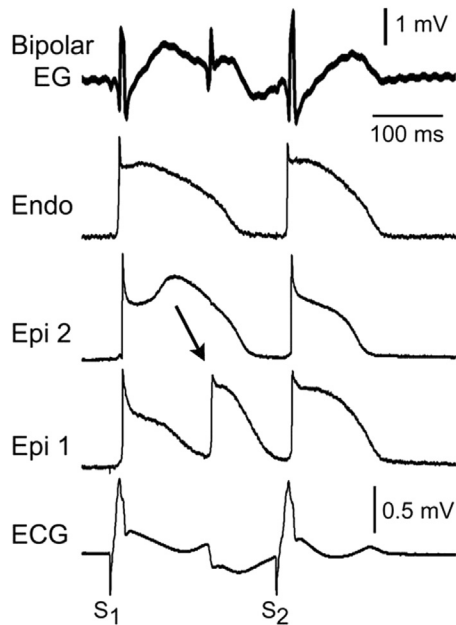


Figure 6 Effects of Stimulated Premature Beat on Action Potentials and Fractionated Bipolar EG in an Experimental Model of BrS

Stimulated premature beat (S_2) restored homogeneity of action potentials and abolished fractionated bipolar EG in this experimental model of BrS. Traces as in Figure 2, all simultaneously recorded from a coronary-perfused right ventricular wedge preparation exposed to NS5806 (5 μ M) and verapamil (2 μ M) to induce the Brugada phenotype. Basic cycle length = 1,000 ms. S_1 - S_2 = 240 ms. **Arrow** shows the propagation of the AP (action potential) dome. Abbreviations as in Figure 1.

premature responses. When due to delayed conduction, the notched appearance should become accentuated with acceleration of rate or prematurity, because delayed conduction

almost always becomes more accentuated at faster rates or with prematurity. When due to repolarization problems, the amplitude of the J wave should diminish due to insufficient time for I_{to} to reactivate, as in the example illustrated in Figure 6. A word of caution: In the presence of drugs with use-dependent actions to inhibit sodium- or calcium-channel current, an opposite response may be observed after an increase in rate or even after a single premature beat. Indeed, in the presence of verapamil (Fig. 6), the use-dependent inhibition of I_{Ca} leads to accentuation of the repolarization defects. The reduced repolarization defect with a single premature beat is due to the fact that the reduction of I_{to} was greater than the reduction of I_{Ca} after a single premature beat.

Another way to discern between depolarization and repolarization defects as a cause of the BrS phenotype is by observing the response to quinidine. The I_{Na} inhibitory effect of quinidine is expected to accentuate late potential and fractionated EG activity if due to a depolarization defect. If, on the other hand, the BrS phenotype is due to a repolarization defect, quinidine via its action to inhibit I_{to} would be expected to reduce or abolish late potential and fractionated EG activity, which is what we observed in our preparations (Fig. 7).

Quinidine and cilostazol have been reported to eliminate SAECG late potentials in patients with Brugada syndrome (17,18) and to exert antiarrhythmic effects in this setting (7,28-32). Our results provide support for the hypothesis that the ameliorative effects of these agents are due to an effect of these two agents to reverse the repolarization defect, thus suppressing concealed phase 2 re-entry and late potential activity (Figs. 7 and 8A). In addition, we demonstrate that milrinone, another PDE-3 inhibitor, is a potent agent capable of exerting ameliorative actions in the

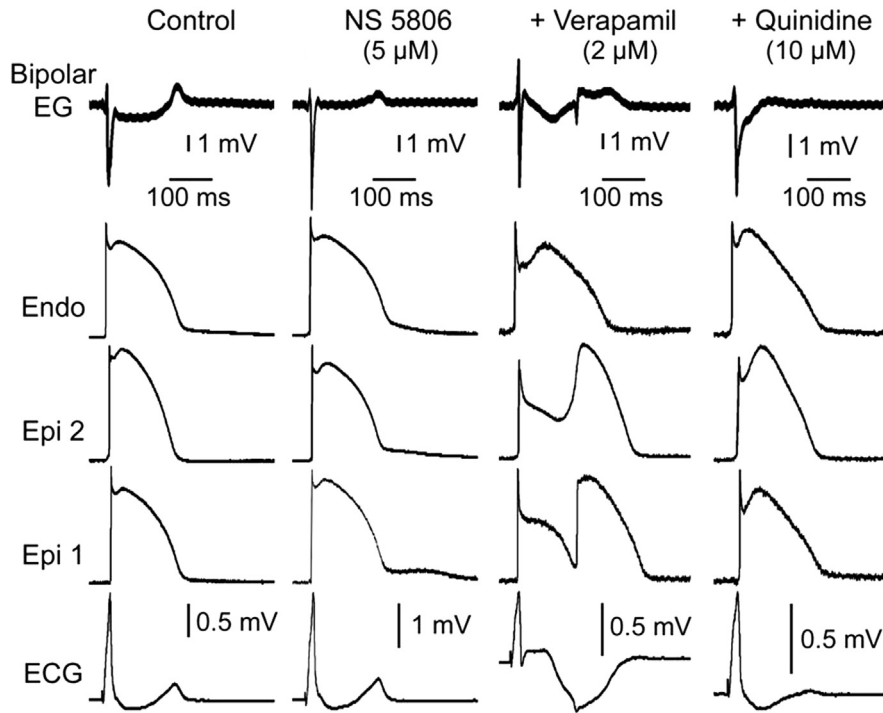


Figure 7

Effects of Quinidine on the Repolarization Defects Involved in Phase 2 Re-Entry and Associated Late Potentials and Fractionated Epi EG Activity in an Experimental Model of BrS

The addition of quinidine (10 μM) to the coronary perfusate reversed the repolarization defects, restored action potential homogeneity, normalized the ECG, and abolished phase 2 re-entry and associated late potentials on the bipolar EG. Traces as in Figure 2, all simultaneously recorded from a coronary-perfused right ventricular wedge preparation exposed to NS5806 (5 μM) and verapamil (2 μM) to induce the Brugada phenotype. Abbreviations as in Figure 1.

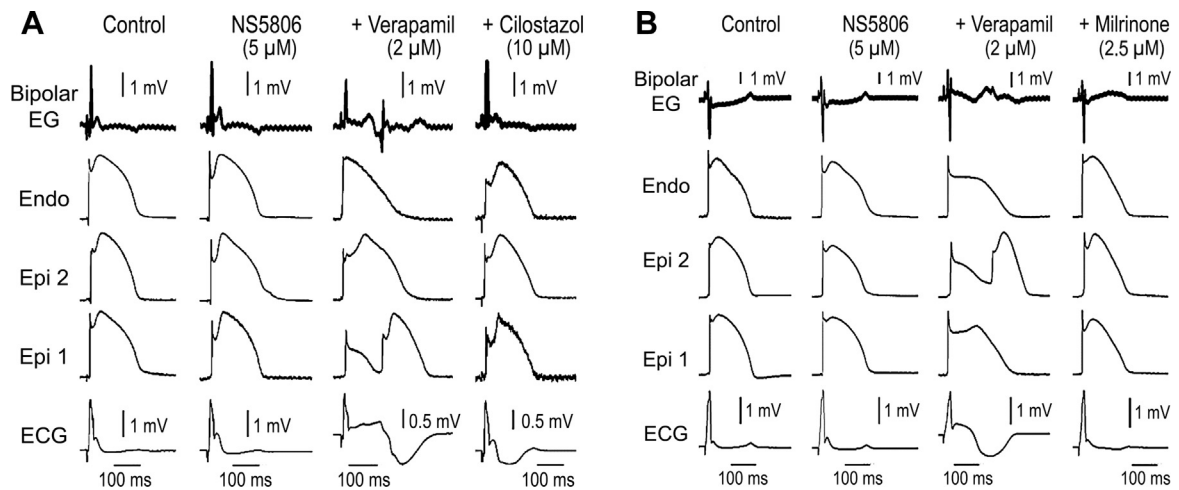


Figure 8

Effects of Cilostazol and Milrinone on the Repolarization Defects Involved in Phase 2 Re-Entry and Associated Late Potentials and Fractionated Bipolar Epi EG Activity in an Experimental Model of BrS

The addition of cilostazol (10 μM) (A) or milrinone (2.5 μM) (B) to the coronary perfusate reversed the repolarization defects, thus restoring the epicardial action potential dome throughout, normalizing the ECG and abolishing phase 2 re-entrant activity and associated late potential and fractionated bipolar Epi EG. Traces as in Figure 2, all simultaneously recorded from a coronary-perfused right ventricular wedge preparation exposed to NS5806 (5 μM) and verapamil (2 μM) to induce the Brugada phenotype. Abbreviations as in Figure 1.

setting of BrS syndrome (Fig. 8B). The ameliorative action of cilostazol and milrinone is likely attributable to the effect of these PDE inhibitors to increase cyclic adenosine monophosphate and thus boost I_{Ca} , leading to a reversal of the repolarization defect and permitting the development of the ECG manifestations of BrS. Quinidine, and cilostazol at higher concentrations (33), exert a direct effect to block I_{to} .

Quinidine is used for primary and secondary prevention and as adjunct therapy for BrS. The ameliorative effect of the PDE inhibitors (especially cilostazol) and quinidine to suppress the BrS phenotype is consistent with clinical findings (28,30,31,34-37).

Finally, if the late potentials recorded in the anterior surface of the RVOT do not reflect delayed conduction, then why does ablation of these sites produce an ameliorative response, as reported by Nademanee et al. (11)? Our hypothesis is that eliminating the cells displaying a high density of I_{to} , known to reside in the RVOT, prevents the development of the repolarization defects involved in the Brugada ECG, phase 2 re-entry, and VT/VF. A lower level of I_{to} is the basis for why female carriers are protected from developing the ECG and arrhythmic manifestations of BrS (38) and is the basis for the actions of quinidine and cilostazol. We are endeavoring to test this hypothesis by ablating the regions of concealed phase 2 re-entry and late potentials in coronary-perfused RV wedge models of BrS. Our preliminary results provide strong support for the hypothesis, showing that ablation of the sites of abnormal repolarization normalizes the ECG and prevents the development of phase 2 re-entry and VT/VF (B. Patocskaï and C. Antzelevitch, unpublished data, January 2014).

Study limitations. As with all data derived from experimental models, extrapolation of the data from in vitro models to clinical practice must be done with due caution.

Conclusions

The present study explored the alternate hypothesis that the late potentials observed on SAECG and fractionated bipolar epicardial EGs are not due abnormal conduction or structural abnormalities in the RV epicardium, but may be associated with the development of abnormal repolarization giving rise to concealed phase 2 re-entry in epicardium. Our results might prove helpful in better understanding the cellular mechanisms underlying late potential and fractionated EG activity in a variety of pathophysiological conditions.

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Reprint requests and correspondence: Dr. Charles Antzelevitch, Masonic Medical Research Laboratory, 2150 Bleecker Street, Utica, New York 13501. E-mail: ca@mmrl.edu.

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