Published in final edited form as:
J Mol Cell Cardiol. 2014 March ; 68: 20–28. doi:10.1016/j.yjmcc.2013.12.012.

Mechanisms Underlying the Development of the Electrocardiographic and Arrhythmic Manifestations of Early Repolarization Syndrome

István Koncz[a,b], **Zsolt Gurabi**[a,b], **Bence Patocskai**[a,b], **Brian K. Panama**[a], **Tamás Szél**[a,b], **Dan Hu**[a], **Hector Barajas-Martínez**[a], and **Charles Antzelevitch**[a,*]

[a]Departments of Molecular Genetics and Experimental Cardiology, Masonic Medical Research Laboratory, 2150 Bleecker St., Utica, NY, 13501, USA

[b]Department of Pharmacology & Pharmacotherapy, University of Szeged, H-6721 Szeged, Dóm tér 12, Hungary

Abstract

Background—Early repolarization pattern in the ECG has been associated with increased risk for ventricular tachycardia/fibrillation (VT/VF), particularly when manifest in inferior leads. This study examines the mechanisms underlying VT/VF in the early repolarization syndrome (ERS).

Method—Transmembrane action potentials (AP) were simultaneously recorded from 2 epicardial and 1 endocardial site of coronary-perfused canine left-ventricular (LV) wedge preparations, together with a pseudo-ECG. Transient outward current (I_{to}) was recorded from epicardial myocytes isolated from inferior and lateral LV of the same heart.

Results—J wave area (pseudo-ECG), epicardial AP notch magnitude and index were larger in inferior vs. lateral wall preparations at baseline and after exposure to provocative agents (NS5806+verapamil+acetylcholine (ACh)). I_{to} density was greater in myocytes from inferior vs. lateral wall (18.4±2.3pA/pF vs. 11.6±2.0pA/pF;$p<0.05$). A combination of NS5806 (7μM) and verapamil (3μM) or pinacidil (4μM), used to pharmacologically model the genetic defects responsible for ERS, resulted in prominent J-point and ST-segment elevation. ACh (3μM), simulating increased vagal tone, precipitated phase-2-reentry-induced polymorphic VT/VF. Using identical protocols, inducibility of arrhythmias was 3-fold higher in inferior vs. lateral wedges. Quinidine (10μM) or isoproterenol (1μM) restored homogeneity and suppressed VT/VF.

Conclusion—Our data support the hypothesis that 1) ERS is caused by a preferential accentuation of the AP notch in LV epicardium; 2) this repolarization defect is accentuated by elevated vagal tone; 3) higher intrinsic levels of I_{to} account for the greater sensitivity of the inferior LV wall to development of VT/VF; 4) quinidine and isoproterenol exert ameliorative effects by reversing the repolarization abnormality.

[*]**Corresponding author:** Charles Antzelevitch, PhD, FACC, FAHA, FHRS Masonic Medical Research Laboratory 2150 Bleecker Street, Utica, NY 13501-1787 Tel: +1-315-735-2217 Fax: +1-315-735-5648 ca@mmrl.edu.

Disclosures: None.

Keywords

J wave syndrome; ventricular fibrillation; transient outward potassium current; parasympathetic tone; electrophysiology

1. Introduction

Early repolarization (ER)[1] pattern in the ECG is characterized by a J point and ST segment elevation, sometimes manifest as a notch or slur on the QRS (J wave). The ER pattern, long thought to be benign, was recently proposed to have a malignant component on the basis of the association of ER with development of ventricular tachycardia and fibrillation (VT/VF) in an experimental model consisting of canine ventricular wedge preparations, thus identifying the ER syndrome (ERS) [1,2]. Validation of this hypothesis came several years later with the demonstration that patients with ER, particularly in the inferior or infero-lateral ECG leads are at a higher risk for VT/VF [3–5]. The co-occurrence of a J point elevation in the right precordial leads (V1–V3) has been linked to a more severe phenotype often associated with the development of electrical storms [4,6].

Recent studies have demonstrated that gain of function mutations in *KCNJ8*, the gene responsible for the pore forming subunit of the ATP-sensitive potassium channel (K_{ATP}), is associated with ERS [7–9]. Loss of function mutations in the α1, β2 and α2δ subunits of the cardiac L-type calcium channel (*CACNA1C, CACNB2, and CACNA2D1*) have also been identified as causative in patients with ERS [10]. Watanabe et al. described [11] loss of function mutations in *SCN5A* in patients with idiopathic ventricular fibrillation associated with early repolarization. Sodium channel blocker challenge resulted in an accentuation of early repolarization and development of VT/VF.

Vagal activity has long been implicated in the development of an ER pattern in the ECG [12,13] and recent clinical observations suggest that parasympathetic tone contributes to the electrocardiographic and arrhythmic manifestations of ERS [14,15]. Sleep is commonly associated with spontaneous VF in patients with ERS [16]. Indeed heart rate spectral analysis has identified a sudden rise in vagal activity just before the development of VF in patients diagnosed with cases of idiopathic VF (IVF) [17].

A number of studies have shown that an ER pattern in the inferior ECG leads (II, III and aVF) is associated with a much higher risk for the development of VT/VF [18,19]. The electrophysiological basis for this distinction is not known and presents a critical gap in our knowledge.

The present study was designed to probe the mechanisms underlying the development of the electrocardiographic and arrhythmic manifestations of ERS, to elucidate the role of vagal influences, and to better understand the basis for the greater susceptibility to VT/VF when the ER substrate resides in the inferior region of the ventricular myocardium. The study was specifically designed to test the hypothesis that an outward shift in the balance of current contributing to the early phase of the left ventricular (LV) epicardial action potential (AP) either via an increase in $I_{K\text{-}ATP}$ or transient outward current (I_{to}) or via a decrease in calcium inward current (I_{Ca}) in LV wedge preparations can recapitulate the ECG and arrhythmic manifestations of ERS. Thus, we sought to test the hypothesis that pharmacologic modeling of the genetic defects associated with ERS, using pinacidil and NS5806 to increase $I_{K\text{-}ATP}$ and I_{to} and verapamil and acetylcholine (ACh) to reduce I_{Ca} or pilsicainide to reduce $I_{Na,}$ could give rise to a substrate capable of inducing phase 2 reentry and polymorphic VT/VF. Finally, we test the hypothesis that increased levels of I_{to} sensitize the ventricular

epicardium to development of ER and ERS and those higher levels of I_{to} in the inferior wall can explain why an ER pattern in the inferior ECG leads is associated with a higher risk for sudden cardiac arrest.

2. Methods

All experiments were carried out in compliance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No 85-23, Revised 1996) and approved by the Institutional Animal Care and Use Committee. Adult dogs (20–35 kg) of either sex were used in this study.

2.1. Wedge Preparations

Detailed methods for isolation, perfusion, and recording of action potentials from coronary-perfused canine ventricular wedge preparations have been reported previously [20,21] and are described in the Online Supplement. All arrhythmias developed spontaneously following exposure to the provocative agents during steady state pacing at cycle length of 1000 to 2000 msec.

2.2. Voltage-clamp studies using enzymatically-dissociated canine cardiomyocytes

Cardiomyocytes were isolated as previously described [22]. I_{to} was measured in isolated left ventricular lateral and inferior epicardial myocytes at 37°C using the whole-cell patch clamp technique [23]. Details of the protocol are presented in the Online Supplement.

2.3. Statistics

Results are expressed as mean ± S.E.M. Statistical comparisons were made using Student's *t*-test for Table 1 and Figure 7 B. Kruskal-Wallis one-way analysis of variance followed by Student-Newman-Keuls post-hoc test was used for Figure 7 C and D.

3. Results

3.1. Early Repolarization ECG pattern is caused by a preferential accentuation of the AP notch in LV epicardium

In the first series of experiments, we sought to examine the effects of I_{to} augmentation on AP and ECG characteristics of coronary-perfused wedge preparations isolated from the inferior and lateral regions of canine LV. Figure 1 shows two examples of the effect of the I_{to} agonist NS5806 (7 μM) to accentuate the epicardial AP notch and to induce J-point elevation, thus generating a prominent ER pattern. Qualitatively similar accentuation of the epicardial AP notch and induction of J-point elevation were obtained in 8 experiments. Because I_{to} is relatively slow to recovery from inactivation, availability of the current is reduced in premature beats, resulting in a diminished phase 1 AP notch. Figure 1B illustrates an example of a reduced epicardial AP notch and J wave associated with a premature beat in another inferior LV wedge preparation. Figure 1C shows a clinical example of this phenomenon recorded from a 16 y/o female with type 3 ERS. A pronounced ER pattern was apparent in the left precordial ECG leads. At the time of the recording the patient was in atrial fibrillation, which was associated with large fluctuations in RR interval. The beat following the abbreviated RR interval exhibited a much reduced J wave and ER pattern, closely mirroring the activity observed in the LV wedge.

3.2. Pharmacologic modeling of genetic mutations associated with early repolarization syndrome

Pharmacologic modeling of the genetic defects known to underlie ERS was evaluated in another series of experiments. A gain of function in $I_{K\text{-}ATP}$ was modeled using the $I_{K\text{-}ATP}$ opener pinacidil. Figure 2 illustrates the electrocardiographic and arrhythmic manifestations resulting from addition of 4 μM pinacidil together with 7 μM NS5806. The combination produced an outward shift in the balance of current in the early phases of the epicardial AP, leading to marked accentuation of epicardial AP notch, giving rise to a prominent ER, heterogeneous loss of the AP dome, and the development of phase 2 reentry-induced VT/VF. Similar changes in transmembrane and ECG activity was recorded in 3 of 6 wedge preparations isolated from the LV wall of the canine heart.

Loss of function of I_{Ca} mutations were pharmacologically modeled using the calcium channel blocker verapamil and I_{Na} mutations were modeled using the sodium channel blockers pilsicainide (5 μM, n=2) or procainamide (50 μM-200 μM, n=3). Figures 3–5 illustrate examples of the effect of a combination of verapamil, pilsicainide and NS5806 to produce a net outward shift in the balance of current in the early phases of the epicardial AP, causing marked accentuation of epicardial AP notch, thus giving rise to a prominent ER, heterogeneous loss of the AP dome, and the development of phase 2 reentry-induced VT/VF. Similar heterogeneous loss of the AP dome leading to development of phase 2 reentry-induced VT/VF was recorded in 5 of 10 wedge preparations isolated from the LV wall of the canine heart. Figure 5B illustrates an example in which the addition of pilsicainide induced VT/VF when a combination of NS5806 + verapamil + ACh failed to do to so.

3.3. Mechanism underlying arrhythmogenic influence of parasympathetic tone in ERS

Vagal influences are known to accentuate the ECG and arrhythmic manifestations in patients with ERS. In a third series of experiments, we examined the basis for these effects by exposing wedge preparations sensitized with a combination of 7 μM NS5806 and 3 μM verapamil to ACh. Figure 4 shows an example in which the combination of NS5806 and verapamil caused marked accentuation of the epicardial AP notch, thus inducing J-point and ST segment elevation, but no arrhythmia developed. Addition of ACh (3 μM), to mimic increased vagal tone, accentuated epicardial AP notch magnitude, increased notch index, facilitated loss of the epicardial AP dome (Table 1), enhanced ST segment elevation and precipitated repeated episodes of phase 2 reentry and polymorphic VT/VF. Similar exacerbation of arrhythmic activity by ACh was observed in 5 out of 11 wedge preparations isolated from LV wall of the canine heart that failed to exhibit arrhythmic activity when exposed to NS5806 (7 μM) and verapamil (3 μM) alone.

3.4. Quinidine and isoproterenol suppress arrhythmogenesis in experimental models of ERS

Quinidine and isoproterenol have been shown to exert ameliorative effects in the J wave syndromes. In another series of experiments, we probed the cellular basis for these effects in our ERS model. We induced the ERS phenotype using a combination of NS5806 (7–15 μM) + verapamil (3 μM) + acetylcholine (3–10 μM). Quinidine (10 μM) and isoproterenol (1 μM) each reversed the repolarization abnormality responsible for ERS by restoring the epicardial AP dome, thus reducing ST segment elevation and suppressing VT/VF (Figures 5 and 6). Quinidine restored electrical homogeneity by restoring the epicardial action potential dome, and thus prevented or suppressed VT/VF in 4 of 4 preparations and isoproterenol in 5 of 6 preparations.

3.5 Cellular and ionic basis for the greater susceptibility of the inferior LV wall to development of VT/VF

A number of clinical studies report that ER in the inferior ECG leads is associated with a higher level of risk of arrhythmic events and sudden cardiac death. In a final series of experiments we probed the cellular and ionic basis for the higher susceptibility of the inferior wall to arrhythmias in the setting of ER. In inferior and lateral wedge preparations isolated from the same dogs, epicardial AP notch magnitude recorded from the inferior wall was greater than that recorded from the lateral wall. At baseline Phase 1 magnitude in inferior and lateral wall preparations was 14.1±1.7 % vs. 8.4±1.2 % of Phase 0 amplitude, $p<0.05$; after application of provocative agents (7 μM NS5806 + 3 μM verapamil + 3 μM ACh) it was 34.4±6.0 % vs. 23.1±4.3 % of Phase 0 amplitude ($p<0.05$, n=6). Notch index at baseline was 270.3±48.3 vs. 149.7±26.3, $p<0.05$, n=5 in inferior and lateral preparations and after application of provocative agents increased to 3015.5±1178.1 vs. 969.4±246.2, respectively (Figure 7C, n=5; p=0.31).

J wave area recorded in wedge preparations from the inferior wall was slightly although not significantly greater than that recorded from the lateral wall (Figure 7D, n=4) under baseline conditions (6.5±2.0 mV × ms vs. 4.4±1.1 mV × ms) and significantly greater after application of provocative agents (7 μM NS5806 + 3 μM verapamil + 3 μM ACh) (60.3±25.6 mV × ms vs. 18.4±0.5 mV × ms; $p<0.05$).

To test the hypothesis that differences in I_{to} contribute to these distinctions in action potential characteristics and pharmacologic responsiveness, we measured I_{to} in epicardial myocytes isolated from the inferior vs. lateral LV wall. Ito density was significantly larger in cells from the inferior vs. lateral wall (18.4 ± 2.3 vs. 11.6 ± 2.0 pA/pF at +40 mV, respectively; $p<0.05$, Figure 7B).

Finally, using identical protocols (7 μM NS5806 + 3 μM verapamil + 3 μM ACh) we found that incidence of phase 2 reentry and VT/VF was 3-fold higher in inferior vs. lateral wedges isolated from the same dogs (Figure 7E).

4. Discussion

4.1. Summary of main findings

The present study advances our understanding of the cellular and ionic basis for the electrocardiographic and arrhythmic manifestations of the early repolarization syndrome, providing data in support of the hypothesis that ERS is caused by a preferential accentuation of the AP notch in LV epicardium; that this repolarization defect is accentuated by cholinergic agonists and that higher intrinsic levels of I_{to} account for the greater vulnerability of the inferior LV wall to VT/VF. Finally, we provide a mechanistic understanding of the ameliorative effects of quinidine and isoproterenol, showing their ability to reverse the repolarization abnormalities responsible for ERS.

4.2. Accentuation of the J wave

Under physiological conditions, the presence of an AP notch in epicardium but not endocardium creates a transmural voltage gradient that can cause the inscription of the J wave of the ECG [24]. Accentuation of this transmural gradient across the right ventricular (RV) wall has been shown to lead to repolarization defects that underlie the ECG and arrhythmic manifestations of Brugada syndrome (BrS) [1]. The present study provides evidence in support of the hypothesis that a similar accentuation of transmural gradients across the LV wall underlies the repolarization abnormalities responsible for ERS, giving rise to J point elevation, distinct J waves, or slurring of the terminal part of the QRS.

4.3. Induction of Phase 2 Reentry

ERS has been associated with gain of function mutations in *KCNJ8* giving rise to an increase in $I_{K\text{-}ATP}$ [7–9] and loss of function mutations in the α1, β2 and α2δ subunits of the cardiac L-type calcium channel (*CACNA1C, CACNB2*, and *CACNA2D1*) leading to a reduction in I_{Ca} [10,25] as well as loss of function mutations in *SCN5A* leading to a decrease in I_{Na}. [11] We pharmacologically modeled these genetic defects using the $I_{K\text{-}ATP}$ agonist pinacidil, the I_{Ca} antagonist verapamil and the I_{Na} antagonists pilsicainide or procainamide. In the setting of a prominent I_{to}, induced using NS5806, both pinacidil and verapamil were found to cause all-or-none repolarization, leading to loss of the AP dome at some epicardial sites but not others, resulting in an epicardial dispersion of repolarization (EDR). Propagation 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 reentry mechanism within the epicardium. Loss of the dome in the epicardium also created a transmural dispersion of repolarization (TDR) giving rise to a vulnerable window across the ventricular wall which, when captured by a closely coupled extrasystole generated in the epicardium, induced VT/VF (Figures 2 and 3). When a combination of NS+verapamil+ACh failed to induce VT/VF, the addition of the sodium channel blocker pilsicainide (Figure 5, panel B) or procainamide caused accentuation of the ER phenotype leading to the development of VT/VF. Consistent with the observation of Watanabe et al, [11] we found that the sodium channel block is capable of accentuating the ER phenotype leading to the induction of VT/VF.

4.4. Cholinergic influence accentuates the repolarization defect

Vagal influences are thought to play a prominent role in the development of life threatening arrhythmias in patients with the early repolarization syndrome [26]. The parasympathetic nervous system has been shown to modulate the manifestation of the J wave and ST segment elevation in patients with ERS [13,14]. Acetylcholine has been shown to exert direct effects on the electrical response of canine ventricular myocardium, preferentially in epicardium [27]. ACh was shown to depress the AP plateau in canine right ventricular epicardium but not endocardium, resulting in an ST-segment elevation and to accentuate the AP notch, thus facilitating loss of the RV action potential dome in the presence of pinacidil or flecainide [1].

In the present study, we mimic vagal activity using ACh and demonstrate its ability to facilitate heterogeneous loss of the dome in LV epicardium in the presence of NS5806 and verapamil, leading to an ST segment elevation and development of phase 2 reentry in the free wall of the LV (Figures 4–7 and Table 1).

Vagal innervation has recently been shown to be quite extensive in the ventricles [28]. Takahashi et al. demonstrated in dogs that vagal efferent fibers cross to the ventricles in the superficial epicardium at the atrioventricular groove in a base to apex direction and, within 1–2 cm of the atrioventricular groove, penetrate the myocardium en route to transmurally innervating the rest of the LV free wall and part of the inter-ventricular septum [29]. Efferent vagal stimulation has also been reported to exert potent negative inotropic effects in dog ventricular myocardium [30] and to exert direct inhibition of I_{Ca} [31]. I_{Ca} inhibition is likely also a consequence of “accentuated antagonism” wherein vagal activity antagonizes the effects of sympathetic stimulation to increase I_{Ca} [32]. Interestingly, cardiomyocytes have been shown to actively secrete ACh and thus amplify vagal influences [33,34]. Adrenergic stimulation was also shown to up-regulate expression levels of cholinergic components [34]. Thus, cholinergic influences on the electrical activity of ventricular myocardium are robust.

4.5. Higher intrinsic levels of I_{to} account for the greater sensitivity of the inferior LV wall to development of VT/VF

Numerous studies have reported a higher risk for the development of arrhythmias and sudden cardiac arrest in patients with an ER pattern in the inferior or infero-lateral leads of the ECG [16,18,35–41]. We investigated the cellular and ionic basis for the greater vulnerability of the inferior region of the LV for development of VT/VF in the setting of ERS. Our findings support the hypothesis that cells in the inferior region of ventricular epicardium possess a higher level of I_{to} than cells in the lateral LV and that this predisposes the inferior region to develop phase 2 reentry and VT/VF when provocative agents are introduced to pharmacologically model the genetic defects known to underlie ERS. Our results are in agreement with those reported by Szentandrassy and co-workers [42] showing AP with a more prominent phase1 in cells isolated from the apical vs. basal regions of the canine and human LV. From the description provided, the apical preparations studied overlapped with what we consider to be the inferior wall of the LV and their basal samples overlap with what we consider to be the lateral wall. Consistent with these findings, I_{to} density as well as expression of genes that encode I_{to}, including KChIP2 and $K_v1.4$, were significantly more abundant in apical vs. basal myocytes. Kv4.3 expression was also higher in apical tissues, although it did not reach statistical significance.

Of note is the fact that I_{to} density at +40 mV in the inferior wall of the LV (18.4±2.3pA/pF) was intermediate between that found in the lateral wall of the LV (11.6±2.0pA/pF;$p<0.05$) and the RV (24.2 ± 0.5pA/pF), the latter known to predispose to the development of the electrocardiographic and arrhythmic manifestations of BrS [43].

It is noteworthy, that apico-basal differences in adrenergic innervation have been reported, with a 10-fold higher concentration of norepinephrine in tissues in the base vs. apex of the LV [44]. It is tempting to speculate that reduced sympathetic activity in the apical regions of the LV contributes to the greater vulnerability of the inferior wall to arrhythmogenesis. This would also be consistent with the well-known ameliorative effect of β adrenergic activity in the setting of ERS [45,46]. Interestingly, Roten et al. [46] reported that in response to β adrenergic stimulation with isoproterenol, J waves may persist in a subset of patients with precordial and inferior J waves, but never in lateral location. This heterogeneous response to isoproterenol may indicate distinctive autonomic and/or cellular and ionic mechanisms in the anterior, lateral and inferior regions of ventricular myocardium.

4.6. Pharmacologic approach to therapy of ERS

Our study demonstrates the ability of quinidine and isoproterenol to reverse the repolarization abnormalities responsible for ERS, thus exerting an ameliorative effect. Both agents act to restore the epicardial AP dome by causing an inward shift in the balance of current; quinidine via its inhibition of I_{to} and isoproterenol by its augmentation of I_{Ca}. These actions restore homogeneity across the left ventricular wall, thus suppressing all arrhythmic activity and eliminating the substrate for the development of phase 2 reentry and VT/VF.

These effects of quinidine and isoproterenol in the LV wedge preparation provide a mechanistic understanding of their beneficial effects in the clinic. A recent multicenter study examined the effectiveness of quinidine and isoproterenol in normalizing the ECG pattern and in suppressing arrhythmia recurrence in patients with ERS [45]. Isoproterenol infusion was reported to immediately suppress electrical storms in 7 of 7 patients. Quinidine was effective in 9 of 9 patients, decreasing recurrent VF from 33±35 episodes to 0 for 25±18 months. Moreover, quinidine normalized the ECG in all cases.

Our experimental findings provide strong rationale for developing agents possessing I_{to} blocking, anticholinergic, as well as $I_{K\text{-}ATP}$ antagonistic properties as possible alternatives to

oral quinidine for chronic treatment of symptomatic ERS in cases in which the drug is not tolerated or produces unacceptable adverse effect.

4.7. Study limitations

As with all data derived from experimental animal models, extrapolation from *in vitro* models to the clinic must be done with great care. It is noteworthy that the RV wedge preparation was the first to identify quinidine and isoproterenol for the treatment of BrS in 1999 [1]. Both agents are used today for primary and secondary prevention and as adjunct therapy for BrS. Thus, the ventricular wedge preparation has been highly predictive of the clinical efficacy of all pharmacologic agents thus far tested. ERS thus far has been associated with loss of function mutations in I_{Ca} and I_{Na} and gain of function mutations in I_{to} and $I_{K\text{-}ATP}$. Our study demonstrates that a loss of function in I_{Ca} or I_{Na} and gain of function in $I_{K\text{-}ATP}$ can contribute to manifestation of the ER phenotype in the setting of an enhanced I_{to}. Additional studies are also clearly needed to probe the mechanism underlying the regional differences in I_{to}, which are responsible for the arrhythmic vulnerability of the inferior wall in the LV as well for disparate response of closely apposed regions to the provocative agents, making phase 2 reentry possible. It is noteworthy that the concentration of ACh needed to precipitate arrhythmic activity *in vivo* is expected to be much lower than that needed in our models because the bradycardia-effect of parasympathetic influence on sinus rate is lacking.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by a postdoctoral fellowship grant from the Heart Rhythm Society to IK (supported by an educational grant from St. Jude Medical), grant HL47678 from NHLBI (CA), NYSTEM grant #C026424 (CA) and the Masons of New York, Florida, Massachusetts, Connecticut, Maryland and Rhode Island.

We are grateful to José M. Di Diego, M.D. for continuous support and personal guidance and to Serge Sicouri, M.D. and Vladislav V. Nesterenko, Ph.D. for helpful discussions and support and to Timothy K. Knilans M.D. for providing us with the ECG recordings. We also gratefully acknowledge the technical assistance of Judy Hefferon, Rebecca Warren and Robert Goodrow Jr.

Abbreviations

ACh	acetylcholine
AP	action potential
BrS	Brugada syndrome
EDR	epicardial dispersion of repolarization
ER	early repolarization
ERS	early repolarization syndrome
I_{Ca}	calcium inward current
I_{to}	transient outward current
IVF	idiopathic ventricular fibrillation
K_{ATP}	ATP-sensitive potassium channel
LV	left ventricle

RV	right ventricle
TDR	transmural dispersion of repolarization
VT/VF	ventricular tachycardia/ventricular fibrillation.

References

[1]. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST segment elevation. Circulation. 1999; 100:1660–6. [PubMed: 10517739]

[2]. Gussak I, Antzelevitch C. Early repolarization syndrome: clinical characteristics and possible cellular and ionic mechanisms. J Electrocardiol. 2000; 33:299–309. [PubMed: 11099355]

[3]. Haissaguerre M, Derval N, Sacher F, Jesel L, Deisenhofer I, De Roy L, et al. Sudden cardiac arrest associated with early repolarization. N Engl J Med. 2008; 358:2016–23. [PubMed: 18463377]

[4]. Nam GB, Kim YH, Antzelevitch C. Augmentation of J waves and electrical storms in patients with early repolarization. N Engl J Med. 2008; 358:2078–9. [PubMed: 18463391]

[5]. Rosso R, Kogan E, Belhassen B, Rozovski U, Scheinman MM, Zeltser D, et al. J-point elevation in survivors of primary ventricular fibrillation and matched control subjects: incidence and clinical significance. J Am Coll Cardiol. 2008; 52:1231–8. [PubMed: 18926326]

[6]. Nam GB, Ko KH, Kim J, Park KM, Rhee KS, Choi KJ, et al. Mode of onset of ventricular fibrillation in patients with early repolarization pattern vs. Brugada syndrome. Eur Heart J. 2010; 31:330–9.

[7]. Haissaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Loussouarn G, et al. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/K_{ATP} channel. J Cardiovasc Electrophysiol. 2009; 20:93–8. [PubMed: 19120683]

[8]. Medeiros-Domingo A, Tan BH, Crotti L, Tester DJ, Eckhardt L, Cuoretti A, et al. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac K(ATP) channel Kir6.1 as a pathogenic substrate for J-wave syndromes. Heart Rhythm. 2010; 7:1466–71. [PubMed: 20558321]

[9]. Barajas-Martinez H, Hu D, Ferrer T, Onetti CG, Wu Y, Burashnikov E, et al. Molecular genetic and functional association of Bugada and early repolarization syndromes with S422L missense mutation in KCNJ8. Heart Rhythm. 2012; 9:548–55. [PubMed: 22056721]

[10]. Burashnikov E, Pfeiffer R, Barajas-Martinez H, Delpon E, Hu D, Desai M, et al. Mutations in the cardiac L-type calcium channel associated J wave sydnrome and sudden cardiac death. Heart Rhythm. 2010; 7:1872–82. [PubMed: 20817017]

[11]. Watanabe H, Nogami A, Ohkubo K, Kawata H, Hayashi Y, Ishikawa T, et al. Electrocardiographic characteristics and SCN5A mutations in idiopathic ventricular fibrillation associated with early repolarization. Circ Arrhythm Electrophysiol. 2011; 4:874–81. [PubMed: 22028457]

[12]. Wilhelm M, Brem MH, Rost C, Klinghammer L, Hennig FF, Daniel WG, et al. Early repolarization, left ventricular diastolic function, and left atrial size in professional soccer players. Am J Cardiol. 2010; 106:569–74. [PubMed: 20691318]

[13]. Marcus RR, Kalisetti D, Raxwal V, Kiratli BJ, Myers J, Perkash I, et al. Early repolarization in patients with spinal cord injury: prevalence and clinical significance. J Spinal Cord Med. 2002; 25:33–8. [PubMed: 11939464]

[14]. Mizumaki K, Nishida K, Iwamoto J, Nakatani Y, Yamaguchi Y, Sakamoto T, et al. Vagal activity modulates spontaneous augmentation of J-wave elevation in patients with idiopathic ventricular fibrillation. Heart Rhythm. 2012; 9:249–55. [PubMed: 21939630]

[15]. Koutbi L, Roussel M, Haissaguerre M, Deharo JC. Hyperpnea test triggering malignant ventricular arrhythmia in a child with early repolarization. Heart Rhythm. 2012; 9:1153–6. [PubMed: 22370246]

[16]. Kawata H, Noda T, Yamada Y, Okamura H, Satomi K, Aiba T, et al. Effect of sodium-channel blockade on early repolarization in inferior/lateral leads in patients with idiopathic ventricular fibrillation and Brugada syndrome. Heart Rhythm. 2012; 9:77–83. [PubMed: 21855521]

[17]. Kasanuki H, Ohnishi S, Ohtuka M, Matsuda N, Nirei T, Isogai R, et al. Idiopathic ventricular fibrillation induced with vagal activity in patients without obvious heart disease. Circulation. 1997; 95:2277–85. [PubMed: 9142005]

[18]. Tikkanen JT, Anttonen O, Junttila MJ, Aro AL, Kerola T, Rissanen HA, et al. Long-term outcome associated with early repolarization on electrocardiography. N Engl J Med. 2009; 361:2529–37. [PubMed: 19917913]

[19]. Junttila MJ, Sager SJ, Tikkanen JT, Anttonen O, Huikuri HV, Myerburg RJ. Clinical significance of variants of J-points and J-waves: early repolarization patterns and risk. Eur Heart J. 2012; 33:2639–43. [PubMed: 22645193]

[20]. Fish JM, Welchons DR, Kim YS, Lee SH, Ho WK, Antzelevitch C. Dimethyl lithospermate B, an extract of danshen, suppresses arrhythmogenesis associated with the Brugada syndrome. Circulation. 2006; 113:1393–400. [PubMed: 16534004]

[21]. Di Diego JM, Sicouri S, Myles RC, Burton FL, Smith GL, Antzelevitch C. Optical and electrical recordings from isolated coronary-perfused ventricular wedge preparations. J Mol Cell Cardiol. 2013; 54:53–64. [PubMed: 23142540]

[22]. Zygmunt AC, Goodrow RJ, Antzelevitch C. Sodium effects on 4-aminopyridine-sensitive transient outward current in canine ventricular cells. Am J Physiol. 1997; 272:H1–H11. [PubMed: 9038916]

[23]. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch. 1981; 391:85–100. [PubMed: 6270629]

[24]. Yan GX, Antzelevitch C. Cellular basis for the electrocardiographic J wave. Circulation. 1996; 93:372–9. [PubMed: 8548912]

[25]. Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, et al. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation. 2007; 115:442–9. [PubMed: 17224476]

[26]. Gross GJ. Early repolarization and ventricular fibrillation: vagally familiar? Heart Rhythm. 2010; 7:653–4. [PubMed: 20153448]

[27]. Litovsky SH, Antzelevitch C. Differences in the electrophysiological response of canine ventricular subendocardium and subepicardium to acetylcholine and isoproterenol. A direct effect of acetylcholine in ventricular myocardium. Circ Res. 1990; 67:615–27. [PubMed: 2397572]

[28]. Ulphani JS, Cain JH, Inderyas F, Gordon D, Gikas PV, Shade G, et al. Quantitative analysis of parasympathetic innervation of the porcine heart. Heart Rhythm. 2010; 7:1113–9. [PubMed: 20381645]

[29]. Takahashi N, Barber MJ, Zipes DP. Efferent vagal innervation of canine ventricle. Am J Physiol. 1985; 248:H89–H97. [PubMed: 3970180]

[30]. DeGeest H, Levy MN, Zieske H, LIPMAN RI. Depression of ventricular contractility by stimulation of the vagus nerves. Circ Res. 1965; 17:222–35. [PubMed: 14338695]

[31]. Yang ZK, Boyett MR, Janvier NC, McMorn SO, Shui Z, Karim F. Regional differences in the negative inotropic effect of acetylcholine within the canine ventricle. J Physiol (Lond). 1996; 492(Pt 3):789–806. [PubMed: 8734990]

[32]. Levy MN. Sympathetic-parasympathetic interactions in the heart. Circ Res. 1971; 29:437–45. [PubMed: 4330524]

[33]. Kakinuma Y, Akiyama T, Sato T. Cholinoceptive and cholinergic properties of cardiomyocytes involving an amplification mechanism for vagal efferent effects in sparsely innervated ventricular myocardium. FEBS J. 2009; 276:5111–25. [PubMed: 19674111]

[34]. Rocha-Resende C, Roy A, Resende R, Ladeira MS, Lara A, de Morais Gomes ER, et al. Non-neuronal cholinergic machinery present in cardiomyocytes offsets hypertrophic signals. J Mol Cell Cardiol. 2012; 53:206–16. [PubMed: 22587993]

[35]. Junttila MJ, Sager SJ, Tikkanen JT, Anttonen O, Huikuri HV, Myerburg RJ. Use {15361}. Eur Heart J. 2012; 33:2639–43. [PubMed: 22645193]

[36]. Noseworthy PA, Tikkanen JT, Porthan K, Oikarinen L, Pietila A, Harald K, et al. The early repolarization pattern in the general population clinical correlates and heritability. J Am Coll Cardiol. 2011; 57:2284–9. [PubMed: 21600720]

[37]. Tikkanen JT, Junttila MJ, Anttonen O, Aro AL, Luttinen S, Kerola T, et al. Early repolarization: electrocardiographic phenotypes associated with favorable long-term outcome. Circulation. 2011; 123:2666–73. [PubMed: 21632493]

[38]. Rosso R, Adler A, Halkin A, Viskin S. Risk of sudden death among young individuals with J waves and early repolarization: putting the evidence into perspective. Heart Rhythm. 2011; 8:923–9. [PubMed: 21295159]

[39]. Rosso R, Glikson E, Belhassen B, Katz A, Halkin A, Steinvil A, et al. Distinguishing “benign” from “malignant early repolarization”: The value of the ST-segment morphology. Heart Rhythm. 2012; 9:225–9. [PubMed: 21914497]

[40]. Rosso R, Halkin A, Viskin S. J waves and early repolarization: do not confuse me with the facts! Heart Rhythm. 2012; 9:1603–4. [PubMed: 22842117]

[41]. Viskin S, Rosso R, Halkin A. Making sense of early repolarization. Heart Rhythm. 2012; 9:566–9. [PubMed: 22120134]

[42]. Szentandrassy N, Banyasz T, Biro T, Szabo G, Toth BI, Magyar J, et al. Apico-basal inhomogeneity in distribution of ion channels in canine and human ventricular myocardium. Cardiovasc Res. 2005; 65:851–60. [PubMed: 15721865]

[43]. Di Diego JM, Sun ZQ, Antzelevitch C. I_{to} and action potential notch are smaller in left vs. right canine ventricular epicardium. Am J Physiol. 1996; 271:H548–H561. [PubMed: 8770096]

[44]. Pierpont GL, DeMaster EG, Cohn JN. Regional differences in adrenergic function within the left ventricle. Am J Physiol. 1984; 246:H824–H829. [PubMed: 6742147]

[45]. Haissaguerre M, Sacher F, Nogami A, Komiya N, Bernard A, Probst V, et al. Characteristics of recurrent ventricular fibrillation associated with inferolateral early repolarization role of drug therapy. J Am Coll Cardiol. 2009; 53:612–9. [PubMed: 19215837]

[46]. Roten L, Derval N, Sacher F, Pascale P, Scherr D, Komatsu Y, et al. Heterogeneous response of J wave syndromes to beta-adrenergic stimulation. Heart Rhythm. 2012; 9:1970–6. [PubMed: 22864265]

Highlights

- We examine the mechanisms underlying ventricular tachycardia/ventricular fibrillation in early repolarization syndrome.
- Preferential accentuation of action potential notch in left ventricle epicardium leads to early repolarization syndrome.
- This repolarization defect is accentuated by elevated vagal tone.
- Higher levels of the transient outward current (I_{to}) contribute to arrhythmic vulnerability of inferior left ventricle wall.
- Quinidine & isoproterenol prevent V early repolarization syndrome by reversing the repolarization abnormality.

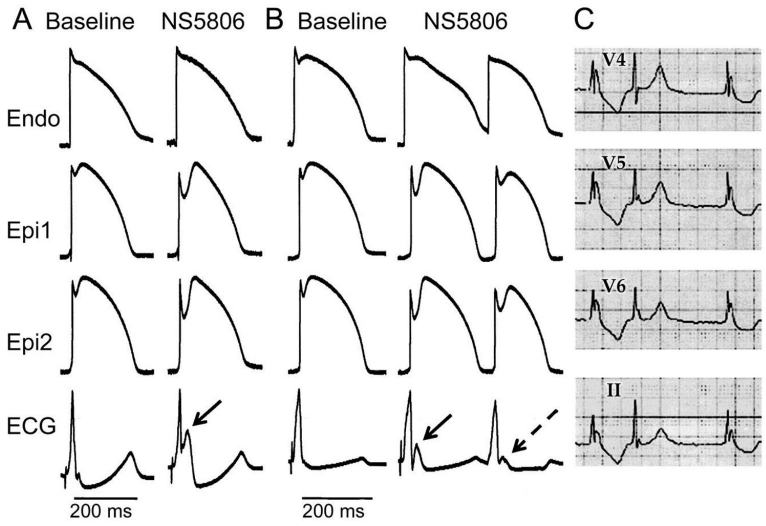

Figure 1.

Augmentation of the transient outward current (I_{to}) promotes early repolarization and J wave manifestation in left ventricle (LV) wedge preparations from the inferior wall of the LV of the canine heart. Each panel shows simultaneous recordings from one endocardial (Endo) and two epicardial (Epi) sites together with a pseudo-ECG. **A**: Recorded under control conditions and 35 min following addition of NS5806 (7 μM); Basic Cycle Length (BCL)=1000 ms. **B**: Recorded from another wedge preparation under control conditions and 30 min after addition of NS5806 (7 μM); BCL=2000 ms. The I_{to} agonist gives rise to prominent J waves (solid arrows) secondary to accentuation of the Epi action potential (AP) notch. A much diminished Epi AP notch and ECG J wave (dashed arrow) is associated with a closely coupled premature beat. **C**: Clinical example of accentuated J wave in a patient with early repolarization syndrome and atrial fibrillation. Note the marked attenuation of the J wave attending the abbreviated RR interval.

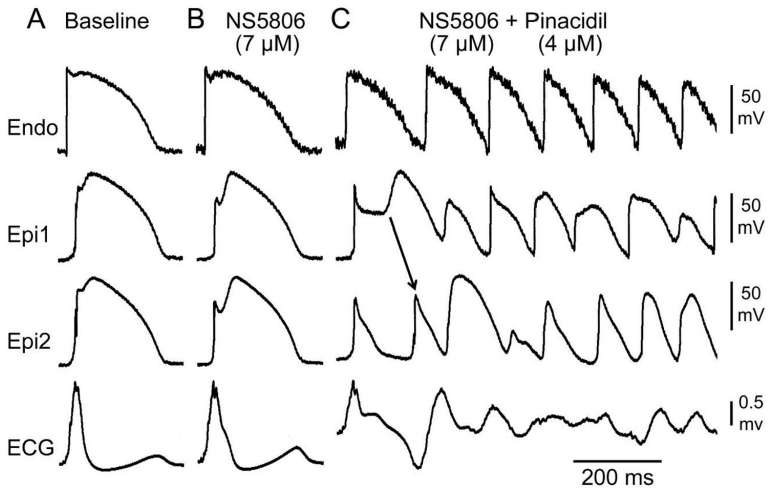

Figure 2.

Augmentation of the increase in ATP-sensitive potassium current ($I_{K\text{-}ATP}$) leads to development of phase 2 reentry and polymorphic ventricular tachycardia (VT). Each panel shows simultaneous recordings from one endocardial (Endo) and two epicardial (Epi) sites together with a pseudo-ECG of a canine left ventricular wedge preparation. **A**: Control; **B** and **C**: The $I_{K\text{-}ATP}$ agonist pinacidil (4 μM) and I_{to} agonist NS5806 (7 μM) were used to pharmacologically model a gain of function mutation in $I_{K\text{-}ATP}$ in the setting of a high level of I_{to}. Phase 2 reentry and polymorphic VT/ ventricular fibrillaton developed 20 min after addition of pinacidil. Basic Cycle Length=1000 ms.

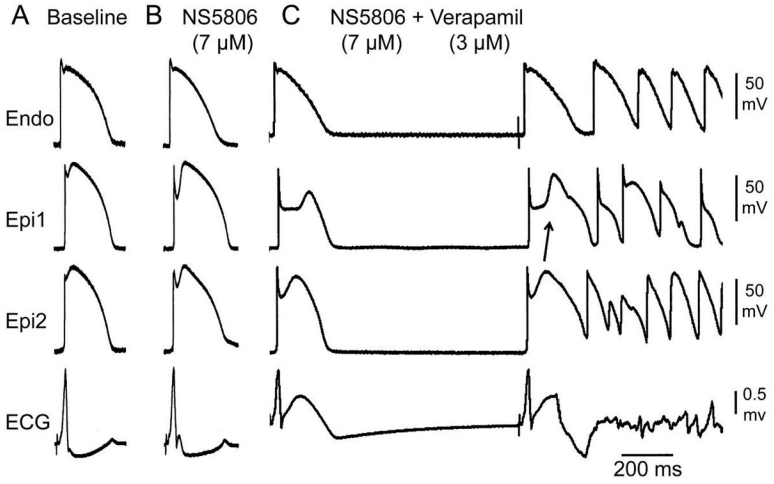

Figure 3.

Calcium channel inhibition leads to development of phase 2 reentry and polymorphic ventricular tachycardia (VT) in a left ventricular wedge preparation. Each panel shows simultaneous recordings from one endocardial (Endo) and two epicardial (Epi) sites together with a pseudo-ECG. **A**: Control; **B**: NS5806 (7 μM) accentuated the Epi action potential (AP) notch causing the appearance of a distinct J wave. **C**: Addition of verapamil (3 μM) to pharmacologically model loss of function calcium channel mutations further accentuated the epicardial AP notch, thus greatly amplifying the J wave, causing it to appear as an ST segment elevation. Phase 2 reentry and polymorphic VT/ ventricular fibrillation developed 15 min after addition of verapamil. Basic Cycle Length=1000 ms.

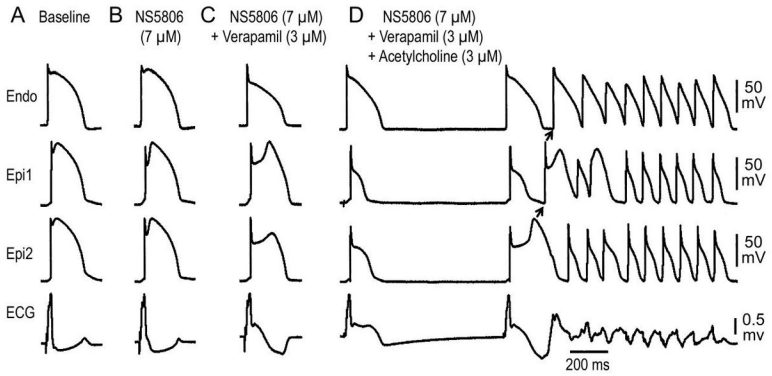

Figure 4.

Acetylcholine (ACh) leads to development of phase 2 reentry and polymorphic ventricular tachycardia (VT) in an experimental model of early repolarization syndrome. Each panel shows simultaneous recordings from one endocardial (Endo) and two epicardial (Epi) sites together with a pseudo-ECG. **A**: Control; **B** and **C**: The combination of verapamil (3 μM) and transient outward current agonist (NS5806, 7 μM) caused an early repolarization pattern to develop in the left ventricular wedge preparation, but no arrhythmogenic substrate. **D**: The addition of ACh, used to mimic increased vagal tone (ACh, 3 μM), led to the development of phase 2 reentry and polymorphic VT/ ventricular fibrillation. Basic Cycle Length=1000 ms.

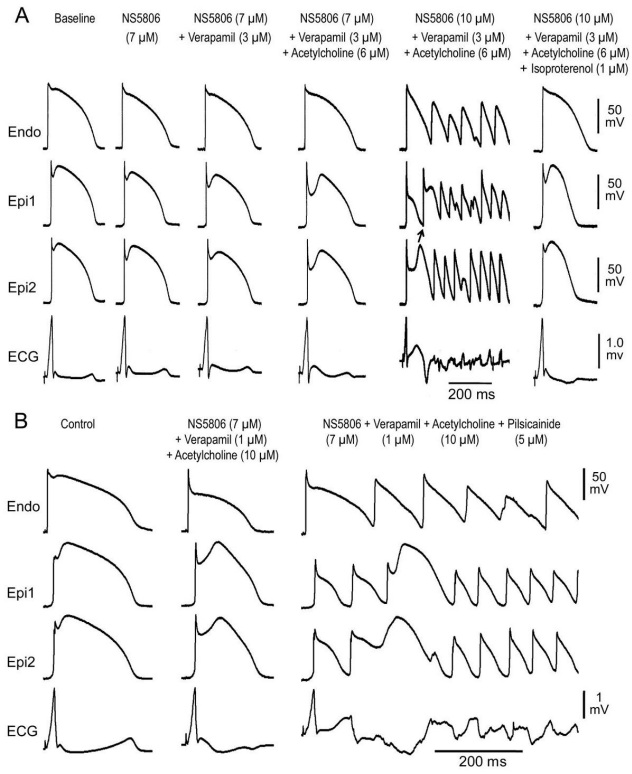

Figure 5.

A: Isoproterenol normalizes early repolarization (ER) pattern and prevents the development of ventricular tachycardia/ventricular fibrillation (VT/VF) in a left ventricular wedge model of ER syndrome. Each panel illustrates transmembrane action potentials (APs) simultaneously recorded from 1 endocardial (Endo) and 2 epicardial (Epi) sites, together with a pseudo-ECG. Exposure to NS5806 (7 μM) accentuated the epicardial AP notch, thus augmenting the J wave. Addition of verapamil (3 μM) further accentuated manifestation of the J wave. Addition of acetylcholine (6 μM) further enlarged the Epi AP notch and J wave. Increasing NS5806 to 10 μM caused heterogeneous loss of the AP dome in the Epi, giving rise to Phase 2 Reentry and VT/VF. F: Isoproterenol (1 μM) restored the Epi AP dome, normalized the ECG and abolished all arrhythmic activity. Basic Cycle Length=1000 ms.

B: When a combination of NS5806+verapamil+ACh failed to induce VT/VF, the addition of the sodium channel blocker pilsicainide caused accentuation of the ER phenotype leading to the development of VT/VF. Basic Cycle Length=500 ms.

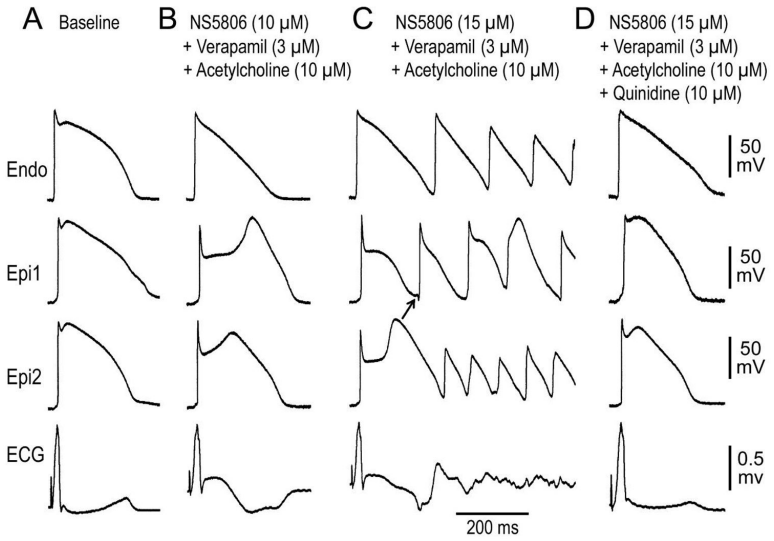

Figure 6.

Quinidine normalizes early repolarization (ER) pattern and suppresses ventricular tachycardia/ventricular fibrillation (VT/VF) in a left ventricular wedge model of ER syndrome. Each panel shows transmembrane action potentials (APs) simultaneously recorded from 1 endocardial (Endo) and 2 epicardial (Epi) sites, together with a pseudo-ECG. **A**: Control; **B**: ER phenotype was induced by NS5806 (10 μM) + Verapamil (3 μM) + Acetylcholine (10 μM); **C**: Increasing NS5806 concentration to 15 μM, led to the development of Phase 2 Reentry, **D**: Recorded 20 min after addition of 10 μM quinidine. Quinidine restored the epicardial AP dome, abolished ER and ST segment elevation and suppressed VT/VF. Basic Cycle Length=1000 ms.

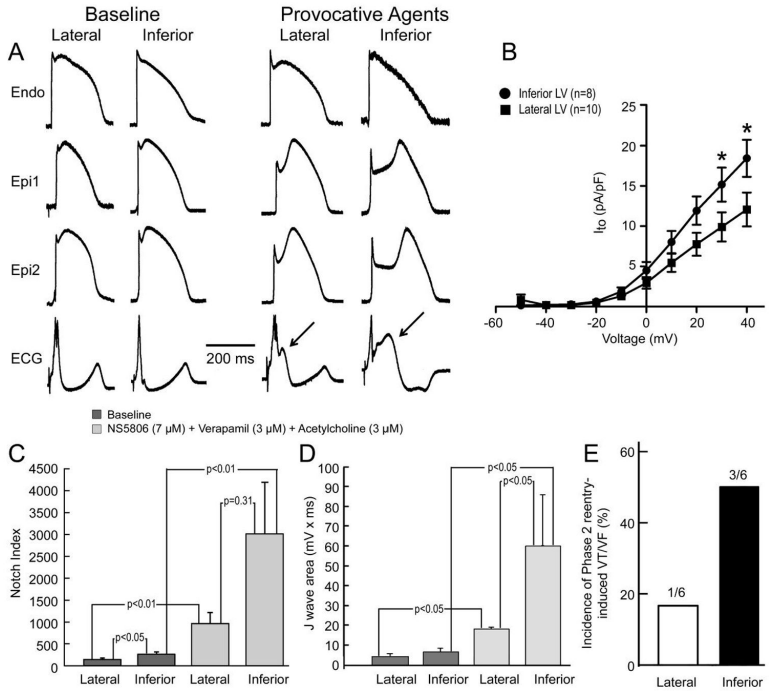

Figure 7.

Basis for greater vulnerability of inferior wall to early repolarization syndrome-mediated arrhythmia. Results shown are from left ventricular (LV) wedge preparations isolated from the inferior and lateral regions of the same hearts. **A:** Under baseline conditions, the action potential (AP) notch is more prominent in the epicardium (Epi) of the inferior wall as compared with the lateral wall and the response (accentuation of AP notch, increase in J wave) to a combination of NS5806 (7 μM) + Verapamil (3 μM) + Acetylcholine (ACh) (3 μM) is much more pronounced in the Epi of the inferior wall. APs shown are recorded from the lateral and the inferior part of the LV of the same dog. Arrows show increased J wave upon application of provocative agents. Endo = endocardial. **B:** Transient outward current density is more pronounced in Epi myocytes isolated from inferior vs. lateral wall. **C:** Notch index of APs recorded from the Epi surface of paired inferior vs. lateral wedge preparations isolated from the same heart under baseline conditions and after exposure to the same combination of provocative agents [NS5806 (7 μM) + Verapamil (3 μM) + ACh (3 μM)]. Results are mean ± S.E.M. **D**: J wave area recorded from paired inferior vs. lateral wedge preparations isolated from the same heart under baseline conditions and after exposure to the same combination of provocative agents [NS5806 (7 μM) + Verapamil (3 μM) + ACh (3 μM)]. Results are mean ± S.E.M. **E:** Incidence of phase 2 reentry –induced VT/VF after exposure of the lateral and inferior wedge preparations isolated from the same heart to the same combination of provocative agents.

Table 1

Effects of provocative agents on transmural and epicardial (Epi) dispersion of repolarization and on action potential (AP) parameters in perfused left ventricular wedge preparations.

	Endo APD_{90} (ms)	Epi1 APD_{90} (ms)	Epi2 APD_{90} (ms)	Endo APD_{50} (ms)	Epi1 APD_{50} (ms)	Epi2 APD_{50} (ms)	Notch Magnitude (% ofPh0)*	Notch Index†	TDR (ms)	EDR (ms)
Control	223.2 ± 2.1	199.7 ± 5.9	201.8 ± 3.9	177.8 ± 3.9	169.5 ± 7.6	170.7 ± 4.2	14.0 ± 3.1	255.2 ± 54.3	17.0 ± 3.5	14.7 ± 2.8
NS 5806 (7 μM)	226.2 ± 5.1	203.7 ± 6.5	207.2 ± 2.6	176.5 ± 7.4	169.3 ± 8.0	176.1 ± 2.5	31.7 ± 4.0	780.8 ± 116.0	17.7 ± 5.3	13.3 ± 2.8
Verapamil (3 μM)	227.0 ± 7.9	225.9 ± 8.3[a]	222.5 ± 4.6[a]	167.9 ± 6.9	188.8 ± 7.9	186.5 ± 3.8[a]	38.9 ± 5.0[b]	2551.9 ± 182.8[b]	21.3 ± 4.4	14.6 ± 6.4
Acetylcholine (3 μM)‡	217.3 ± 3.1	129.9[c] ± 17.2	246.3 ± 26.6	157.1 ± 2.1	69.1[c] ± 17.8	202.7 ± 26.4	42.2 ± 5.1	4654.5 ± 894.0	80.8[c] ± 15.5	111.2[c] ± 24.1

APD_{90}; APD_{50}= action potential durations at 90%and 50% repolarization. Results are mean ± S.E.M. a=p < 0.05, b=p < 0.01 vs control, c=p < 0.05 vs NS5806 + verapamil combination. Basic cycle length =1000 ms; n=5.

*Epicardial AP notch data (notch magnitude and notch index) measured before loss of the AP dome at epicardial sites.

†Notch index = Notch Magnitude × (Ph 0 to Ph 2 interval) which approximates the area of the notch.

‡Epicardial dispersion of repolarization (EDR) and transmural dispersion of repolarization (TDR) reported here were measured in cases at which the addition of Acetylcholine caused loss of the AP dome at EPI 1 site but no EPI 2, with the development of Phase 2 Reentry.