Amplified Transmural Dispersion of Repolarization as the Basis for Arrhythmogenesis in a Canine Ventricular-Wedge Model of Short-QT Syndrome

Fabrice Extramiana, MD; Charles Antzelevitch, PhD

Background—The short-QT syndrome is a new clinical entity characterized by corrected QT intervals <300 ms and a high incidence of ventricular tachycardia (VT) and fibrillation (VF). Gain-of-function mutations in the gene for outward potassium currents have been shown to underlie the congenital syndrome. The present study examined the cellular basis of VT/VF in an experimental model associated with short QT intervals created with a potassium channel activator.

Methods and Results—Transmembrane action potentials from epicardial and M regions, 4 transmural unipolar electrograms, and a pseudo-ECG were simultaneously recorded in canine arterially perfused left ventricular wedge preparations. At a basic cycle length of 2000 ms, pinacidil (2 to 3 μmol/L) abbreviated the QT interval from 303.7±5.4 to 247.3±6.9 ms (mean±SEM, P<0.0001). The maximal transmural dispersion of repolarization (TDRmax) increased from 27.0±3.8 to 64.9±9.2 ms (P<0.01), and an S2 applied to the endocardium induced a polymorphic VT (pVT) in 9 of 12 wedge preparations (P<0.01). Addition of isoproterenol (100 nmol/L, n=5) led to greater abbreviation of the QT interval, a further increase in TDRmax (from 55.4±13.7 to 69.7±8.3 ms), and more enduring pVT. TDRmax was correlated significantly with the Tpeak-Tend interval under all conditions. The effects of pinacidil were completely reversed by glybenclamide (10 μmol/L, n=4) and partially reversed by E4031 (5 μmol/L, n=5), which prevented induction of pVT in 3 of 5 preparations.

Conclusions—Our data suggest that heterogeneous abbreviation of the action potential duration among different cell types spanning the ventricular wall creates the substrate for the genesis of VT under conditions associated with short QT intervals. (Circulation. 2004;110:3661-3666.)

Key Words: ventricles ■ tachycardia ■ arrhythmia ■ fibrillation ■ electrophysiology

The short-QT syndrome is a relatively new clinical entity characterized by short QT intervals in the ECG, the absence of structural heart disease, a familial history of sudden cardiac death, and major (resuscitated cardiac arrest, syncope) or minor (palpitations, dizziness, atrial fibrillation) arrhythmic events.1–6

From a theoretical point of view, the shortening of ventricular repolarization can be the consequence of either an increase in repolarizing currents or a decrease in depolarizing currents during the plateau and/or phase 3 of repolarization. Our group recently described the first mutation associated with the short-QT syndrome.5 A missense mutation involving a substitution of lysine for asparagine in position 588 of HERG (KCNH2) was found to cause a remarkable gain of function in the rapidly activating delayed rectifier current, IKr.

A distinctive ECG feature of the short-QT syndrome is the development of tall, peaked, symmetrical T waves and relatively long Tpeak-Tend intervals, indicative of augmented transmural dispersion of repolarization (TDR). Previous studies involving the canine arterially perfused wedge preparation have demonstrated the arrhythmogenic role of increased TDR under long-QT conditions as well as in the Brugada syndrome.7–12 In this study, we made use of the canine left ventricular (LV) wedge preparation to test the hypothesis that abbreviation of the QT interval is associated with an increase in TDR, which creates the substrate for reentry responsible for the development of life-threatening ventricular tachycardia/fibrillation (VT/VF). Because an IKr activator is unavailable, we chose to use the ATP-sensitive potassium current (IK-ATP) activator pinacidil to augment outward currents.

Methods

Adult (ie, >1 year old) male dogs weighing 20 to 25 kg were anticoagulated with heparin and anesthetized with sodium pentobarbital (30 to 35 mg/kg IV). The chest was opened via a left thoracotomy, and the heart was excised, placed in Tyrode’s solution, and transferred to a dissection tray. Transmural LV wedges with dimensions of ~12×25×12 mm were dissected from the mid-to-apical anterior region of the LV wall, and a diagonal branch of the left anterior descending coronary artery was cannulated and perfused with Tyrode’s solution. The composition of the Tyrode’s solution was (in mmol/L) as follows: NaCl 129, KCl 4, NaH2PO4 0.9, NaHCO3 20, CaCl2 1.8, MgSO4 0.5, and d-glucose 5.5 (pH 7.4). All
experiments were performed in conformance with the guidelines of The Institutional Animal Care Committee of The Masonic Medical Research Laboratory.

The ventricular-wedge preparations were allowed to equilibrate in the chamber for 2 hours while being paced at basic cycle lengths (BCLs) of 2000 ms with Ag bipolar electrodes placed in contact with the endocardial surface. The temperature of the perfusate was maintained at 35°C.

Transmembrane action potentials were recorded from the epicardial surface and subendocardial M-cell regions by using floating microelectrodes. Four transmural, unipolar electrograms were recorded and used to measure activation recovery intervals (ARIs). ARI1 was recorded from the subendocardium, ARI2 was recorded from the subepicardium, and ARI2 and ARI3 were equally spaced within the midmyocardium. Each unipolar recording was differentiated, and the ARI approximating the action potential duration (APD) at each site was measured as the interval between the time of the minimum first derivative (V_{min}) of the QRS deflection and the maximum first derivative (V_{max}) of the T wave. The repolarization time (RT) was defined as ARI+activation time (from stimulus artifact to V_{max}). The absolute value of the maximal RT difference was considered as the maximal transmural dispersion of repolarization (TDRmax). A transmural pseudo-ECG was recorded by using 2 AgCl half-cells placed 1 cm from the epicardial (+) and endocardial (-) surfaces of the preparation and along the same axis as the transmembrane and unipolar recordings. T_{max-T_{end}} was measured from the peak to the end of the T wave in the case of an upright T wave and from the nadir to the end of the T wave in the case of a negative T wave.

After control recordings were obtained, pinacidil (2 to 3 μmol/L) was added to the coronary perfusate alone or in association with either isopropenol (100 μmol/L), E4031 (5 μmol/L), or glybenclamide (10 μmol/L). Experiments were performed in 12 different hearts. Control and pinacidil (2 to 3 μmol/L) conditions were tested in 12 experiments. Isopropenol (100 μmol/L) was added to pinacidil in 5 experiments. Glybenclamide (10 μmol/L) was added to pinacidil in 4 experiments (in 1 experiment after isopropenol washout). E4031 (5 μmol/L) was added to pinacidil in 5 experiments (in 1 experiment after isopropenol washout) All measured values returned to presisperpenol values after isopropenol washout.

VT inducibility was examined under each condition (control, drugs, BCL 600 ms, and BCL 2000 ms). An S2 extrastimulus was applied starting at an S1-S2 coupling interval equivalent to the longest APD recorded, decreasing in 10-ms steps until the refractory period was reached.

Statistics

Summary data are reported as mean±SEM. Statistical analysis was performed with a paired t test for the 12 replicates recorded under control conditions and after pinacidil. We used Friedman’s test for the 3 secondary experiments (pinacidil plus isopropenol, glybenclamide, or E4031). Proportions were compared with McNemar’s test for paired data.

Results

Differences in the time course of repolarization of the epicardial and M-cell regions of the LV wall are responsible for the inscription of the ECG T wave and the normal and pathophysiological characteristics of the TDR. These characteristics of the LV wall are illustrated in Figure 1. Each panel shows transmembrane action potentials simultaneously recorded from the epicardial and deep subendocardial M-cell regions of the arterially perfused LV wedge preparation, together with 4 unipolar electrodes and an ECG. The QT interval was 313 ms and the TDR was 41 ms under control conditions. Figure 1B was recorded after the activation of I_{KATP} by pinacidil (2 μmol/L). Pinacidil produced a preferential abbreviation of the M-cell action potential, leading to abbreviation of the QT interval (213 ms), inversion of the T wave due to repolarization of the epicardium after the M region, and amplification of the TDR to 67 ms. The addition of isopropenol (100 μmol/L) produced a further abbreviation of the QT interval to 195 ms and an augmentation of the TDR to 92 ms.

Figure 2 illustrates the dose-dependent effect of pinacidil on the QT interval and TDR. Pinacidil produced a dose-dependent abbreviation of QT but a prolongation of TDR. Programmed electrical stimulation (S2 applied to the endocardium) failed to induce VT either under control conditions or after 1 or 2 μmol/L pinacidil but succeeded in precipitating polymorphic ventricular tachycardia (pVT) after 3 μmol/L pinacidil. TDRmax increased to 79 ms at the highest concentration of pinacidil.

In 12 replicate experiments, 2 to 3 μmol/L pinacidil abbreviated the QT interval from 303.7±5.4 to 247.3±6.9 ms (P<0.0001). TDRmax increased from 27.0±3.8 to 64.9±9.2 ms (P<0.01) as a result of a greater abbreviation of ARI1 or AR12 versus AR13 or AR14 (maximum abbreviation: AR11 or AR12, 90.8±26.2 ms; AR13 or AR14, 61.5±17.8 ms; P<0.05). Conduction, as assessed by the time interval between the stimulus artifact and the V_{max} of the epicardial electrogram, did not change after pinacidil (32.6±1.69 versus 33.8±2.07 ms under control conditions). Arrhythmia was never inducible under control conditions. After pinacidil (2 to 3 μmol/L), pVT was induced in 9 of 12 preparations (P<0.01).

Figure 3 illustrates the response of individual experimental preparations to pinacidil-induced TDRmax and pVT. The 3 preparations in which pVT could not be induced were those
that exhibited the smallest increase in TDRmax. Programmed electrical stimulation induced pVT in all preparations in which TDRmax was increased to values >40 ms.

The QT-interval abbreviation unaccompanied by an increase in TDR was not sufficient to induce pVT. Reduction of the BCL to 600 ms abbreviated the QT interval under control conditions to values similar to those obtained with pinacidil at a BCL of 2000 ms (242.4 ± 3.8 versus 247.3 ± 6.9 ms). TDRmax under these conditions reached 39.7 ± 3.7 ms and pVT was not inducible, suggesting that QT abbreviation alone was not enough to permit the induction of pVT.

Induction of VT/VF in other sudden death syndromes, including catecholaminergic VT and the long-QT syndrome, is facilitated by exercise and other sympathetic stimuli. In another series of experiments, we examined the influence of β-adrenergic stimulation in the form of isoproterenol. Figure 4 illustrates the effect of isoproterenol on arrhythmogenicity in this experimental. The QT interval and TDR were 313 and 41 ms, respectively, under control conditions and 213 and 67 ms, respectively, after 2 μmol/L pinacidil. Programmed electrical stimulation (S1-S2=150 ms) induced a brief episode of pVT. The addition of isoproterenol (100 nmol/L) led to a further abbreviation of the QT interval (195 ms), a further increase of TDRmax (92 ms), and a more enduring pVT (precipitated at an S1-S2 of 110 ms).

Figure 5 and the Table summarize the results of 5 similar experiments. VT could not be induced under control conditions. With pinacidil alone, pVT was induced in 3 of 5 preparations. After the addition of isoproterenol, pVT was induced in 5 of 5 preparations.

Figure 6 displays the relation between TDRmax, the Tpeak-Tend interval, and the susceptibility to induced pVT. The correlation between TDRmax and the Tpeak-Tend interval was significant.
Figure 5. Isoproterenol (n=5) accentuates effect of pinacidil to increase TDRmax and induce VT in 5 experiments. BCL=2000 ms. Dotted line separates conditions under which pVT was inducible (above dotted line) from conditions that failed to permit induction of pVT (below dotted line). Abbreviations are as defined in text.

(glybenclamide 320.6
7.9† 326.0
0.8 285.2
9.3† 37.6
8.0† 280.0
8.7† 28.4
5.2
13.3† 307.0
P
0.05 vs control, †
0.05 vs pinacidil. P
0.05 vs control, †
0.05 vs pinacidil. Epi indicates epicardial; M, M cell. All other abbreviations are as defined in text. All values are mean±SEM in ms.

Effect of Pinacidil Alone and in Association With Isoproterenol, E4031, or Glybenclamide on APD at 90% Repolarization (APD90), Tpeak–Tend Interval, ARI, and TDRmax

<table>
<thead>
<tr>
<th>BCL 2000</th>
<th>APD90, M</th>
<th>APD90, Epi</th>
<th>QT</th>
<th>Tpeak–Tend</th>
<th>ARI1</th>
<th>ARI2</th>
<th>ARI3</th>
<th>ARI4</th>
<th>TDRmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>289.0±10.5</td>
<td>242.6±7.1</td>
<td>300.6±8.1</td>
<td>28.7±3.0</td>
<td>278.7±9.5</td>
<td>273.4±11.5</td>
<td>272.2±8.3</td>
<td>242.3±5.0</td>
<td>28.9±7.8</td>
</tr>
<tr>
<td>Pinacidil</td>
<td>222.0±6.1</td>
<td>191.6±5.8</td>
<td>235.3±12.5</td>
<td>57.4±9.7</td>
<td>192.5±22.3</td>
<td>208.3±12.8</td>
<td>201.9±10.9</td>
<td>184.9±4.9</td>
<td>55.4±13.7</td>
</tr>
<tr>
<td>Pinacidil + isoproterenol</td>
<td>132.7±12.4*</td>
<td>140.5±13.6*</td>
<td>158.2±14.8*</td>
<td>69.4±5.6*</td>
<td>95.4±9.2*</td>
<td>110.3±14.8*</td>
<td>124.4±21.6*</td>
<td>115.3±19.6*</td>
<td>69.7±8.3*</td>
</tr>
<tr>
<td>Friedman’s test (n=5)</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Control</td>
<td>297.6±6.3</td>
<td>248.1±5.6</td>
<td>308.0±4.7</td>
<td>31.1±3.5</td>
<td>285.8±6.1</td>
<td>286.5±4.1</td>
<td>272.5±6.0</td>
<td>246.0±3.2</td>
<td>29.0±5.5</td>
</tr>
<tr>
<td>Pinacidil</td>
<td>232.0±13.3</td>
<td>194.0±8.6</td>
<td>251.2±12.9</td>
<td>54.7±11.0</td>
<td>190.6±24.7</td>
<td>225.5±16.8*</td>
<td>216.1±13.3*</td>
<td>191.3±5.7</td>
<td>61.0±10.4*</td>
</tr>
<tr>
<td>Pinacidil + E4031</td>
<td>300.1±12.2†</td>
<td>241.1±21.0</td>
<td>309.5±6.2†</td>
<td>30.7±8.4</td>
<td>268.8±20.1</td>
<td>279.6±9.1</td>
<td>264.1±6.0</td>
<td>238.4±13.2†</td>
<td>53.5±10.6</td>
</tr>
<tr>
<td>Friedman’s test (n=5)</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.09</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Control</td>
<td>296.8±10.8</td>
<td>263.7±13.1</td>
<td>315.9±10.5</td>
<td>29.8±2.5</td>
<td>287.7±6.4</td>
<td>261.6±8.2</td>
<td>272.1±7.6</td>
<td>252.3±7.6</td>
<td>30.6±7.0</td>
</tr>
<tr>
<td>Pinacidil</td>
<td>245.1±9.4</td>
<td>225.0±15.6</td>
<td>253.2±5.2</td>
<td>62.1±19.1</td>
<td>241.4±22.9</td>
<td>163.7±23.1</td>
<td>208.8±11.9</td>
<td>197.5±13.2</td>
<td>89.8±16.2</td>
</tr>
<tr>
<td>Pinacidil + glybenclamide</td>
<td>320.6±8.0†</td>
<td>280.0±7.9†</td>
<td>326.0±8.7†</td>
<td>28.4±5.4</td>
<td>310.2±13.3†</td>
<td>307.0±8.8</td>
<td>285.2±8.4†</td>
<td>260.0±9.3†</td>
<td>37.6±5.2</td>
</tr>
<tr>
<td>Friedman’s test (n=4)</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P=0.17</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 6. Relation between Tpeak–Tend interval and TDRmax. Solid black line is linear regression line, and dotted line separates conditions associated with inducible pVT from those not associated with inducible pVT. pVT could be induced with TDRmax values >50 ms. n=5. Abbreviations are as defined in text.
a preferential abbreviation of the LV M cells located in the deep subendocardium. The ionic basis for this preferential abbreviation of the M-cell APD is unknown. One hypothesis is that this is a consequence of a nonuniform density of \( I_{K_{ATP}} \) or other currents or exchangers across the ventricular wall. The heterogeneous response to pinacidil observed in our experimental series is inconsistent with a greater role for \( I_{K_{ATP}} \) in epicardium versus endocardium, as has been suggested for the feline heart.\(^{19}\)

Although previous studies have shown that pinacidil facilitates the induction of VF under both normoxic conditions and conditions of ischemia/reperfusion,\(^{20,21}\) relatively little information regarding the mechanism of its arrhythmogenesis has been provided. Abbreviation of the effective refractory period by \( I_{K_{ATP}} \) openers has been shown to increase ventricular vulnerability to reentry and to accelerate its rate in a model of VF in superfused canine right ventricular epicardial slices.\(^{22}\)

Our results indicate that pinacidil-induced abbreviation of repolarization in the LV is associated with an increased TDR, a well-known substrate for the development of pVT. Although the abbreviated wavelength (product of effective refractory period and conduction velocity) expected under these conditions is insufficient by itself to form the substrate for reentry, this action of the drug does serve to reduce the threshold at which TDR can permit pVT to \( \approx 50 \) ms. It is noteworthy that under long-QT conditions, this value is \( \approx 90 \) ms in the LV wedge preparation.\(^{13,23}\)

Pinacidil is a specific activator of the \( I_{K_{ATP}} \) channel. Pinacidil’s effects on APD, ARI, and TDR were all reversed by glybenclamide, suggesting that these actions of the drug are largely attributable to \( I_{K_{ATP}} \) activation, despite the fact that glybenclamide is not a specific inhibitor of \( I_{K_{ATP}} \). The variability in drug potency to abbreviate the APD and increase the TDR in different preparations may be due to breed- or age-related differences in the sensitivity of the \( I_{K_{ATP}} \) channel or to intrinsic differences in net repolarizing current.

Isoproterenol was found to amplify the actions of pinacidil to preferentially abbreviate the M-cell APD. Among its many actions, isoproterenol increases slowly activating delayed rectifier current (\( I_{Kr} \)) and in the absence of pinacidil produces a greater abbreviation of the epicardial and endocardial action potential, where \( I_{Kr} \) is relatively large.\(^{24,25}\) The greater abbreviation of the M-cell APD in the presence of pinacidil may be secondary to cAMP-mediated phosphorylation of the \( I_{K_{ATP}} \) channel,\(^{16}\) leading to potentiation of the action of pinacidil. This potentiation of \( I_{K_{ATP}} \) activation by isoproterenol may underlie the effect of catecholamines to increase the risk of life-threatening arrhythmias under ischemic conditions and may contribute to the protective effect of \( \beta \)-blockers.\(^{26,27}\)

The \( I_{Kr} \) blocker E4031 partially reversed the effect of pinacidil. pVT could still be induced at a BCL of 2000 ms in 1 preparation and at a BCL of 600 ms in 2 additional preparations. The lack of protection of E4031 at the faster pacing rate may be due to the well-known reverse rate-dependent prolongation of APD observed with most \( I_{Kr} \) blockers.\(^{28}\)

We found a good association between the level of TDR\(_{\text{max}}\) and the inducibility of pVT. The critical role of TDR as a substrate for functional reentry has been demonstrated in models of prolonged repolarization (congenital and acquired long-QT syndrome,\(^{7–9,29}\) hypertrophy,\(^{30}\) and heart failure).\(^{31}\) In addition, epicardial and transmural dispersion of repolarization seems also to be a key mechanism of arrhythmias in the Brugada syndrome.\(^{10–12}\) To the best of our knowledge, this is the first demonstration of a role for transmural heterogeneity of repolarization under conditions associated with short QT intervals in the ECG.

The pinacidil model of the short-QT syndrome, although mechanistically related, is phenotypically different from the clinical syndrome caused by a gain of function of \( HERG \) (SQT1), the gene that encodes \( I_{Kr} \), or the short-QT syndrome recently described by Belloq and coworkers\(^{32}\) and shown to be due to a gain of function in \( KCNQ1 \) (SQT2), the gene that encodes \( I_{Kr} \). In these 2 syndromes, the ECG of affected individuals often manifests tall, peaked, symmetrical T waves rather than inverted T waves, as predicted by the present model involving activation of \( I_{K_{ATP}} \). The prolonged T\(_{\text{peak}}\)–T\(_{\text{end}}\) observed in SQT1 and SQT2 points to a prolonged TDR as the arrhythmogenic substrate. Thus, the present model is consistent with the known clinical phenotypes, in that abbreviation of the QT interval is associated with a very significant accentuation of TDR. In a review of the short-QT syndrome in 2002, we suggested \( I_{Kr} \) and \( I_{K_{ATP}} \) as 2 of our 4 principal gene candidates.\(^{2}\) The other 2 were \( I_{K_{ATP}} \) and acetylcholine-activated potassium current. The wedge model also mimics the clinical syndrome in its ability to develop pVT in response to programmed electrical stimulation. Although both pVT and monomorphic VT have been reported to be associated with the short-QT syndrome,\(^{33}\) we have not as yet observed monomorphic VT in the wedge. Our data provide an important proof of concept relative to the role of TDR in arrhythmogenesis under conditions associated with premature repolarization of the ventricles and short QT intervals. It is tempting to speculate that this mechanism may play a role in the development of the short, coupled variant of torsade de pointes as well as ischemia-induced arrhythmias, because \( I_{K_{ATP}} \) activation is an important component of ischemia. Potassium channel activators have been proposed as potential antiarrhythmic agents in the long-QT syndrome. Our data suggest a potential proarrhythmic effect of \( I_{K_{ATP}} \) activation.

Acknowledgment

This study was supported by grant HL47678 from the NHLBI (to C.A.) and grants from the American Heart Association (to C.A.), Fédération Française de Cardiologie (to F.E.), and New York State and Florida Grand Lodges F and AM. We are grateful to Judy Hefferon for her expert technical assistance and Dr Jeffrey Fish for his valuable help.

References


Amplified Transmural Dispersion of Repolarization as the Basis for Arrhythmogenesis in a Canine Ventricular-Wedge Model of Short-QT Syndrome
Fabrice Extramiana and Charles Antzelevitch

Circulation. 2004;110:3661-3666; originally published online November 29, 2004;
doi: 10.1161/01.CIR.0000143078.48699.0C
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/110/24/3661

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/