Chapter 6

Interventricular dispersion in repolarization causes bifid T waves in dogs with dofetilide-induced LQT syndrome

Veronique M.F. Meijborg, Samuel Chauveau, Michiel J. Janse, Evgeny P. Anyukhovsky, Peter R. Danilo Jr., Michael R. Rosen, Tobias Opthof, Ruben Coronel

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ABSTRACT

Background: Long QT2 (LQT2) syndrome is characterized by bifid (or notched) T waves, whose mechanism is not understood.

Objective: The purpose of this study was to test whether increased interventricular dispersion of repolarization induces bifid T waves.

Methods: We simultaneously recorded surface ECG and unipolar electrograms at baseline and after dofetilide in a canine model of dofetilide-induced LQT2 (6 male mongrel dogs). Standard ECG variables, T wave duration, and moments of peaks of bifid T waves (Tp1 and Tp2) were correlated with moments of local repolarization. Epicardial electrograms were recorded over the left ventricular (LV) and right ventricular (RV) anterior walls (11x11 electrode grid, 5-mm interelectrode distance). In 5 of the 6 hearts we also recorded intramural unipolar electrograms (n= 4-7 needles per heart). In each unipolar recording, we determined activation time, repolarization time (RT) and activation-recovery interval. In addition, we studied RT response to heart rate changes.

Results: Dofetilide prolonged QT and QTc, induced bifid T waves in 4 of 6 animals, and prolonged RT heterogeneously in LV and RV, resulting in increased interventricular and LV intraventricular RT dispersion. Dofetilide did not induce a disparate response in activation-recovery interval across the transmural axis. Dofetilide-induced separation of RT across the RV-LV interface concurred with the moments of T wave peaks. Dofetilide-induced steepening of restitution slopes was larger in LV than RV.

Conclusion: Dofetilide-induced bifid T waves result from interventricular RT dispersion.
INTRODUCTION

The long QT (LQT) syndrome is characterized by a prolonged QT interval and is associated with sudden cardiac death. The congenital LQT syndromes are classified according to their associated gene mutations. Because genetic screening lacks sensitivity and QT prolongation is absent in approximately 25% of LQT patients with a mutation, other markers have been identified to improve diagnosis. LQT syndrome type 2 (LQT2) typically manifests bifid (or notched) T waves, which change in morphology during abrupt heart rate increases provoked by sudden standing.

LQT2 is associated with a mutation in the HERG gene (KCNH2), which encodes the alpha-subunit of the ion channel carrying the rapidly activating component of the delayed rectifier potassium current IKr. Loss of function of Ikr channels leads to prolonged action potentials and QT interval. The mechanism of bifid (or notched) T waves of LQT2 patients is not known. The relation with sudden standing suggests that altered restitution may be involved in the genesis of the bifid T wave, because restitution determines the amount of heart rate-induced changes in repolarization, which is the main determinant for T wave morphology.

Volders et al showed a gain of function of the slowly activating component of the delayed rectifier potassium current Iks in canine cardiomyocytes during Ikr block, and higher Iks densities in RV cardiomyocytes than LV cardiomyocytes. Because Iks is primarily responsible for adaptation of action potential duration to altered cycle length, spatial differences in Iks are accentuated and alter restitution properties during Ikr block. Therefore, we postulated that during Ikr block interventricular differences in repolarization become more prominent and may lead to a bifid T wave. We tested this hypothesis in a canine LQT2 model with Ikr channel blocker dofetilide infusion and sudden heart rate changes. Our data shed light on the mechanism responsible for bifid (or notched) T waves in LQT2 patients.

METHODS

The experimental protocol complied with the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health Publication 85-23, revised 1996) and was approved by the Stony Brook University Animal Care and Use Committee.

Surgical preparation

Six male mongrel dogs (weight 23-26 kg) were preanesthetized with propofol (6-8 mg/kg IV), intubated, artificially ventilated, and anesthetized with a mixture of isoflurane (2%-3.5%) and oxygen. A left thoracotomy was performed at the fifth intercostal space,
and the heart was suspended in a pericardial cradle (see Supplementary Material for more details).

**Drug administration protocol**
After the baseline recordings, dofetilide was infused intravenously, and rate-corrected QT ( QTc, Bazett formula \(^{17}\) ) was continuously monitored. When QTc prolongation was at least 25%, we stopped the infusion and identified this dofetilide dose as the bolus. We then commenced dofetilide infusion at a rate= bolus/hour. The total dofetilide dose varied among dogs from a 12-18 µg/kg bolus followed by a 12-18 µg/kg/hour infusion. This protocol induced stable QTc prolongation, that is, QTc before pacing was similar to that measured after completion of the pacing protocols (580±34 ms vs 564±28 ms [mean±SEM], \(P>0.05\), paired t test).

**Electrophysiologic study**
Electrophysiologic study was performed at baseline and during dofetilide infusion. A grid with 11x11 electrodes (5-mm interelectrode distance) was sutured to the left ventricular (LV) and right ventricular (RV) anterior walls to obtain local epicardial electrograms (see Supplemental Figure S1). In addition, 4-7 intramural plunge needles (each containing 4 electrode terminals, 4-mm interelectrode distance) were inserted in the LV and RV wall in each heart of 5 dogs. Simultaneously, 6 surface ECG leads (I, II, III, aVR, aVL, aVF) were recorded.

Recordings were performed during atrial pacing (A-pace; left atrial appendage) and ventricular pacing (V-pace, anterobasal LV; see Supplemental Methods for details on pacing protocols). In brief, during V-pacing the stimulation protocol incorporated successive sequences of at least 300 beats: first at basic cycle length (S1), then at a shorter cycle length (S2), and then again at S1 (S1-S2-S1). S2 was shortened in 30-ms steps until S2 approached the T wave end. During A-pacing, only the S1-S2-S1 sequence with the shortest possible S2 cycle length was used.

The following surface ECG measurements were made: RR interval, QRS duration, QT interval and Bazett-corrected QT interval ( QTc). We also determined moments of T wave peaks (T\(_{p1}\)= first peak; and T\(_{p2}\)= second peak for bifid T waves) relative to QRS onset. T wave duration (T-width) was determined using the tangent method and averaged over all leads.

Activation time (AT) and repolarization time (RT) were determined at each recording site as the interval from QRS onset or ventricular pacing spike on ECG to the minimum derivative of the local QRS and maximum derivative of the local T wave,\(^{18}\) respectively. Local activation-recovery intervals (ARI) – a measure of local action potential duration – was defined as RT – AT.\(^{18,19}\) Diastolic interval (DI) was calculated as preceding RR interval – RT. Dispersion of repolarization time (dRT) within a region was determined as
maximum – minimum RT. Interventricular dRT was defined as mean LV RT – mean RV RT, and LV apicobasal dRT was defined as mean LV apex RT – mean LV base RT. Signal analysis was performed offline using a custom-made data analysis program written in Matlab 2006b (The MathWorks Inc., Natick, MA).20

We evaluated beat-to-beat RT responses to RR transition and constructed steady-state ARI-DI restitution lines (last complex of S2 sequence) from V-pacing recordings. Details of acquisition and analysis are provided in the Supplemental Methods.

Statistics
Continuous variables are presented as mean±SEM. A paired t test was used to compare variables between baseline and dofetilide, between LV and RV, and between LV base and LV apex. Correlations between T-width and RT dispersion were tested with Pearson r. Transmural dispersion and responses in ARI were tested by paired t tests with Bonferroni correction. P≤0.05 was considered statistically significant.

RESULTS
Surface ECG
Figure 1 shows the typically negative T waves in canine ECG leads II, III, and AVF21 at baseline and during dofetilide infusion (atrial pacing). During dofetilide infusion, the T waves in leads II, III, aVL, and aVF were bifid, not unlike those in the limb leads and mainly precordial leads in the LQT2 patient.8 Table 1 lists the ECG characteristics during sinus rhythm and slow A-pacing. In all dogs, dofetilide significantly increased RR intervals during sinus rhythm and prolonged QT and QTc intervals during sinus rhythm and atrial pacing. Bifid T waves appeared in 4 of 6 dogs. In the other 2 dogs, steady-state QTc prolongation did not reach 25% and T-width widening was smallest.

<table>
<thead>
<tr>
<th>Table 1: ECG variables during sinus rhythm and A-pacing.</th>
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<tbody>
<tr>
<td><strong>Sinus Rhythm</strong></td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>RR, ms</td>
</tr>
<tr>
<td>QRS, ms</td>
</tr>
<tr>
<td>QT, ms</td>
</tr>
<tr>
<td>QTc</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>505 ± 24</td>
</tr>
<tr>
<td>76 ± 1</td>
</tr>
<tr>
<td>266 ± 4</td>
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<tr>
<td>376 ± 6</td>
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</table>

Values are given as mean ± SEM.
Chapter 6

Repolarization maps

AT and RT were obtained from 118-120 epicardial recording sites per heart. Figures 2A and 2C show typical repolarization maps at baseline and during dofetilide (slow A-pacing). At baseline, repolarization starts at the RV apex and ends at the LV apex. During dofetilide, repolarization still proceeds from RV apex to LV apex, although interventricular RT dispersion is larger compared to baseline (note crowded isochrones). With dofetilide, RV repolarization occurs substantially earlier than LV repolarization. Dispersion is smaller within RV than LV regions. Increased interventricular RT dispersion and smaller RT dispersion within RV compared to LV occurred during dofetilide in 4 of 6 hearts and was smaller or absent in animals without bifid T waves after dofetilide infusion. Activation maps were similar between baseline and dofetilide (see Supplemental Figure S2). Table 2 summarizes data on activation and repolarization during slow A-pacing for the entire heart, the RV, and the LV. RT and dispersion in RT increased during dofetilide. The prolonging effect on RTs was about twice as large in LV as in RV. RT dispersion increased only in LV, not in RV. Interventricular and LV apicobasal RT dispersion was also increased.

At faster pacing rates (Figures 2B and 2D), the repolarization pattern was similar to that at slower rates, although RTs were shorter because of physiologic adaptation (restitution) to higher heart rate. During V-pacing, similar changes in repolarization occurred.

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**Figure 1:** T waves on an ECG of a dog at baseline and dofetilide.

**Repolarization maps**

AT and RT were obtained from 118-120 epicardial recording sites per heart. Figures 2A and 2C show typical repolarization maps at baseline and during dofetilide (slow A-pacing). At baseline, repolarization starts at the RV apex and ends at the LV apex. During dofetilide, repolarization still proceeds from RV apex to LV apex, although interventricular RT dispersion is larger compared to baseline (note crowded isochrones). With dofetilide, RV repolarization occurs substantially earlier than LV repolarization. Dispersion is smaller within RV than LV regions. Increased interventricular RT dispersion and smaller RT dispersion within RV compared to LV occurred during dofetilide in 4 of 6 hearts and was smaller or absent in animals without bifid T waves after dofetilide infusion. Activation maps were similar between baseline and dofetilide (see Supplemental Figure S2). Table 2 summarizes data on activation and repolarization during slow A-pacing for the entire heart, the RV, and the LV. RT and dispersion in RT increased during dofetilide. The prolonging effect on RTs was about twice as large in LV as in RV. RT dispersion increased only in LV, not in RV. Interventricular and LV apicobasal RT dispersion was also increased.

At faster pacing rates (Figures 2B and 2D), the repolarization pattern was similar to that at slower rates, although RTs were shorter because of physiologic adaptation (restitution) to higher heart rate. During V-pacing, similar changes in repolarization occurred,
but with smaller effects in interventricular RT dispersion, probably because of a slower and opposite activation sequence (see Supplemental Figure S3 and Supplemental Table).

Larger repolarization dispersion during dofetilide led to biphasic T waves in local epicardial recordings at the LV-RV border zone. Figure 3 shows an example of the transition of T wave morphology in epicardial electrograms recorded from RV to LV across the anterior wall, in which biphasic T waves are obvious (asterisks). As a result, transition from RV to LV (recordings 5 and 6) manifests a clear jump in RT. This phenomenon occurred in 5 of 6 hearts.

The first T wave peak (Tp1) was significantly later during dofetilide than at baseline (Table 2). In 4 of 6 dogs, a second T wave peak (Tp2) was observed. There was a high

**Figure 2:** Repolarization maps from a dog during atrial pacing at different cycle lengths, at baseline and during dofetilide. **A:** Baseline. Last beat of slow pacing rate sequence (RR=500 ms). **B:** Baseline. Beat 3 of fast pacing rate sequence (RR=300 ms). **C:** Dofetilide. Last beat of slow pacing rate sequence (RR=650 ms). **D:** Dofetilide. Beat 3 of fast pacing rate sequence (RR=550 ms). See text for discussion. HR= heart rate.
Table 2: Electrophysiologic variables during slow A-pacing.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Dofetilide</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>No. of animals</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>AT duration, ms</td>
<td>43 ± 3</td>
<td>39 ± 2</td>
<td>0.09</td>
</tr>
<tr>
<td>RT, ms</td>
<td>215 ± 5</td>
<td>313 ± 24</td>
<td>0.01</td>
</tr>
<tr>
<td>RT_RV, ms</td>
<td>201 ± 5*</td>
<td>270 ± 16*</td>
<td>0.01</td>
</tr>
<tr>
<td>RT_LV, ms</td>
<td>226 ± 5</td>
<td>348 ± 32</td>
<td>0.01</td>
</tr>
<tr>
<td>dRT, ms</td>
<td>48 ± 5</td>
<td>128 ± 25</td>
<td>0.02</td>
</tr>
<tr>
<td>dRT_RV, ms</td>
<td>24 ± 4</td>
<td>23 ± 4*</td>
<td>0.75</td>
</tr>
<tr>
<td>dRT_LV, ms</td>
<td>23 ± 1</td>
<td>74 ± 10</td>
<td>0.02</td>
</tr>
<tr>
<td>dRT_inter, ms</td>
<td>25 ± 4</td>
<td>79 ± 19</td>
<td>0.03</td>
</tr>
<tr>
<td>dRT_apicobasal, ms</td>
<td>15 ± 2</td>
<td>43 ± 10</td>
<td>0.03</td>
</tr>
<tr>
<td>Tp1, ms</td>
<td>214 ± 7†</td>
<td>287 ± 18‡</td>
<td>0.03</td>
</tr>
<tr>
<td>Tp2, ms</td>
<td></td>
<td>354 ± 23§ (n=4)</td>
<td></td>
</tr>
<tr>
<td>T-width, ms</td>
<td>99 ± 6</td>
<td>172 ± 25</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM.

AT duration= maximum activation time; RT= repolarization time; dRT= dispersion of repolarization time; dRT_apicobasal= left ventricular apicobasal dispersion of repolarization time; dRT_inter= interventricular dispersion of repolarization time; LV= left ventricle; RV= right ventricle; T-width = T wave duration; Tp1= first T wave peak; Tp2= second T wave peak.

* P<0.05 for RV vs LV
† P= NS for correlation between RT_RV and Tp1 at baseline (R²=0.30)
‡ P< 0.01 for correlation between RT_RV and Tp1 during dofetilide (R²=0.98)
§ P< 0.01 for correlation between RT_LV and Tp2 during dofetilide (R²=0.98)

Figure 3: Example of transition of epicardial electrograms from right ventricle (RV) to left ventricle (LV) during atrial pacing (RR=550 ms) and dofetilide infusion. The repolarization time (RT) map (bottom) is copied from Figure 2D. Numbers of recordings correspond to electrode positions on RT map. Red dots indicate time of local repolarization (dV/dt max). Note that T waves in LV and RV are large and opposite in direction, and that at intermediate recording sites T waves are biphasic, with a second maximum dV/dt (red unfilled circles, remote repolarization).
correlation between Tp1 and Tp2 with mean RV and LV repolarization, respectively (Table 2), although there was no correlation of Tp1 with either mean RV or LV repolarization at baseline. Figure 4A shows a typical repolarization map at baseline and during dofetilide with moments of T wave peaks (black lines). In all hearts with bifid T waves, both Tp isochrones coincided with the RV-LV border. Figure 4B shows a highly significant correlation between T-width and interventricular RT dispersion during dofetilide. Bifid T waves (asterisks) occurred at the largest interventricular RT dispersions. The correlation between T-width and LV apicobasal RT dispersion during dofetilide was low and not significant. When heart rate increased, interventricular RT dispersion reduced and bifid T wave morphology attenuated (see Supplemental Figure S4).

**Figure 4:** A: Repolarization maps together with moments of T wave peaks (black lines, Tp1 and Tp2) of a heart at baseline (left) and during dofetilide (right), during slow atrial pacing. B: Relation between T wave width (T-width) and mean interventricular or left ventricular apicobasal repolarization time dispersion (dRT_inter, dRT_apicobasal, respectively) in all hearts during dofetilide and atrial pacing. Note that the greater the interventricular repolarization time dispersion, the wider the T wave.
Regional restitution lines

Supplemental Figure S5 shows beat-to-beat responses of RT after a change in pacing rate. As a result of fast pacing RTs in LV shortened in an alternating fashion, whereas little or no adaption of RTs occurred in RV. To quantify the heart rate-induced response in repolarization, we reconstructed restitution lines per region (RV, LV, LV apex, and LV base) at baseline and dofetilide. Figure 5A shows typical regional restitution data in 1 heart at baseline and during dofetilide (lower and upper boxes, respectively). Restitution lines were constructed as steady-state ARI vs DI. Solid lines represent the averaged restitution lines of electrodes within a region. At baseline, restitution slopes were different between regions (LV vs RV, and LVbase vs LVapex), although differences were small. During dofetilide, restitution slopes were larger, also between regions, particularly between LV and RV. Figure 5B summarizes restitution data for all experiments (n=6). At baseline, RV restitution slopes were significantly smaller than LV slopes, and LV slopes were smaller at the base than at the apex. Differences in slopes between LV and RV and between LV apex and base were significantly larger during dofetilide than baseline.

\[
\begin{align*}
\text{DOFETILIDE} & \\
\text{RV} & \quad \text{ARI} = 0.13 \times \text{DI} + 210.81 \\
\text{LV} & \quad \text{ARI} = 0.69 \times \text{DI} + 194.33 \\
\text{LV}_{\text{base}} & \quad \text{ARI} = 0.57 \times \text{DI} + 174.51 \\
\text{LV}_{\text{apex}} & \quad \text{ARI} = 0.76 \times \text{DI} + 218.04 \\
\text{BASELINE} & \\
\text{RV} & \quad \text{ARI} = 0.09 \times \text{DI} + 132.39 \\
\text{LV} & \quad \text{ARI} = 0.21 \times \text{DI} + 140.54 \\
\text{LV}_{\text{base}} & \quad \text{ARI} = 0.17 \times \text{DI} + 141.28 \\
\text{LV}_{\text{apex}} & \quad \text{ARI} = 0.23 \times \text{DI} + 137.22
\end{align*}
\]

**Figure 5:** A: Mean restitution lines of different areas in a heart at baseline (bottom) and during dofetilide (top). There is a major shift in the lines after dofetilide, with a much steeper slope of left ventricle (LV) compared to right ventricle (RV). ARI= activation-recovery interval; DI= diastolic interval. B: Mean restitution slopes were averaged for all 6 animals, and differences between LV and RV and between LVapex and LVbase were tested (paired t test, * $P<0.05$, top). Regional slope differences (LV-RV and LVapex-LVbase) between baseline and dofetilide were tested as well (paired t test, * $P<0.05$, bottom).
Transmural repolarization

It has been suggested that \( I_{Kr} \) block leads to a disparate response in ARI across the LV wall.\(^{22,23} \) This may contribute to a change in T wave morphology. Therefore, we determined whether the effect of dofetilide was different across the LV wall. As shown in the Supplemental Results and Supplemental Figure S6 ARIs were shorter at the epicardium than at the endocardium both at baseline and during dofetilide without midmural maxima. Furthermore, dofetilide-induced changes in ARI were homogeneous across the LV wall.

DISCUSSION

The appearance of bifid (or notched) T waves in dogs with dofetilide-induced LQT2 is related to increased interventricular dispersion of repolarization and steepening of the restitution slope that is larger in LV than RV. The dofetilide effect on ARIs across the ventricular walls is homogeneous.

Dofetilide effect

The dofetilide-induced increase in QT and QTc intervals, RTs, and dispersion of repolarization is consistent with earlier reports.\(^{24} \) The repolarization-prolonging effect of dofetilide was larger in LV than RV. Intraventricular RT dispersion increased in LV alone. This may be explained by the higher \( I_{Kr} \) density in RV than LV,\(^ {15} \) which plays a dominant role in repolarization after \( I_{Kr} \) blockade.\(^ {14} \) As a consequence, less repolarizing current (\( I_{Ks} \)) will be available in LV, resulting in greater prolongation of LV than RV repolarization.

After dofetilide, we observed a steeper restitution slope in LV than in RV, which is inconsistent with the anticipation that a higher \( I_{Ks} \) density in RV encompasses a steeper restitution slope than in LV. However, DIs in RV were approximately 50 ms larger than in LV and therefore may be nearer to the plateau of its restitution curve.\(^ {25} \) Additionally, methodologic considerations (use of linear instead of a higher-order function and not pacing at minimum possible DI) may have led to underestimation of the maximum steepness of the slope.

When heart rate was increased by pacing during dofetilide, an alternation in RT occurred during the first beats of the rapid rate in LV only (see Supplemental Figure S4).\(^ {26} \) Our data suggest that this change is due to the steeper restitution slope and the shorter DIs in LV compared to RV.

Bifid T waves

We observed bifid T waves in 4 of 6 dogs after dofetilide infusion. This is consistent with results of another study in dofetilide-treated dogs.\(^ {27} \) The T waves had a negative polarity
in most leads, typical of canine hearts, and in contrast with positive T waves in most leads in the human ECG. This discrepancy is likely explained by the different thoracic heart position and thorax shape of the dog. Additionally, there may be a species difference in the repolarization sequence. Furthermore, in LQT2 patients bifid (or notched) T waves have a low amplitude, in contrast with our observations. However, Zhang et al. showed that the amplitude of bifid T waves was variable (SD 0.25 mV) and that its distribution was skewed and therefore must have included some high voltage T waves. Although T wave morphology is recognizable for both congenital and acquired LQT, bifid T waves have a low incidence in acquired LQT patients. However, this low incidence may be associated with smaller QTc prolongation compared to our study, due to a more prudent drug challenge in patients.

Clinically, the first peak of the T wave usually has a higher amplitude in right precordial leads, whereas the second peak of the T wave has a higher amplitude in left precordial leads (Figure 2 in Zhang et al.). Therefore, we reasoned that the first T wave peak is spatially associated with RV repolarization and that the second T wave peak with LV repolarization. Our data confirm that RV repolarization is early, whereas LV repolarization is late (Figures 2-4). The disparate repolarization of RV and LV during dofetilide may result in 2 separated T waves, which, by superimposition, may lead to a bifid morphology. Accordingly, we demonstrated that isochrones of the T wave peaks (Tp1 and Tp2) did concur with the end of RV repolarization and the first LV repolarization (Figure 4A). Moreover, T-width did correlate with mean interventricular dispersion of repolarization (Figure 4B). This indicates that interventricular dispersion of repolarization play a role in generating both components of the bifid T wave. LV apicobasal RT dispersion and difference in ARI restitution slopes also were increased and may contribute to the bifid T wave as well. However, interventricular differences were approximately 1.5–2 times larger. Also, the relationship between LV apicobasal RT dispersion and T-width was weaker. Therefore, we suggest that LV apicobasal RT dispersion has no prominent role in bifid T wave genesis.

In children without detectable heart disease, the incidence of bifid T waves in leads V2 and V3 is high (18.3%) and usually concurs with normal QTc intervals, likely excluding repolarization pathology. Calabrò et al. suggested that this phenomenon depends on the hypothetical figure-of-eight shape T-loop on the horizontal plane, which may disappear with aging because of changes in the ventricular repolarization process. Thus, an age-dependent shift in the balance between Ik and Iks may explain the bifid T wave morphology in the young. Indeed, our voltage-clamp study demonstrated that in the majority of LV cardiomyocytes from young dogs, only Ik is functionally expressed, whereas Iks and Ikf are both present in adult myocardium.

Bifid (or notched) T waves can also be observed in patients with alcoholic cardiomyopathy or in those with central nervous system disease. Interestingly, the latter study
suggested that notched T waves may be related to divergence of RT of 2 ventricular cell populations, due to alterations in sympathetic tone. This is in line with the disparate repolarization of the RV and LV that we observed.

Nevertheless, some precaution should be taken when comparing our model to LQT2 patients. Our model is essentially an “acquired” LQT2 model (i.e., acute $I_{Kr}$ block) and may differ from “congenital” LQT2 patients (i.e., chronic $I_{Kr}$ absence), in which remodeling of the balance in $I_{Kr}$ and $I_{Ks}$ may have played a role in the mechanism. Also, remodeling of other repolarizing currents may influence the mechanism. In addition, contrary to man, the T wave in dogs typically is negative in the “standard” leads. Therefore, extensive electrophysiologic mapping in LQT2 patients is needed to fully corroborate that a similar mechanism holds for the human bifid T wave.

Viskin et al$^{10}$ showed that bifid (or notched) T waves exaggerate during sudden standing and increased heart rates. In contrast, in our study, bifid T waves attenuated at faster rates. However, an exaggeration or attenuation of bifid T waves may depend on how fast the heart rate is before standing (Figure 6, left or right to intersection of LV and RV slopes). Also, we did not incorporate autonomic nerve modulations occurring during sudden standing, and these may also play a role in bifid T waves.

**Figure 6:** Schematic restitution lines (activation-recovery interval [ARI] versus RR) at baseline and during dofetilide for 2 regions within the heart. Lines were based on our data (solid lines) and extrapolation using literature (dashed lines). LV= left ventricle; RV= right ventricle.

### Transmural dispersion of repolarization

Antzelevitch et al$^{22,23}$ have demonstrated a disparate response of an $I_{Kr}$ blocker on action potential duration across the LV wall in a canine wedge preparation. Our study in open-chested dogs showed that dofetilide-induced changes in ARI were homogeneous across the LV wall. The discrepancy in transmural response may be due to the use of a different pharmacological agent and, probably more importantly, the use of different preparations. It has been suggested that the sodium-blocking effect of propofol may reduce
heterogeneity of repolarization. However, propofol was used only to induce anesthesia (see Supplementary Methods), so it is unlikely that it influenced repolarization at the time of recording. Furthermore, we did observe a large increase in interventricular RT dispersion, and there is no compelling reason to assume that transmural RT dispersion would be selectively decreased.

**Implications for arrhythmogenesis**

Restitution of the RV had a flatter slope in our experiments than that of the LV, particularly after dofetilide infusion. This may have consequences for arrhythmogenesis. Dofetilide alters the repolarization properties of myocardial tissue such that RV and LV restitution shift away from each other (schematically illustrated in Figure 6). We could only construct restitution lines over a limited range of cycle lengths, due to LV pacing (solid lines). The rest of the line was extrapolated using theory from the literature (broken lines). During dofetilide infusion, interventricular RT dispersion is largest during long pacing cycle lengths. In this condition, a short coupled premature beat occurring in the RV will be able to conduct through the RV but will find activation block at the LV due to refractoriness (Figure 6), especially after a preceding pause. This is in line with the reported long-short sequence preceding torsade de pointes.

The schematic restitution lines in Figure 6 may also explain alterations of bifid (or notched) T wave morphology during heart rate changes. For example, a transition in heart rate will cause a shift on the x-axis resulting in a change in interventricular dispersion in ARI (i.e., change in interventricular RT dispersion), which in turn may determine the presence and morphology of bifid T waves.

**CONCLUSION**

The dofetilide-induced bifid (or notched) T wave is correlated with the large dispersion of repolarization between RV and LV.

**ACKNOWLEDGEMENTS**

We thank Ya-Ping Jiang, MD, Tania Rahim, MS, and Ira S. Cohen, MD, PhD, for technical support during the experiments.
REFERENCES


DATA SUPPLEMENT

Supplemental Methods

Surgical preparation
Six male mongrel dogs weighing 23-26 kg were preanesthetized with propofol (6-8 mg/kg i.v., 60-90 minutes before measurements were made), intubated, artificially ventilated and anesthetized with a mixture of isoflurane (2% - 3.5%) and oxygen. Two peripheral intravenous catheters were inserted for administration of fluids and drugs. A femoral artery catheter was used to monitor blood pressure continuously. A left thoracotomy was performed at the fifth intercostal space, and the heart was suspended in a pericardial cradle. To provide atrial and ventricular stimulation, bipolar silver electrodes were sewn to the left atrial appendage and the anterobasal left ventricle, respectively. Body temperature was monitored via a thermistor probe placed deep within the thorax and maintained at 36-37°C via a heating pad and heating lamp.

Experimental setup
Supplemental Figure S1 show a schematic of the experimental setup. A grid with 11x11 electrodes (5-mm interelectrode distance) and 9-10 intramural plunge needles were used to obtain local electrograms. Simultaneously, surface ECGs were obtained.

Figure S1: Schematic of the experimental setup. Block pulses at left atrium and left ventricular base indicate the location of stimulating electrodes. An 11x11 electrode grid was positioned over the anterior left ventricle (LV) and right ventricle (RV). Plunge needles were inserted at several locations surrounding the 11x11 grid. To determine electrophysiologic variables for different regions within the heart, we selected groups of electrodes of the 11x11 grid that represent these regions. In the figure the selections are indicated by the colored boxes.
Pacing protocol
The pacing protocol incorporated pacing at the atrium (A-pacing) or at the ventricle (V-pacing). During V-pacing each ventricular stimulus was preceded by a stimulus at the left atrial appendage with an interval of 20 to 30 ms, to avoid influences of decremental AV conduction on ventricular rhythms and prevent coincidence of the remote P-wave with T waves in local unipolar electrograms. The V-pacing stimulation protocol incorporated a S1-S2-S1 sequence with at least 300 beats per cycle length. The S1 was the basic cycle length (just faster than intrinsic sinus rate) and S2 was at a shorter cycle length than S1. S2 was sequentially shortened by 30 ms until S2 approached the end of the T wave. Following we performed the A-pacing stimulation protocol, which was a repetition of the S1-S2-S1 sequence with the shortest possible S2 cycle length performed during V-pacing.

Steady-state restitution lines
Steady-state restitution lines (last complex of S2 sequence) were constructed from ARI and DI data obtained during V-pacing. First we calculated a linear regression line \( ARI = \text{slope} \times DI + \text{intercept} \) per epicardial electrode. For each region (RV, LV, LV base, and LV apex) we determined a mean restitution line as the average slope and intercept of all lines within a group of epicardial electrodes (Supplemental Figure S1: 33 electrodes for RV, 44 for LV of which 16 for LV base and 16 for LV apex. In one dog, RV area was small, permitting use of only 22 electrodes).

Acquisition
Local unipolar electrograms were recorded using a 256-channel amplifier (BioSemi, 24 bit dynamic range, 122.07 nV bit step, total noise 0.5 µV); sampling frequency of 2048 Hz (bandwidth \([-3\text{dB}]\) DC-400 Hz). The active common mode electrode was positioned in the subcutaneous fat at the sternal incision site. Stimulation pulse amplitude was 1.5 x stimulation threshold and pulse width \(=2\) ms. We used an EMKA system (version 1.8) to simultaneously record 6-lead ECGs throughout each experiment. Sampling frequency was 1000 Hz with filtering with a high-pass 0.05 Hz and a low-pass 200 Hz.

Supplemental Results

Activation: A-pacing versus V-pacing
The activation during A-pacing started at the RV apical region and ended at the LV or RV basal region. During V-pacing activation was in the opposite direction, starting always at the LV basal region and ended at the RV apical region. An example of activation maps during A-pacing and V-pacing is demonstrated in Supplemental Figure S2. This figure demonstrates that the activation sequence is opposite in direction between the two
pacing modes. It demonstrates also that activation is slower and occurs later after the start of activation within the heart (defined as the beginning of the QRS complex or pacing artefact). Activation did not differ between baseline and dofetilide.
Repolarization during V-pacing

Supplemental Figure S3A and S3C show typical examples (same dog as in Figure 3) of repolarization maps at baseline and during dofetilide during V-pacing at a slow pacing rate (RR 500 ms and RR 650 ms, respectively). At baseline, repolarization started in the LV basal region and ended in 3 animals in the LV apical region (as in the figure) and in

**Figure S3**: Examples of repolarization maps of the dog in Figure 2 during ventricular pacing (V-pace) at different cycle lengths, at baseline and during dofetilide. **A**: Baseline. Last beat of the slow pacing rate sequence (RR= 500 ms). **B**: Baseline. Beat 10 of the fast pacing rate sequence (RR= 300 ms). **C**: Dofetilide. Last beat of the slow pacing rate sequence (RR= 650 ms). **D**: Dofetilide. Beat 10 of the fast pacing rate sequence (RR= 550 ms).
3 animals in the RV apical region. During dofetilide infusion, interventricular RT dispersion is increased and repolarization starts at the RV basal region and ends in all animals at the LV apex. However, due to stimulation from the LV base repolarization at LV base remains substantially early, leading to smaller interventricular dispersion in repolarization compared to repolarization during A-pacing after dofetilide (see Figure 3C and 3D).

Supplemental Table summarizes the data on activation and repolarization times and dispersion in repolarization during V-pacing at a slow rate for the entire heart, the RV and the LV. It shows similar results as during A-pacing (Table 2), although differences in repolarization between the LV and RV were less prominent.

**Supplemental Table:** Electrophysiologic variables during ventricular pacing (V-pacing) at slow heart rate.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Dofetilide</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>AT duration, ms</td>
<td>108 ± 2</td>
<td>105 ± 3</td>
<td>0.25</td>
</tr>
<tr>
<td>RT, ms</td>
<td>265 ± 5</td>
<td>360 ± 20</td>
<td>0.01</td>
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<tr>
<td>RT_RV, ms</td>
<td>266 ± 6</td>
<td>336 ± 12</td>
<td>0.01</td>
</tr>
<tr>
<td>RT_LV, ms</td>
<td>263 ± 5</td>
<td>377 ± 28</td>
<td>0.01</td>
</tr>
<tr>
<td>dRT, ms</td>
<td>40 ± 2</td>
<td>102 ± 19</td>
<td>0.01</td>
</tr>
<tr>
<td>dRT_RV, ms</td>
<td>20 ± 3 *</td>
<td>22 ± 6 *</td>
<td>0.50</td>
</tr>
<tr>
<td>dRT_LV, ms</td>
<td>39 ± 3</td>
<td>88 ± 16</td>
<td>0.01</td>
</tr>
<tr>
<td>dRT_inter, ms</td>
<td>6 ± 2</td>
<td>45 ± 15</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM.

AT duration = maximum – minimum activation time; RT = repolarization time; dRT = dispersion in repolarization time; dRT_inter = interventricular dispersion of repolarization time; LV = left ventricle; RV = right ventricle.

* P<0.05 for RV vs LV

Response in bifid T wave morphology to heart rate changes

Supplemental Figure S4 shows a typical response in bifid T wave morphology with corresponding repolarization times to a change in heart rate during atrial pacing and dofetilide. The first beat at slow heart rate (RR650) show a clear bifid T wave on the ECG. During the following beats at a faster heart rate (RR550), the RT dispersion between the LV and RV decreased (bottom panel) and led to a less pronounced morphology of the bifid T wave (top panel).

RT response to abrupt heart rate changes

We assessed the response in repolarization to changes in heart rate. Supplemental Figure S5A and S5B show typical RT responses in 5 selected epicardial recordings (2 at RV epicardium, base and apex; and 3 at LV epicardium, base, central, and apex) during transitions of cycle length (long [1 complex] – short [11 complexes] – long [11 complexes]) in absence and presence of dofetilide. At baseline (Supplemental Figure S5A), the transi-
tion in pacing rate (RR= 500 ms to 300 ms) led to shortening of repolarization times of approximately 30 ms in LV and 20 ms in RV. RTs prolonged after the return to slow pacing. However, during dofetilide infusion (Supplemental Figure S5B), the response to transitions in pacing rate (RR= 650 to 550 ms) differed substantially between LV and RV. In the LV, RTs shortened as a result of fast pacing and this occurred in an alternating fashion. In contrast, little or no adaption of RTs occurred in RV in response to a change in pacing rate. The difference in adaptation is emphasized in the epicardial electrograms of the first 6 complexes (last slow and first 5 fast) in Supplemental Figure S5C for one LV and one RV recording site (asterisks in Supplemental Figure S5B).

Transmural dispersion of repolarization
It has been suggested that I_{Kr} block leads to a disparate response in ARI across the LV wall.\textsuperscript{1,2} This may contribute to a change in T wave morphology. Therefore, we determined whether the effect of dofetilide was different across the LV wall. For testing transmural ARI dispersion and ARI responses we selected needles without missing data (i.e., 4 electrograms per needle) both at baseline and during dofetilide. Supplemental Figure S6A shows 11 transmural ARI profiles obtained in 4 hearts. ARIs were shorter at the epicar-
Bifid $T$ waves in LQT2

During both baseline and during dofetilide, repolarization time (RT) was shorter at the epicardium than at the endocardium both at baseline and during dofetilide without midmural maxima. We also calculated for each transmural recording site the dofetilide-induced change in ARI (ARI dofetilide – ARI baseline). The changes in ARIs were homogeneous.

**Figure S5**: A typical response of repolarization time (RT) to a change in heart rate (V-pacing: slow-fast and fast-slow) at baseline (A) and dofetilide (B). The RT responses in 5 epicardial recordings (2 at RV epicardium [red] and 3 at LV epicardium [blue]) are shown. The x-axes show the following RT complex numbers: last of slow pacing sequence – first 10 and last of fast pacing sequence – first 10 and last of returning slow pacing sequence. The y-axes show RT. C: Epicardial recordings from LV (blue) and RV (red) of sequential complexes (last of slow and first 5 of fast pacing rate), aligned on the QRS complex. Note the large changes in LV repolarization (ascending slope of the $T$ wave) compared to minimal changes in RV.
across the LV wall, with minor difference (10 ms) between epicardium and endocardium. As a consequence, the RT profiles were almost flat between epicardium and endocardium at baseline and during dofetilide (Supplemental Figure S6B). Obviously, differences in RT between endocardium and any transmural site, including the epicardium, cannot explain the occurrence of bifid T waves in the presence of dofetilide.

Supplemental References
