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Calcium oscillations and T-wave lability precede ventricular arrhythmias in acquired long QT type 2

Jan Němec¹, Jong J. Kim², Beth Gabris³, and Guy Salama^{3,4}

¹Cardiovascular Institute, Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

²Department of Bioengineering, School of Engineering, University of Pittsburgh, Pittsburgh, PA 15261

³Department of Cell Biology and Physiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

Abstract

Background—Alternans of intracellular Ca^{2+} (Ca_i) underlies T-wave alternans, a predictor of cardiac arrhythmias. A related phenomenon, T-Wave Lability (TWL), precedes *Torsade de Pointes* (TdP) in patients and animal models with impaired repolarization. However, the role of Ca_i in TWL remains unexplored.

Methods—Action potentials (APs) and Ca_i transients, (CaT s) were mapped optically from paced Langendorff female rabbit hearts ($n=8$) at 1.2s cycle length, after AV node ablation. Hearts were perfused with normal Tyrode's solution then with dofetilide (0.5 μM) and reduced $[\text{K}^+]$ (2 mM) and $[\text{Mg}^{2+}]$ (0.5 mM) to elicit long QT type 2 (LQT2). Lability of EKG, voltage and Ca_i signals were evaluated during regular paced rhythm, before and after dofetilide perfusion.

Results—In LQT2, lability of Ca_i , voltage and EKG signals increased during paced rhythm, before the appearance of early afterdepolarizations (EADs). LQT2 resulted in AP prolongation and multiple (1-3) additional Ca_i upstrokes, while APs remained monophasic. When EADs appeared, Ca_i rose before voltage upstrokes at the origins of propagating EADs. Interventions (i.e. ryanodine and thapsigargin, $n=3$ or low $[\text{Ca}]_o$ and nifedipine, $n=4$) that suppressed Ca_i oscillations also abolished EADs.

Conclusions—In LQT2, Ca_i oscillations (Ca_iO) precede EADs by minutes, indicating that they result from spontaneous sarcoplasmic reticulum Ca^{2+} release rather than spontaneous I_{CaL} reactivation. Ca_iO likely produce oscillations of Na/Ca exchange current, I_{NCX} . Depolarizing I_{NCX} during the AP plateau contributes to the generation of EADs by re-activating Ca^{2+} -channels that have recovered from inactivation. TWL reflects CaT s and APs lability that occur before EADs and TdP.

⁴To whom all correspondence should be addressed: Guy Salama, University of Pittsburgh, School of Medicine, 200 Lothrop Street, S 312 Biomedical Science Tower, Pittsburgh, PA 15261, gsalama@pitt.edu; Phone: 412-648-9354; Fax: 412-383-6517. JN and JK contributed equally

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Key Words and Abbreviations

Electrophysiology; Calcium Oscillations; Long QT syndrome, (LQTS); T-Wave Lability, TWL; Simultaneous optical mapping of voltage and calcium; impaired repolarization

Introduction

The prolongation of ventricular action potential duration (APD) and QT interval often leads to life-threatening polymorphic ventricular tachycardia (VT) that exhibit a characteristic EKG appearance known as *Torsade de Pointes* (TdP). Acquired long QT (LQT) is of critical importance because of its prevalence in the clinical setting. Several factors (e.g. medications, electrolyte abnormalities and heart failure) impair ventricular repolarization and may lead to lethal arrhythmias. LQTS is also of conceptual importance, because the congenital form represents a “pure global repolarization disease”,¹ which demonstrates a direct link between repolarization delay and sudden cardiac death (SCD).² Although the molecular defects leading to prolonged APDs in congenital LQTS have been elucidated in remarkable detail,³ the mechanism by which impaired repolarization causes VT on the tissue level remains less clear.⁴⁻⁶ Two hypotheses, which are not mutually exclusive, have been proposed. First, prolonged APDs result in triggered activity in the form of early afterdepolarizations (EADs).² Second, spatially heterogeneous APD prolongation leads to increased dispersion of refractoriness and may form a substrate for functional reentry.^{3, 4}

Clinically, prolonged APD is reflected on the surface EKG as QT interval prolongation. Several forms of temporal repolarization instability, such as microvolt T wave alternans (mTWA)^{7,8} and increased QT interval variability⁹⁻¹¹ (QTV), have been linked to SCD in clinical and experimental settings. Multiple lines of evidence suggest that Ca_i alternans leads to APD alternans and mTWA.¹²⁻¹⁴ However, arrhythmias during impaired repolarization are classically associated with bradycardia or pauses, whereas mTWA is usually a tachycardia-induced phenomenon. Microvolt TWA is not frequently observed in LQT,^{15,16} and may not be necessary for induction of LQT-related arrhythmias. On the other hand, non-alternans T wave lability (TWL) precedes TdP in LQTS patients^{17,18} as well as in animal models of prolonged repolarization.^{19,20} TWL refers to beat-to-beat changes in T wave morphology, which do not follow an alternans (i.e. ABAB...) pattern. It appears to be a better predictor of arrhythmia than the absolute degree of repolarization delay.

The mechanisms underlying non-alternans repolarization lability are a matter of speculation and the possible role of abnormal Ca^{2+} handling in this phenomenon remains unexplored. It is possible that TWL is caused by the same process that drives EADs, but generates depolarizations of insufficient amplitude to trigger propagating waves.

EADs have been classically attributed to the spontaneous reactivation of the L-type Ca^{2+} current, I_{CaL} , during the abnormally long APD and slow AP downstroke.^{21,22} Alternatively, spontaneous Ca^{2+} release from the sarcoplasmic reticulum (SR) during phase 2 or phase 3 of the AP can further depolarize the plateau potential through the activation of the electrogenic Na/Ca exchanger (NCX).⁵ A similar mechanism has been documented as the trigger of delayed afterdepolarizations (DADs), but its role for EADs remains controversial. In cryoablated rabbit hearts, simultaneous mapping of transmembrane voltage (V_m) and cytoplasmic free Ca^{2+} (Ca_i) in drug-induced LQT2 showed that a rapid increase in Ca_i precedes the rise of V_m at the first sites that fire EADs on the epicardium.²³ The dynamic relationship between Ca_i and V_m during an EAD supports the notion that SR Ca^{2+} overload and spontaneous SR Ca^{2+} release activate an inward NCX current (I_{NCX}) which triggers I_{CaL} to produce EADs. However, EADs initiated by oxidative stress (with hydrogen peroxide, H_2O_2 0.2-1 mM) were attributed to

Ca^{2+} /Calmodulin kinase (CaMKII) activation which increased I_{CaL} , impaired inactivation of I_{CaL} and of voltage-gated Na^+ current (I_{Na}).²⁴ Still, SR Ca^{2+} release should not be excluded as the trigger of EADs because H_2O_2 acts at numerous targets, including ryanodine receptor, NCX and SR Ca^{2+} pump.

In this report, we investigated the role of Ca_i dynamics on TWL in a non-cryoablated rabbit model of LQT2 using simultaneous measurements of Ca_i transient (CaT), AP and EKG during paced rhythms and focused on events that precede ventricular ectopy.

Methods

Heart preparations

New Zealand White rabbits (female, 60-120 days old) were anesthetized with pentobarbital (35 mg / kg I.V.) and anti-coagulated with heparin (200 U/ kg I.V.). The heart was perfused with Tyrode's solution (mM): 130 NaCl, 24 NaHCO_3 , 1.0 MgCl_2 , 4 KCl, 1.2 NaH_2PO_4 , 50 dextrose, 1.25 CaCl_2 , gassed with 95% O_2 –5 % CO_2 . The atrioventricular node was destroyed with electrocautery to control heart rate. To minimize motion artifact, blebbistatin (5-10 μM for ~ 15 min) was added to the perfusate. The heart was immobilized in a chamber and stained with a voltage-sensitive dye (RH 237: 200 μl of 1 mg/ml dimethyl sulfoxide, DMSO) and loaded with a Ca^{2+} indicator (Rhod-2 AM, 200 μl of 1 mg/ml DMSO). Epicardial bipolar pseudo-EKG was continuously monitored. Epicardial pacing with a unipolar electrode on the right ventricle was performed at cycle length 1.2 s (50 bpm; bradycardia for rabbit hearts). After baseline recordings, LQT2 was imposed by perfusing with Tyrode's containing dofetilide (250-500 nM, Pfizer), a selective I_{Kr} blocker and lowering K^+ and Mg^{2+} concentrations by 50 %.²⁵ This investigation conformed to the current *Guide for Care and Use of Laboratory Animals* published by the National Institutes of Health.

Optical apparatus

The optical apparatus based on two photodiode arrays has been described previously.²⁶ The anterior surface of the heart was illuminated with a 520 ± 30 nm excitation beam and the fluorescence was passed through a dichroic mirror (660 nm) to focus the Rhod-2 and RH 237 fluorescence images on two 16×16 photodiode arrays (Hamamatsu Corp. C4675–103). Outputs from the arrays were amplified, digitized at 1 kHz frequency and stored in computer memory, along with surface EKG.

Data analysis

Automatic measurement of APD and CaT duration (CaTD) from all pixels was used to calculate APD and CaTD dispersion, defined as the standard deviation of APD /CaTD values. Activation time at each site was calculated from $(dF_v/dt)_{\text{max}}$ of the local AP upstroke, and APD (CaTD) at each site was the interval from $(dF_v/dt)_{\text{max}}$ to the recovery of V_m (Ca_i) to 10% of baseline (APD₉₀ or CaTD₉₀). Isochronal maps of APD and CaT duration were generated as previously described.²⁶

In addition, custom-software was created in C++ (Microsoft Visual Studio 6.0, Microsoft Corp., Redmond, WA) for data analysis. Signals were digitally low-pass filtered (3-pole Bessel filter, 20Hz) and baseline fluctuations were subtracted with a smooth cubic spline. Optical signals were evaluated from 5 pixels (2-8, 8-2, 8-8, 15-8, 8-15; the numbers stand for the x-y pixel coordinates of the 16×16 array). Simultaneous EKG, V_m and Ca_i signals were displayed and durations were measured with electronic calipers. During each scan (typically lasting 32 s), the interval from pacing stimulus to end of the T wave (the “QT interval” equivalent) and the local duration of V_m and Ca_i signals were measured in 2 beats and averaged. The number of Ca_i peaks per AP was determined visually and were averaged over 2 beats from 5 pixels in

each of the 32 s scans. Except for the dispersions of APD and CaTD, the average value taken over these 5 pixels were used for subsequent analysis.

The lability of T waves, V_m and Ca_i signals was calculated as previously reported.¹⁶ Briefly, the EKG, V_m or Ca_i signals from each beat (unless excluded due to poor signal quality or subsequent PVC) were superimposed using the stimulation artifact, and the root-mean-square of the beat-to-beat differences in signal amplitude measured at corresponding time points of the repolarization segment (150 ms to 900 ms after the stimulus) were calculated. The maximal root-mean-square value (corresponding to the most labile time point) was normalized to the amplitude of the signal-averaged QRS complex (defined as the maximum minus the minimum value, evaluated 10-900 ms after stimulation artifact, thus including the QRS complex). The reported T wave, V_m and Ca_i lability values are taken as a natural logarithm of the normalized root-mean-square values (Table 1).

The correlation coefficient between V_m and Ca_i signal values during the repolarization segment of the last paced beat not followed by EAD were calculated from 5 pixels as described above and averaged. Again, these values were compared to the corresponding values prior to dofetilide perfusion. Unless described otherwise, two-tailed paired Student t-test (Excel, Microsoft Corp., Redmond, WA) was used to compare signal values obtained from baseline data segments with data segments after dofetilide perfusion, but before the onset of ventricular ectopy. P-values of <0.05 were considered statistically significant.

Results

TdP was induced by dofetilide perfusion in all hearts (n=8) in < 10 minutes and was preceded by short-coupled premature ventricular beats (PVBs). PVBs on the EKG corresponded to EADs on optical V_m tracings. In all cases, the onset of EADs was preceded by prolongation of APD (415 ± 118 vs. 678 ± 235 ms; $p < 0.01$) and of CaT duration (474 ± 144 vs. 702 ± 227 ms; $p < 0.005$) as compared to controls. The QT interval determined from epicardial EKG also prolonged markedly (481 ± 105 vs. 603 ± 172 ms; $p < 0.02$). Occasionally, ectopic ventricular beats with late coupling and V_m tracing suggestive of DADs were observed.

Beat-to-beat lability of repolarization, AP and CaT

TWL increased in all experiments prior to the onset of arrhythmia (-4.061 ± 0.944 vs. -2.894 ± 0.950 ; $n = 8$, $p < 0.002$). TWL corresponded to discernible changes of T wave morphology during regular rhythms preceding ventricular arrhythmias (Fig. 1). In contrast, macrovolt T wave alternans was never observed before the onset of ventricular ectopy (n=8). Similarly, the beat-to-beat lability of optical V_m signals increased prior to VT onset (-3.436 ± 0.492 vs. -2.994 ± 0.779 ; $p < 0.05$). There was a trend towards an increase in CaT lability prior to VT onset which did not reach statistical significance (-3.338 ± 0.372 vs. -2.970 ± 0.647 ; $p = 0.084$). However, at sites of EAD origin, CaT lability was significantly higher compared to baseline for the 2 consecutive beats preceding the onset of EADs (-4.049 ± 0.685 vs. -3.356 ± 1.033 ; $p < 0.05$). Table 1 summarizes these EKG, V_m and Ca_i parameters before and after the induction of LQT2 but before the onset of ectopic beats.

CaT oscillations

Besides beat-to-beat lability, CaT exhibited other forms of instability. Normal CaTs were monophasic (a single Ca_i peak followed by a recovery to baseline) with Ca_i rising ~10 ms after the rise of V_m . Occasionally, a small secondary rise in Ca_i appeared during phase 3 of the AP at a few sites. Perfusion with dofetilide increased the complexity of CaT kinetics in all hearts, with the appearance of multiple Ca_i peaks (Fig. 2 and 3). The average number of CaT peaks occurring during a single AP increased significantly prior to the onset of EADs (1.332 ± 0.387

vs. 2.120 ± 0.469 ; $p < 0.002$). Interestingly, up to 4 distinct Ca_i peaks could be seen in some experiments, while the corresponding local AP remained monophasic, with a smooth downstroke during phase 2 and 3 (Fig. 2B). Ca_i oscillations (Ca_iO) preceded the onset of EADs in all experiments.

Figure 4A illustrates a marked spatial heterogeneity of V_m and Ca_i signals during dofetilide perfusion, but before the onset of EADs. An isochronal map of APD_{90} shows a marked APD prolongation (pixel 1) that decreased anisotropically. Before the onset of EADs, the correlation between V_m and Ca_i signals during repolarization decreased in all experiments (0.9593 ± 0.0231 vs. 0.8967 ± 0.0644 ; $p < 0.02$, 2-tailed sign test), representing an increasing dissociation between the oscillatory Ca_i signal and the monophasic shape and time-course of APs. In paced beats immediately preceding appearance of EADs, the spatial dispersion of CaT duration (CaTD_{90}) exceeded the dispersion of APD_{90} (65 ± 29 vs. 52 ± 35 ms; $p < 0.02$).

During a normal AP, CaTs are tightly controlled by V_m and AP upstrokes preceded the rise of Ca_i with a delay of 10 ± 2 ms.²³ With the appearance of premature ventricular beats, EAD upstrokes often coincided with a secondary CaT peak. In 3 out of 8 experiments, the 1st EAD occurred outside of the field-of-view of the optical maps. In 2 out of 5 experiments where the 1st EAD fell in the field-of-view of the array, the 2nd rise of Ca_i preceded the 2nd V_m upstroke by 3-5 ms, and this temporal relationship reversed gradually as the EAD propagated away from its origin (Fig. 5). In 3 out of 8 experiments, EAD upstrokes preceded Ca_i upstrokes but with shorter V_m - Ca_i delays of 5-7 ms compared to delays during normal APs. Ca_iO preceding EAD upstrokes (Fig. 2B, 3C and 3D) were routinely observed during complex ectopy, such as bigeminy and trigeminy and VT runs.

As illustrated in Figure 6, a bigeminy pattern on EKG corresponded to a Ca_iO with an EAD during each beat (Fig. 6A). Similarly, trigeminy corresponded to beat-to-beat alternations of a large Ca_iO with an EAD followed by a smaller Ca_i oscillation without an EAD on the next beat (Fig. 6B). Non-sustained runs of polymorphic VT corresponded to long CaT with multiple Ca_iO (Fig. 6C).

TdP is suppressed by interventions which abolish Ca_iO

To corroborate the role of spontaneous SR Ca^{2+} release in eliciting EADs and TdP, several interventions were tested to reduce SR Ca^{2+} load in attempts to suppress Ca_iO and the generation of TdP. As shown in Figure 7, nifedipine ($5 \mu\text{M}$) (A) and low external Ca^{2+} ($100 \mu\text{M}$) (B) suppressed Ca_i oscillations, EADs and TdP ($n=4$ for each intervention), although APD remained prolonged. Restoration of original Ca^{2+} resulted in reappearance of Ca_iO and TdP. In other experiments ($n=3$), the hearts were perfused with Tyrode's containing ryanodine and thapsigargin ($10 \mu\text{M}$ and 200 nM , respectively) to deplete SR prior to perfusion with dofetilide. In the presence of ryanodine and thapsigargin, dofetilide prolonged APD, but Ca_iO and TdP failed to occur.

Discussion

The main finding of this report is that under LQT2 conditions, CaT oscillations occur in ventricular myocardium during regular rhythm. They are not caused by oscillations of membrane potential, which they precede by minutes. When EADs do appear, they usually follow CaT upstroke at the site of EAD origin.

Although Ca_i oscillations under LQT conditions have been reported before, they were always described in the presence of EADs and often understood as a consequence of I_{CaL} reactivation with consequent calcium-induced calcium release. Since the timing of Ca_i and V_m upstrokes is often similar and their relationship may be spatially heterogeneous, this explanation was

difficult to disprove. Here, we show that CaT oscillations consistently occur minutes before the appearance of EADs, at the time when AP downstroke remains smooth and monophasic. Interventions which abolish CaT oscillations also abolish TdP. These findings indicate that the secondary CaT peaks are caused by instabilities of intracellular Ca^{2+} handling caused by AP prolongation. These instabilities in turn promote the appearance of EADs and, eventually, TdP.

CaT oscillations drive EADs

The driving role of Ca_i oscillations in EAD generation is supported by the analysis of CaT and AP relationship at the site of EAD focus. At the origins of EADs, the rise of Ca_i preceded the EAD depolarization ($n=2/5$). At sites remote from EAD foci, V_m preceded Ca_i , which is expected if voltage is responsible for EAD propagation. At sites of earliest EAD upstroke, Ca_i followed the EAD depolarization in 3/5 hearts; even then, the short V_m - Ca_i delays (< 7 ms) suggested that normal voltage-driven SR Ca^{2+} release did not occur. In such cases, the EAD most likely emanated from deeper in the myocardial wall and propagated to the epicardial surface. In cryoablated rabbit hearts where a thin layer of epicardium survives, Ca_i elevation always preceded the EAD depolarization.²³ The cryoablation studies support the interpretation that when EAD depolarizations precede Ca_i elevation, the site of earliest EAD upstrokes correspond to epicardial breakthrough of a transmural depolarization wavefront. The higher spatial dispersion of CaTD compared to APD dispersion just before the onset of EADs also supports the notion that CaTs are not under the control of APs.

Mechanisms linking CaT oscillations to EADs

The most likely mechanism linking secondary CaT upstrokes to membrane depolarization is the NCX current, I_{NCX} , consistent with reports that NCX blockers suppress EADs and TdP.²⁷ We speculate that CaT oscillations are driven by spontaneous Ca^{2+} release from an overloaded SR. In LQT2, the long AP plateau increases Ca^{2+} influx via $I_{\text{Ca,L}}$ because the voltage-dependent component of inactivation is incomplete and long APDs also reduce the driving force for Ca^{2+} efflux via NCX. These changes indirectly increase SR Ca^{2+} uptake due to the increase of sarcolemmal Ca^{2+} entry and the suppression of Ca^{2+} efflux mechanisms. Consistent with this view, nifedipine, low external Ca^{2+} and ryanodine/thapsigargin eliminated CaT oscillations, EADs and TdP. Thus, our data indicate that spontaneous Ca^{2+} release from an overloaded SR network is the primary cause of ventricular arrhythmias in LQT2.

In dofetilide-induced LQT2, Ca_iO always appeared before EADs and TdP. Although all Ca_iO are expected to elicit I_{NCX} oscillations, not all Ca_iO produced EADs because the ability of I_{NCX} (due to a Ca_iO) to sufficiently depolarize the cell membrane and produce an EAD depends on several factors: i) the magnitude of the Ca_i elevation which determines the magnitude of I_{NCX} , ii) V_m during the plateau phase, a determinant of the magnitude of repolarizing K^+ currents: I_{K1} , residual I_{Kr} and I_{Ks} , iii) the time-point along phase 2 of the AP when I_{NCX} rises, a determinant of the number of L-type Ca^{2+} channels that have recovered from inactivation and can be reactivated and iv) the activation of the opposing repolarizing K^+ currents (I_{Kr} and I_{Ks}) are also time-dependent. The data provide compelling evidence that I_{NCX} is the most reasonable mechanism for the generation of EADs through L-type Ca^{2+} channels re-activation but it cannot entirely exclude a contribution from spontaneous $I_{\text{Ca,L}}$ re-activation.

Non-alternans TWL is caused by the lability of ventricular APs, which is preceded by Ca_iO and CaT lability. Ca_iO are not fully synchronized (Fig. 4) and could contribute to AP lability through reverse Ca_i - V_m coupling. In contrast to TWL, alternans of CaT, AP or TWA was never observed before the onset of ectopy.

Spontaneous SR Ca²⁺ release and arrhythmias

Abnormal Ca²⁺ handling has been implicated in arrhythmogenesis in numerous pathologies, ²⁸ including digoxin toxicity²⁹ and catecholaminergic polymorphic ventricular tachycardia. ^{30,31} VT is triggered by DADs in both of these conditions. DADs are caused by spontaneous SR Ca²⁺ release during diastole and membrane depolarization by I_{NCX}.³² However, the role of spontaneous systolic Ca²⁺ release from SR in the generation of EADs continues to be debated.³³ Ca_iO in the form of sparks and waves have been reported in a wide range of cardiac preparations, ranging from isolated myocytes to intact perfused hearts, and have been implicated as an arrhythmogenic mechanism.³⁴ In a guinea-pig model of ischemia/reperfusion, spontaneous Ca_iO appeared to drive ventricular ectopy.³⁵ Reperfusion arrhythmias thus represent another clinically relevant situation involving spontaneous Ca²⁺ release from overloaded SR. The link between delayed repolarization, SR overload, CaT oscillations and TdP may help the development diagnostic and treatment strategies in patients with long QT syndrome.

Conclusions

In LQT2, APD prolongation promotes CaT oscillations, which precede the appearance of EADs. The data provide a mechanistic explanation for TWL and enhance our understanding of arrhythmogenesis in long QT syndrome. This may lead to improved clinical management of patients with impaired repolarization.

Limitations

Dofetilide was used at a relatively high dose to elicit drug-induced LQT2 because lower doses were less reliable at eliciting TdP. Our LQT2 model exhibits an extreme degree of impaired repolarization compared to most clinical situations such that its clinical relevance must be validated. However, it has the advantage of reproducible TdP induction during an acute study.

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Figure 1.

Prolonged repolarization induces TWL

Examples of EKG recordings during pacing at 50 beats per min: (A) Control and (B) LQT2. T-wave morphology is constant in A, but changes on a beat-to-beat basis in B. EKG lability (green traces) is plotted before (C) and during LQT2 (D) and is superimposed on signal-averaged EKG (red traces). The Y-axis for lability is expanded 10-fold with respect to signal-averaged EKG. TWL is calculated as the logarithm of maximum EKG lability measured during the repolarization phase and normalized with respect to the amplitude of signal-averaged QRS. TWL is essentially absent in C and highly pronounced in D. Maximum lability occurs at ~470 ms after the pacing stimulus in this case.

Figure 2.

Ca_iO precede EADs

Simultaneous recordings of V_m (blue) and Ca_i (red) before (**A**) and during LQT2 (**B,C**).

A: V_m and Ca_i are shown at slow (top) and fast (bottom) sweep speeds and both exhibit monophasic time-courses.

B: V_m and Ca_i are shown at slow (top) and fast (bottom) sweep speeds. In the first 2 APs, LQT2 condition prolonged APD and elicited Ca_i oscillations during the paced beats with no V_m instabilities. The 3rd AP exhibited multiple Ca_i peaks which were occasionally coincident with EADs.

C: An example of TdP onset following the 5th paced beat. The EKG (top trace) and the optical traces of V_m and Ca_i (bottom traces) were recorded simultaneously. The Ca_iO precede the first EADs at this site.

Figure 3.

Time-dependent oscillations of Ca_i and the evolution of EADs

V_m (blue) and Ca_i (red) measurements (**left**) during pacing (**A, B**) and ventricular escape rhythms (**C, D**) and the corresponding phase-plots (**right**).

A. In control, V_m and Ca_i are monophasic and similar in shape. **B:** LQT2 for 3 min, a 2nd rise of Ca_i appears during the AP plateau while V_m remains free of EADs. **C:** LQT2 for 6 min promotes more complex Ca_i O that are associated with a single EAD. In this pixel, the Ca_i upstroke precedes the V_m upstroke. **D:** LQT2 for 9 min, 2 consecutive EADs follow each AP upstroke. Prominent Ca_i upstrokes precede EAD upstrokes.

Figure 4.

Spatial and temporal heterogeneity of CaT and APs during LQT2

A: Isochronal Map of APD₉₀ (A (a), **top left**) from the anterior surface in LQT2; the field-of-view of the array is shown (see inset d) and isochronal lines are 50 ms apart (see color scale). Ca_i signals recorded at each site are depicted in the symbolic map of the photodiode array (b). Simultaneous V_m and Ca_i from a single-beat are superimposed for pixels labeled **1, 2 and 3** on the maps and are shown at fast sweep speed (c). Marked spatial heterogeneities of Ca_iO appear; with the highest number of Ca_iO found at sites with the longest APDs and decrease in going to sites with shorter APDs.

B: EKG, V_m and Ca_i recordings from pixel (**1**) for 30 s show a gradual APD prolongation associated with an increasing number of Ca_iO and a rise of diastolic Ca_i . The right panel shows a shorter segment of the signals (in **green box** on left) with better time resolution.

Figure 5.

Propagation of V_m and Ca_i upstrokes during an EAD

A: Isochronal activation map of an ectopic beat (EAD) occurring during an escape rhythm. The origin of the EAD is at site 1. Isochronal lines are 3 ms apart. Note that another independent wavefront emanates from the base of the heart.

B: V_m and Ca_i tracings from sites 1 and 3 in (A). The 1st AP and CaT are monophasic at site 1; during the 2nd AP, there is a distinct 2nd Ca_i peak without an EAD (arrow). On the 3rd beat, an EAD appears with sufficient magnitude to propagate, as in A.

C: The temporal relationship between Ca_i and V_m signals are shown at higher resolution at sites 1-3 as labeled in A. At the site of EAD origin (1), Ca_i upstroke precedes V_m upstroke (8 ms); at site 2, V_m is coincident with Ca_i and at site 3, remote from the EAD origin, V_m precedes Ca_i (3 ms).

Figure 6.

EKG recordings of complex ectopy: Bigeminy and Trigeminy correspond to Ca_iO

A: EKG recording of paced rhythm with bigeminy. Each paced beat (**green arrows**) is followed by a ventricular ectopic beat (**red arrow**). T-waves on EKG signals are indicated by **black arrows**. Note that neither V_m nor Ca_i recover to baseline before the ectopic beat; in this sense, the paced/ectopic beat can be understood as a single complex AP.

B: An episode of trigeminy from the same experiment. Two paced beats are followed by an ectopic beat. The optical tracings indicate that alternans between a short monophasic paced AP and a “bigeminal” AP underlies the trigeminal pattern.

C: Simultaneous EKG and Ca_i tracing with brief runs of polymorphic VT. Pacing rate was 50 bpm with 2:1 capture. Each run of polymorphic VT corresponds to a single CaT with multiple secondary Ca_iO .

Figure 7.

Effect of Reduced SR Ca^{2+} load on Ca_iO and TdP

TdP was induced with LQT2 solution then nifedipine (5 μM) was added (A) or external Ca^{2+} was lowered (100 μM) (B) in the perfusate. Both interventions attenuated Ca_iO and terminated TdP despite continuous dofetilide perfusion and marked APD prolongation.

Table 1

Summary of EKG, V_m and Ca_i parameters under control and LQT2 conditions. The asterisk indicates that Ca_i lability was calculated from 2 beats preceding EAD onset, at the site of EAD origin.

Parameter	Baseline	Dofetilide, before Ectopy	p
APD (ms)	415±118	678±235	p<0.01
CaTD (ms)	474±144	702±227	p<0.005
QT (ms)	481±105	603±172	p<0.05
# of Ca_i peaks	1.332±0.387	2.120±0.469	p<0.002
T wave lability	-4.061±0.944	-2.894±0.950	p<0.002
AP lability	-3.436±0.492	-2.994±0.779	p<0.05
Ca_i lability	-3.338±0.372	-2.970±0.647	p=0.084
Ca_i lability*	-4.049±0.685	-3.356±1.033	p<0.05
$V_m \times Ca_i$ correlation	0.9593±0.0231	0.8967±0.0644	p<0.02