Published in final edited form as:

Heart Rhythm. 2012 January ; 9(1): 115–122. doi:10.1016/j.hrthm.2011.08.014.

Bi-stable Wave Propagation and Early Afterdepolarization-Mediated Cardiac Arrhythmias

Marvin G. Chang, MS^{1,*}, Daisuke Sato, PhD^{1,*,#}, Enno de Lange, PhD^{1,*}, Jong-Hwan Lee^{1,4}, Hrayr S. Karagueuzian, PhD¹, Alan Garfinkel, PhD^{1,2}, James N. Weiss, MD^{1,3}, and Zhilin Qu, PhD¹

¹Department of Medicine (Cardiology), David Geffen School of Medicine at University of California, Los Angeles, California 90095, USA

²Department of Integrative Biology and Physiology, David Geffen School of Medicine at University of California, Los Angeles, California 90095, USA

³Department of Physiology, David Geffen School of Medicine at University of California, Los Angeles, California 90095, USA

⁴Department of Anesthesiology and Pain Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Abstract

BACKGROUND—In normal atrial and ventricular tissue, the electrical wavefronts are mediated by the fast sodium current (I_{Na}), while in sino-atrial and atrioventricular nodal tissue, conduction is mediated by the slow L-type calcium current ($I_{Ca,L}$). However, it has not been shown whether the same tissue can exhibit both the I_{Na} -mediated and the $I_{Ca,L}$ -mediated conduction.

OBJECTIVE—To test the hypothesis that bi-stable cardiac wave conduction, mediated by I_{Na} and $I_{Ca,L}$, respectively, can occur in the same tissue under conditions promoting early afterdepolarizations (EADs), and to study how this novel wave dynamics is related to the mechanisms of EAD-mediated arrhythmias.

METHODS—Computer models of two-dimensional (2D) tissue with a physiologically detailed action potential model were used to study the bi-stable wave dynamics. Theoretical predictions were tested experimentally by optical mapping in neonatal rat ventricular myocyte monolayers.

RESULTS—In the same 2D homogeneous tissue, we could induce spiral waves that are mediated by either I_{Na} or $I_{Ca,L}$ under conditions in which the action potential model exhibited EADs. This bi-stable wave propagation behavior was similar to bi-stability shown in many other nonlinear systems. Since the bistable states are also excitable, we call this novel behavior bi-excitability. In a 2D heterogeneous tissue, the $I_{Ca,L}$ -mediated spiral wave meanders, giving rise to a twisting electrocardiographic QRS axis, resembling torsade de pointes, while the co-existence and interplay between the I_{Na} -mediated wavefronts and $I_{Ca,L}$ -mediated wavefronts give rise to polymorphic ventricular tachycardia. We also present experimental evidence for bi-excitability

© 2011 The Heart Rhythm Society. Published by Elsevier Inc. All rights reserved.

Correspondence to: Zhilin Qu, PhD, Department of Medicine (Cardiology), David Geffen School of Medicine at UCLA. A2-237 CHS, 650 Charles E. Young Drive South, Los Angeles, CA 90095, Tel: 310-794-6050, Fax: 310-206-9133, zqu@mednet.ucla.edu.

*These authors contributed equally

#Current address: Department of Pharmacology, University of California, Davis, California 95616

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

under EAD-promoting conditions in neonatal rat ventricular myocyte monolayers exposed to BayK8644 and isoproterenol.

CONCLUSIONS—Under EAD-prone conditions, both I_{Na} -mediated conduction and $I_{Ca,L}$ -mediated conduction can occur in the same tissue. This novel wave dynamics may be responsible for certain EAD-mediated arrhythmias, such as torsade de pointes and polymorphic ventricular tachycardia.

Keywords

Early afterdepolarizations; reentry; bi-excitability; bi-stability; torsade de pointes

Introduction

In normal ventricular and atrial tissue, the upstroke of the action potential is caused by the activation of the sodium (Na) current (I_{Na}), which mediates impulse conduction through both the atrium and the ventricles, with L-type calcium (Ca) current ($I_{Ca,L}$) playing a passive follower role. In the sinoatrial or atrioventricular nodal tissue, however, the upstroke of the action potential is caused by the activation of $I_{Ca,L}$ and thus impulse conduction is mediated by $I_{Ca,L}$. $I_{Ca,L}$ -mediated conduction can also occur in ischemic ventricular muscle in which the resting potential is depolarized due to extracellular potassium (K) accumulation so that I_{Na} no longer contributes to the upstroke of the action potential¹, or when gap junction coupling is severely decreased^{2, 3}.

In this study, we show a novel wave propagation behavior that allows both I_{Na} -mediated conduction and $I_{Ca,L}$ -mediated conduction to coexist in the same homogeneous tissue under identical conditions. In other words, in the same tissue, both I_{Na} -mediated wave and $I_{Ca,L}$ -mediated waves with distinct propagation properties, and the two types of waves can switch from one to the other. This behavior is similar to a toggle switch in a typical bi-stable system⁴, which has been demonstrated to regulate important physiological properties in many biological systems^{5, 6}. Here we show that bi-stable wave behavior may occur in cardiac tissue when the tissue is prone to early afterdepolarizations (EADs). EADs are abnormal depolarizations in the repolarizing phase of the action potential, which occur in many disease conditions⁷⁻⁹, such as long QT syndromes (LQTS) and heart failure. EADs are thought to cause arrhythmias by promoting reentry through increasing dispersion of action potential duration (APD) and by causing premature ventricular complexes (PVCs) which induce reentry or triggered activity. It is widely accepted that EADs are associated with torsade de pointes (TdP)^{7, 10-14}, a type of polymorphic ventricular tachycardia (PVT) at rates ranging from 150 to 300 beats per minute. TdP has a distinctive electrocardiographic (ECG) appearance in which the QRS axis slowly undulates, and usually terminates spontaneously^{11, 15, 16}. However, mechanisms linking EADs to TdP remain incompletely understood.

Using computer simulations of two-dimensional (2D) homogeneous tissue, we first document the presence of bi-stable wave behavior when EADs are present in the action potential. We then show that in a 2D heterogeneous tissue model, a meandering $I_{Ca,L}$ -mediated spiral wave can give rise to the classic heart rate and ECG appearance of TdP. We also show that the dynamic interactions between $I_{Ca,L}$ -mediated and I_{Na} -mediated wavefronts arising in the same tissue give rise to an ECG appearance of PVT. Finally, we present experimental evidence for bi-excitability in neonatal rat ventricular myocyte (NRVM) monolayers.

Methods

Computer simulation

Computer simulations were carried out in models of isolated myocytes and 2D tissue. The voltage of the single cell is governed by an ordinary differential equation: $C_m dV/dt = -(I_{ion} + I_{sti})$, where C_m is the membrane capacitance of the myocyte, I_{ion} is the total ionic current, and I_{sti} the stimulation current. The 2D tissues were modeled using a mono-domain model with voltage described by the partial differential equation: $\partial V/\partial t = -I_{ion}/C_m + D(\partial^2 V/\partial x^2 + \partial^2 V/\partial y^2)$. The action potential models were modified from the rabbit ventricular model developed previously^{17, 18}, which are described in detail in the online supplemental materials. To generate large amplitude EADs that can propagate in tissue, we modified many of the parameters from the original rabbit ventricular model, such as increase in $I_{Ca,L}$ as well as I_{Ks} . Increasing in $I_{Ca,L}$ and I_{Ks} is consistent with the known effects of BayK8644 and isoproterenol. The detailed parameters are presented in the Table in the supplemental materials.

Cultured NRVM monolayers

NRVM monolayers were isolated by standard methods¹⁹ and plated on 22-mm diameter circular cut polyvinyl chloride coverslips (PGC Scientifics, Frederick, Md). Briefly, hearts harvested from 2- to 3-day-old neonatal Sprague-Dawley rats were digested with collagenase (0.02%; Worthington Biochemical Corp, Lakewood, NJ) and pancreatin (0.06%; Sigma-Aldrich, St Louis, Mo). Myocytes were isolated with the use of a Percoll (Pharmacia Biotech AB, Uppsala, Sweden) gradient and plated at a density of 10^6 cells/mm³ per coverslip. Monolayers were cultured for 4 to 5 days to ensure full confluence (by phase contrast microscopy) and homogeneous electrical propagation before experimental use. Specimens were perfused with oxygenated Tyrode's solution at 37°C. NRVM monolayers were stained for optical voltage recording by immersion into oxygenated Tyrode's solution (in mmol/L: 136 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.33 NaH₂PO₄, 1 MgCl₂, 10 HEPES, and 10 glucose; pH 7.3) containing the voltage dye RH-237 (5 μ mol/L for 5 minutes) at room temperature²⁰. We used electron-multiplying, back-illuminated, cooled charge-coupled device cameras (Photometrics Cascade 128+; 128 $\times$ 128 pixels), acquiring at 0.6 to 5 ms per frame. Signals were digitized with 16 bits of precision and processed offline as described previously²⁰. Conduction velocity was obtained as described previously²⁰. The use and care of the animals in these experiments were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles.

Results

Bi-stable wave conduction in a homogeneous tissue model

In normal ventricular tissue, the resting potential of a myocyte is around -80 mV. When a depolarizing stimulus rapidly elevates the voltage close to >-60 mV, the fast inward current I_{Na} is activated and depolarizes the myocyte to positive potentials, after which I_{Na} quickly inactivates (Fig. 1A). Another inward current ($I_{Ca,L}$), with an activation threshold around -40 mV and slower inactivation, subsequently maintains the action potential plateau. The positive membrane voltage activates outward K currents on a still slower time scale, which then repolarize the myocyte back to the resting voltage. It is well-known that normal ventricular tissue can only support a single type of traveling wave or spiral wave, namely, conduction mediated by I_{Na} ^{1, 3}. If I_{Na} is blocked, $I_{Ca,L}$ is generally insufficient to support wave propagation in normal ventricular tissue unless other electrophysiological properties are also altered.

Under diseased conditions⁷⁻⁹, such as LQTS or heart failure, in which outward currents are reduced and/or inward currents are increased (and also have their kinetics altered), EADs can occur during the repolarizing phases of the action potential, as shown in Fig. 1B. These membrane oscillations (EADs) are caused by $I_{Ca,L}$ reactivation competing with the slow component of the delayed rectifying K currents (I_{Ks}). Interestingly, when these same cells that exhibit EADs at the single cell level are coupled together to form a 2D homogeneous tissue, the tissue becomes capable of reentry mediated by either I_{Na} or $I_{Ca,L}$ depending on the initial conditions. This is unlike normal ventricular tissue or the AV node that can only support one type of propagation at a time, mediated either by I_{Na} (with $I_{Ca,L}$ following passively), or by $I_{Ca,L}$ (in absence of I_{Na}), irrespective of the initial conditions.

Figure 2 shows the two types of spiral waves in our simulation of a 2D homogeneous tissue composed cells modeled with the action potential shown in Fig. 1B. Figure 2A (Online Movie I) shows a spiral wave mediated by I_{Na} . This spiral wave was induced by a cross-field stimulation protocol in which an S1 stimulus was applied at the left edge of the tissue. When approximately half of the tissue repolarized to the resting potential, an S2 stimulus was applied at the left-lower quarter of the tissue (see Online Movie I), as typical for the induction of a spiral wave in a normal tissue. The cycle length of this I_{Na} -mediated spiral wave is short (<180 ms) and voltage repolarizes fully to ~ -80 mV between beats. While both I_{Na} and $I_{Ca,L}$ are present in this case, the rotor and wavefront propagation is mediated by I_{Na} . Alternatively, an $I_{Ca,L}$ -mediated spiral wave (Figure 2B and Online Movie II) can be induced in the same tissue. The cycle length of this spiral wave is much longer (~ 560 ms) and voltage only repolarizes partially to a voltage between -50 mV and -40 mV between beats. Since the voltage remains above -50 mV in this case, I_{Na} remains fully inactivated and only $I_{Ca,L}$ is present to mediate the wave propagation. It should be noted that the induction of the $I_{Ca,L}$ -mediated spiral wave in a homogeneous tissue is not as straightforward as the initiation of an I_{Na} -mediated spiral wave. Using the cross-field stimulation protocol, the spiral wave in Fig. 2B could only be induced after certain S1 beats, using a more premature and weaker S2 stimulus. The action potential in the S1 beat is long, and the S2 must be applied before the action potential repolarizes full and weak enough to avoid causing full repolarization (see Fig. 4). In contrast to homogeneous tissue, however, $I_{Ca,L}$ -mediated reentry can be easily induced in heterogeneous tissue by the EADs with a single S1 stimulus (see Online Fig. S1).

Note that the two types of spiral waves occur in the *identical* homogeneous tissue, depending only on the different initial conditions (i.e. the specific stimulation protocol), unrelated to the tissue substrate itself. The property that the two stable steady states exist under the same parameter conditions of the tissue substrate is a well-known phenomenon called “bi-stability” in nonlinear dynamics⁴, and has been demonstrated in many biological systems^{5, 6}. Since the bi-stable states in our model are also excitable states, we call this property *bi-excitability*.

Co-existence of I_{Na} -mediated and $I_{Ca,L}$ -mediated conduction in the same homogeneous tissue

Bi-stable systems are able to toggle between one state and another in a switch-like manner, but generally do not exhibit both states at the same time. In our system, a mixture of the two types of conduction can co-exist in the same homogenous tissue if the refractoriness or the repolarization reserve of the cell is altered. Figure 3 (and Online Movie III) shows an example in which the central core region of the spiral wave is driven by $I_{Ca,L}$ -mediated rotor (in which I_{Na} is inactivated). However, along the arm of the spiral wave, fast I_{Na} -mediated wavefronts (indicated by arrows) occur concomitantly with slow $I_{Ca,L}$ -mediated wavefronts.

Prevention of full repolarization in a single cell

Since single cells that exhibit EADs can repolarize fully to a resting voltage of -80 mV (Fig. 1B), a question arises: what prevents the full repolarization of an $I_{Ca,L}$ -mediated spiral wave in tissue (Fig. 2B)? The major difference between a single uncoupled cell and a coupled cell in the tissue is the presence of electrotonic current flow, which by inference must be the key factor preventing full repolarization of the cells in tissue. To demonstrate this point, we paced a single cell periodically with an electrical pulse 10 ms in duration and stimulus strength $2 \mu\text{A}$ (similar to the duration and amplitude of the diffusive current in Fig. 2B), the action potential remained depolarized without fully repolarizing (Fig. 4A). The pacing cycle length of this electrical pulse that prevents full repolarization has to be close to the period of the EADs (~ 500 ms, see Fig. 1B), and above a certain stimulus threshold (Fig. 4B). The requirement that the pacing cycle length of this electrical pulse be close to the period of EADs and above a certain stimulus threshold in order to prevent full repolarization of the cell may also explain the occasional full repolarization resulting in the I_{Na} wavefronts seen in Fig. 3. In other words, if the action potential properties are changed so that the cycle length of the $I_{Ca,L}$ -mediated spiral wave is not close to the EAD period of the single cell, the diffusive current is unable to maintain the EADs oscillations, and full repolarization may occur as in the case of Fig. 3.

TdP and PVT due to $I_{Ca,L}$ -mediated and I_{Na} -mediated conductions in a heterogeneous tissue model

The $I_{Ca,L}$ -mediated spiral wave shown in Fig. 2 was a stable spiral wave and thus periodic, which gave rise to monomorphic ventricular tachycardia on the simulated ECG. However, cardiac tissue is heterogeneous, exhibiting transmural K current and Ca transient gradients^{21, 22}. Here we model heterogeneity by linearly increasing I_{Ks} from the center of the tissue to the tissue border (see Fig. 5 legend). In this heterogeneous tissue (Fig. 5A), the $I_{Ca,L}$ -mediated spiral wave was unstable and its core meandered, giving rise to the classic ECG appearance of TdP. While meandering I_{Na} -mediated spiral waves have been previously proposed as a mechanism of TdP²³, their frequency ($5\text{--}15$ Hz) is typically much faster than that of TdP ($2\text{--}5$ Hz). Since the frequency of a meandering $I_{Ca,L}$ -mediated spiral wave is much slower, they may represent a better candidate for TdP.

In patients with LQTS¹⁶, the classic TdP pattern is often observed in some surface ECG leads, and PVT in other leads. To explore the relationship of these different ECG patterns to bi-excitability, we simulated a larger heterogeneous tissue in which the outer region had greater repolarization reserve (achieved by increasing the conductance of I_{Ks}), as seen in the outer M-cell region of the heart. In this larger tissue, an $I_{Ca,L}$ -mediated spiral wave formed in the central region while the outer region exhibited both $I_{Ca,L}$ -mediated and I_{Na} -mediated wavefronts at varying locations at different times (Fig. 5B and Online Movie IV). In this case, the position of the ECG electrode (Fig. 5C) relative to the $I_{Ca,L}$ -mediated spiral wave determined whether a TdP-like or PVT-like ECG was observed. If the electrode was close to the central $I_{Ca,L}$ -mediated spiral wave, TdP was recorded, but if the electrode was positioned either higher above the tissue, or in the periphery away from the central region, a PVT-like ECG was recorded due to the shifting contributions of the $I_{Ca,L}$ -mediated and I_{Na} -mediated wavefronts. These results provide a novel explanation for the genesis of TdP on some ECG leads via a meandering $I_{Ca,L}$ -mediated spiral wave, and PVT on other ECG leads via the irregular appearance of the $I_{Ca,L}$ -mediated and I_{Na} -mediated spiral wave wavefront in the surrounding tissue.

Experimental evidence of bi-excitability

To seek evidence of bi-excitability in real cardiac tissue, we carried out optical mapping studies in cultured NRVM monolayers. To facilitate the initiation of reentry, we placed an

obstacle in the center of the monolayer (Figs. 6A and B). Stable reentry (frequency ~5 Hz) was induced by a burst of rapid pacing during superfusion with normal Tyrode's condition. BayK8644 and isoproterenol were then added to the superfusate, and the reentry continued without a significant change in rotation period (Fig. 6A and Online Movie V). To perturb the system, a second burst of rapid pacing was then applied, which converted the original reentry into a new type of reentry (Fig. 6B and Online Movie VI) with a much slower frequency (~2 Hz). This new type of reentry was insensitive to the Na channel blocker TTX, but was eliminated by the L-type Ca channel blocker nitrendipine (See Online Fig. S2). Since the new type of reentry was induced by pacing alone, without changing the intrinsic electrophysiological properties of the monolayer (e.g. by adding a new drug), this behavior demonstrates that the same tissue can exhibit two distinct types of reentrant waves for different initial conditions (pre- and post the second episode of rapid burst pacing), as predicted by the computer simulations in Fig. 2. As in the computer simulations, we found that inducing both types of waves in the same tissue is nontrivial, and obtained only 3 episodes of such bistable waves out of 54 monolayers. However, when the BayK8644 and isoproterenol concentrations were increased, we could easily induce nitrendipine-sensitive reentry without I_{Na} -mediated reentry in the same monolayers, with the frequencies of rotation ranging from 1.5 Hz to 3 Hz in different monolayers. Presumably, the higher BayK8644 and isoproterenol concentrations impeded full repolarization to the resting potential under these conditions, preventing I_{Na} -mediated wavefronts formation.

In another NRVM monolayer, we observed $I_{Ca,L}$ -mediated propagation arising from bursts of EAD-mediated focal activity that failed completely repolarize between beats (Fig. 6C and Online Movie VII) following application of BayK8644 and isoproterenol. Note that the conduction velocity of the first wave, arising from fully repolarized tissue during the initial upstroke, was 30 cm/s, but progressively slowed to 4 cm/s during the subsequent waves mediated by $I_{Ca,L}$ during the EAD upstrokes, as seen in the space-time plot. This is consistent with a transition from I_{Na} -mediated propagation to $I_{Ca,L}$ -mediated propagation, due to the progressive incomplete repolarization during successive beats during EAD bursts, as evident in the optical trace above.

Discussion and conclusions

Bi-stability is a widely-known behavior in nonlinear dynamics⁴, and has been demonstrated in many biological systems^{5, 6}. In this study, we show a novel bi-stable behavior of an excitable medium, in which cardiac tissue prone to EADs becomes capable of exhibiting two types of wave propagation with distinct conduction velocities and rotation frequencies, mediated either by I_{Na} or by $I_{Ca,L}$, respectively. The two types of waves can switch or co-exist simultaneously, even in completely homogeneous tissue. We also provided experimental evidence of bi-excitability in cultured NRVM monolayers exposed to BayK8644 and isoproterenol. In the computer model, the $I_{Ca,L}$ -mediated spiral wave is stable in homogeneous tissue, but in heterogeneous tissue, the $I_{Ca,L}$ -mediated spiral wave meanders, which gives rise to both the heart rate and appearance of TdP on some ECG leads, while the interaction between the I_{Na} -mediated and $I_{Ca,L}$ -mediated propagating wavefronts in the outer arm of the $I_{Ca,L}$ -mediated spiral wave produce an appearance of PVT on other ECG leads.

EADs and bi-excitability

It is known that cardiac conduction can be mediated by either I_{Na} or $I_{Ca,L}$, depending on the characteristics of the tissue substrate. In general, to support I_{Na} -mediated conduction, cardiac tissue needs to have a resting potential more negative than -60 mV (as seen in normal atrial and ventricular myocardium). Conversely, to support $I_{Ca,L}$ -mediated propagation, cardiac tissue requires a resting potential more depolarized than -60 mV, such

as during ischemia or in the sinoatrial or atrioventricular nodes where the resting membrane potential is significantly elevated, and $I_{Ca,L}$ is solely responsible for the upstroke of the action potential. It is also important to note that $I_{Ca,L}$ -mediated propagation may serve as the sole source of propagation in substrates with reduced gap junction coupling (such as the healed infarct border zone²) despite any change in the resting membrane potential. Thus, normal tissue can only support one type of propagation at a time, and that changing from I_{Na} -mediated wave to $I_{Ca,L}$ -mediated wave propagation requires changing the cellular or tissue properties.

The novel behavior identified in the present study, however, shows that when EADs are present, the same homogeneous tissue becomes capable of supporting both I_{Na} -mediated and $I_{Ca,L}$ -mediated waves. The switching from one wave type to the other does not require a change in cellular or tissue properties. This is a typical bi-stable behavior^{5,6}, and since the two states are also excitable, we call this behavior bi-excitability. Note that the conditions for bi-excitability to occur is not simply that the action potential exhibits EADs, but that the take-off potential of EADs be low enough and their amplitude large enough (as seen in Fig 1B) to facilitate the propagation of EADs in tissue. If the takeoff potential of the EADs is too high and the amplitude too small (such as in the case of phase-2 EADs shown in many previous studies^{9,18,24,25}), the EAD is unable to propagate and the tissue is unable to exhibit bi-excitability. The take-off potential of EADs is multifactorial, and involves a complex interaction between amplitude and kinetics of $I_{Ca,L}$ properties and repolarizing currents such as I_{Ks} . Thus, $I_{Ca,L}$ -mediated reentry in our model can be prevented if I_{Ks} is either too large (promoting full repolarization) or too small (such that the EAD take-off potential is too high to support propagation in tissue), relative to $I_{Ca,L}$.

Bi-excitability and cardiac arrhythmias

In this study, we show that a meandering $I_{Ca,L}$ -mediated rotor is responsible for the appearance of TdP in some ECG leads. In addition, the rate of TdP, which typically ranges from 150 to 300 beats per minute (or 2.5 Hz to 5 Hz) in clinical settings^{11,16}, is much more consistent with a meandering $I_{Ca,L}$ -mediated spiral wave than an I_{Na} -mediated spiral wave. Our simulations of $I_{Ca,L}$ -mediated spiral waves in Fig. 2B and Fig. 5 exhibit a frequency of 120 beats per minute (2 Hz), which is close to the lower end of the clinically observed rate of TdP. By enhancing $I_{Ca,L}$ -mediated excitability in our model, the rate can be matched to the upper end as well. In contrast, the heart rate of I_{Na} -mediated reentry shown in Fig. 2A is 330 beats per minute (5.5 Hz), which is also consistent with I_{Na} -mediated reentry in human whole heart simulations²⁶, and other computational and experimental studies that show both I_{Na} -mediated reentry and VF (under conditions of normal excitability) occurring at frequencies ranging from 5 to 20 Hz²⁷⁻²⁹.

In addition to providing a novel explanation for the characteristic undulating QRS morphology observed on ECG that is consistent with its relatively slow rate (150–300 bpm) during episodes of TdP, bi-excitability may also explain the clinically observed phenomenon of spontaneous termination of TdP and degeneration to VF. Most episodes of TdP terminate spontaneously, and only a small portion degenerates to VF to cause sudden cardiac death^{11,15,16}. One possibility is that during TdP, hypotension-induced ischemia leads to opening of the ATP-sensitive K channels, which increases repolarization reserve, thereby suppressing the excitability of the $I_{Ca,L}$ -mediated excitable state. This could lead to termination of the $I_{Ca,L}$ -mediated spiral wave (or an $I_{Ca,L}$ -mediated focus as shown in Fig. 6C), and thus the termination of TdP, or degeneration to VF if the repolarization pattern were sufficiently heterogeneous to initiate I_{Na} -mediated spiral waves.

Limitations

Although we believe that the general mechanisms outlined in this study can provide useful insight into the mechanisms of TdP and EAD-related arrhythmias in long QT syndrome and other diseased conditions, such as heart failure, further theoretical and experimental studies will be required to establish their physiological relevance. A condition required for bi-excitability to occur is EAD propagation, and to generate propagating EADs, a Ca-activated non-selective cation current ($I_{ns(Ca)}$) was added to the action potential model¹⁸. Although this current has been identified in different species^{30, 31}, its existence is controversial and its physiological or pathophysiological roles remain unclear. Nevertheless, EADs have been shown to be able to propagate in cardiac tissue¹⁴, and large magnitude EADs have been observed³², therefore, no matter which ionic mechanism is responsible for EAD propagation, our observation of bi-excitability may still occur in cardiac tissue as long as the EADs can propagate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Guillaume Calmettes for technical assistance.

This work is supported by NIH/NHLBI P01 HL078931, R01 HL103662, NIH/MSTP T32 GM008042 and NIH T32 GM065823 (to M.G.C.), a postdoctoral fellowship award from the American Heart Association, Western States Affiliate (to D.S.), a fellowship award for advanced researchers from the Swiss Foundation for Grants in Biology and Medicine (E.D.L), and the Laubisch and Kawata Endowments.

References

1. Shaw RM, Rudy Y. Electrophysiologic effects of acute myocardial ischemia: A mechanistic investigation of action potential conduction and conduction failure. *Circ Res.* 1997; 80:124–138. [PubMed: 8978331]
2. Chang MG, Zhang Y, Chang CY, et al. Spiral waves and reentry dynamics in an in vitro model of the healed infarct border zone. *Circ Res.* 2009; 105:1062–1071. [PubMed: 19815825]
3. Shaw RM, Rudy Y. Ionic mechanisms of propagation in cardiac tissue: Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling. *Circ Res.* 1997; 81:727–741. [PubMed: 9351447]
4. Strogatz, SH. *Nonlinear dynamics and Chaos: with applications to physics, biology, chemistry, and engineering.* Cambridge: Westview Press; 2000.
5. Gardner TS, Cantor CR, Collins JJ. Construction of a genetic toggle switch in *Escherichia coli*. *Nature.* 2000; 403:339–342. [PubMed: 10659857]
6. Sha W, Moore J, Chen K, et al. Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts. *Proc Natl Acad Sci U S A.* 2003; 100:975–980. [PubMed: 12509509]
7. Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell.* 2001; 104:569–580. [PubMed: 11239413]
8. Li GR, Lau CP, Ducharme A, Tardif JC, Nattel S. Transmural action potential and ionic current remodeling in ventricles of failing canine hearts. *Am J Physiol Heart Circ Physiol.* 2002; 283:H1031–1041. [PubMed: 12181133]
9. Saucerman JJ, Healy SN, Belik ME, Puglisi JL, McCulloch AD. Proarrhythmic consequences of a KCNQ1 AKAP-binding domain mutation: computational models of whole cells and heterogeneous tissue. *Circ Res.* 2004; 95:1216–1224. [PubMed: 15528464]
10. Roden DM. Drug-induced prolongation of the QT interval. *N Engl J Med.* 2004; 350:1013–1022. [PubMed: 14999113]

11. El-Sherif N, Turitto G. Torsade de pointes. *Curr Opin Cardiol*. 2003; 18:6–13. [PubMed: 12496496]
12. Antzelevitch C. Ionic, molecular, and cellular bases of QT-interval prolongation and torsade de pointes. *Europace*. 2007; 9(Suppl 4):iv4–15. [PubMed: 17766323]
13. Liu J, Laurita KR. The mechanism of pause-induced torsade de pointes in long QT syndrome. *J Cardiovasc Electrophysiol*. 2005; 16:981–987. [PubMed: 16174020]
14. Choi BR, Burton F, Salama G. Cytosolic Ca^{2+} triggers early afterdepolarizations and Torsade de Pointes in rabbit hearts with type 2 long QT syndrome. *J Physiol-London*. 2002; 543:615–631. [PubMed: 12205194]
15. Krikler DM, Curry PV. Torsade De Pointes, an atypical ventricular tachycardia. *Br Heart J*. 1976; 38:117–120. [PubMed: 1259825]
16. Drew BJ, Ackerman MJ, Funk M, et al. Prevention of torsade de pointes in hospital settings: a scientific statement from the American Heart Association and the American College of Cardiology Foundation. *Circulation*. 2010; 121:1047–1060. [PubMed: 20142454]
17. Mahajan A, Shiferaw Y, Sato D, et al. A rabbit ventricular action potential model replicating cardiac dynamics at rapid heart rates. *Biophys J*. 2008; 94:392–410. [PubMed: 18160660]
18. Sato D, Xie LH, Sovari AA, et al. Synchronization of chaotic early afterdepolarizations in the genesis of cardiac arrhythmias. *Proc Natl Acad Sci U S A*. 2009; 106:2983–2988. [PubMed: 19218447]
19. Rohr S, Scholly DM, Kleber AG. Patterned growth of neonatal rat heart cells in culture. Morphological and electrophysiological characterization. *Circ Res*. 1991; 68:114–130. [PubMed: 1984856]
20. de Diego C, Pai RK, Dave AS, et al. Spatially discordant alternans in cardiomyocyte monolayers. *Am J Physiol Heart Circ Physiol*. 2008; 294:H1417–1425. [PubMed: 18223190]
21. Laurita KR, Katra R, Wible B, Wan X, Koo MH. Transmural heterogeneity of calcium handling in canine. *Circ Res*. 2003; 92:668–675. [PubMed: 12600876]
22. Akar FG, Rosenbaum DS. Transmural electrophysiological heterogeneities underlying arrhythmogenesis in heart failure. *Circ Res*. 2003; 93:638–645. [PubMed: 12933704]
23. Pertsov AM, Davidenko JM, Salomonsz R, Baxter WT, Jalife J. Spiral waves of excitation underlie reentrant activity in isolated cardiac muscle. *Circ Res*. 1993; 72:631–650. [PubMed: 8431989]
24. Tran DX, Sato D, Yochelis A, Weiss JN, Garfinkel A, Qu Z. Bifurcation and Chaos in a Model of Cardiac Early Afterdepolarizations. *Phys Rev Lett*. 2009; 102:258103. [PubMed: 19659123]
25. Clancy CE, Rudy Y. Linking a genetic defect to its cellular phenotype in a cardiac arrhythmia. *Nature*. 1999; 400:566–569. [PubMed: 10448858]
26. Ten Tusscher KH, Hren R, Panfilov AV. Organization of ventricular fibrillation in the human heart. *Circ Res*. 2007; 100:e87–101. [PubMed: 17540975]
27. Xie F, Qu Z, Yang J, Baher A, Weiss JN, Garfinkel A. A simulation study of the effects of cardiac anatomy in ventricular fibrillation. *J Clin Invest*. 2004; 113:686–693. [PubMed: 14991066]
28. Valderrabano M, Yang JZ, Omichi C, et al. Frequency analysis of ventricular fibrillation in swine ventricles. *Circ Res*. 2002; 90:213–222. [PubMed: 11834715]
29. Choi BR, Nho W, Liu T, Salama G. Life span of ventricular fibrillation frequencies. *Circ Res*. 2002; 91:339–345. [PubMed: 12193467]
30. Ehara T, Noma A, Ono K. Calcium-activated non-selective cation channel in ventricular cells isolated from adult guinea-pig hearts. *J Physiol*. 1988; 403:117–133. [PubMed: 2473193]
31. Giles W, Shimoni Y. Comparison of sodium-calcium exchanger and transient inward currents in single cells from rabbit ventricle. *J Physiol*. 1989; 417:465–481. [PubMed: 2621606]
32. Damiano BP, Rosen MR. Effects of pacing on triggered activity induced by early afterdepolarizations. *Circulation*. 1984; 69:1013–1025. [PubMed: 6705157]

Figure 1. Normal and abnormal cardiac depolarizations of single-cell models

A. The voltage and major inward (I_{Na} and $I_{Ca,L}$) and outward (I_{Ks} and I_{Kr}) currents versus time in the normal action potential model¹⁷, illustrating the depolarization and consequential activation and inactivation of different currents. The pacing cycle length is 500 ms. **B.** Same as A but from an action potential model generating EADs (the details of action potential model are shown in the online supplemental methods), showing that during EADs, I_{Na} maintains at zero while $I_{Ca,L}$ oscillates for depolarizations at the repolarizing phase. The pacing cycle length is 2743 ms.

Figure 2. Bi-stable spiral waves in a homogeneous tissue model

A. An I_{Na} -mediated spiral wave in a homogeneous 2D tissue induced by cross-field stimulations (left), with traces of voltage, I_{Na} , and $I_{Ca,L}$ versus time at a representative location (right). **B.** An $I_{Ca,L}$ -mediated spiral wave (left) induced by a different cross-field stimulation pattern in the same tissue, with traces of voltage, I_{Na} , and $I_{Ca,L}$ versus time from the same site as in A (right).

Figure 3. Co-existence of I_{Na} -mediated and $I_{Ca,L}$ -mediated conductions

Voltage snapshots (left), and traces of voltage and I_{Na} versus time at two locations (right) during the spiral wave. Note intermittent full repolarization at site b, but not site a. Arrows indicate wavefronts mediated by I_{Na} . The action potential model was modified from that of Fig. 2 by altering the Ca-activated nonselective cation current ($I_{ns(Ca)}$) (see supplemental materials).

Figure 4. Prevention of full repolarization in a single cell by periodic stimulation

A. Voltage versus time for periodic pacing (PCL=500 ms) with a weak stimulus (2 μ A and 10 ms, as shown in the inset), the first beat was applied before full repolarization. For comparison, the red line is the voltage trace without the periodic pacing and black line the one with periodic pacing (pacing is marked by the short vertical lines). **B.** APD distribution (color scale) versus stimulation strength (y-axis) and pacing period (x-axis). White region is the region where the action potential does not recover to the resting potential during the 100 s simulation period.

Figure 5. Bi-excitability causing TdP and PVT in heterogeneous tissue model

A. Left: Voltage snapshot showing a meandering $I_{Ca,L}$ -mediated spiral wave (the spiral tip is shown by the blue trace) in a 4.5×4.5 cm heterogeneous tissue. Right: Pseudo-ECG showing a classic TdP pattern. The location of ECG electrode is marked by white circle and is 1.8 cm above the tissue. **B.** Voltage snapshots in a larger 9×9 cm heterogeneous tissue showing an $I_{Ca,L}$ -mediated spiral wave in the center, with co-existing I_{Na} -mediated fast wavefronts (white arrows) and $I_{Ca,L}$ -mediated slow wavefronts in the outlying regions. **C.** Pseudo-ECG from electrodes at different locations and heights above the tissue. The white circles labeled as “a” and “b” in the left panel mark the locations in the x-y plane. The distances of “a” and “b1” are 0.9 cm above the tissue, “b2” is 1.8 cm above the tissue; and “b3” is 4.5 cm above the tissue. The action potential model is the same as in Fig. 2 except that the maximum conductance of I_{Ks} was 0.5 times the value of Fig. 2 in the tissue center and increased linearly from 0.5 to 2 times at the radius of 3 cm (dashed circle) and then was kept at 2 times in the outer region. The same action potential model and heterogeneity were used in A.

Figure 6. Bi-excitable wave propagation in experiments of NRVM monolayers superfused with BayK8644 and isoproterenol

A. Snapshot of reentry (left) around a central obstacle and a local trace of optical voltage (right) in a monolayer, in the presence of BayK8644 (5 nM) and isoproterenol (100 nM). **B.** Same monolayer, after rapid burst pacing induced a slower reentry. **C.** Voltage snapshot (left) and optical voltage trace (right) from a confluent monolayer after BayK8644 (50 nM) and isoproterenol (1 μ M) showing focal excitations. A spontaneous action potential is followed by an EAD-mediated burst, which never fully repolarizes between beats. A line scan (along the white dashed line in the voltage snapshot above) of the first eight beats is shown below, illustrating the progressive slowing of conduction, due to a shift from I_{Na^+} to $I_{Ca,L}$ -mediated propagation.