Intracellular Ca\(^{2+}\) waves, afterdepolarizations, and triggered arrhythmias

Yohannes Shiferaw\(^1\), Gary L. Aistrup\(^2\), and J. Andrew Wasserstrom\(^2*\)

\(^1\)Department of Physics and Astronomy, California State University (Northridge), Northridge, CA, USA; and \(^2\)Department of Medicine (Cardiology) and the Feinberg Cardiovascular Research Institute, Northwestern University Feinberg School of Medicine, 310 E. Superior St., Morton 7-607, Chicago, IL 60611, USA

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1. Introduction

Clinical studies have shown that sudden death is initiated by an illtimed propagated ectopic beat that leads to fibrillation.\(^1\)–\(^4\) However, the mechanism underlying these focal excitations is not completely understood. Experimental studies have demonstrated that abnormal calcium (Ca\(^{2+}\)) cycling is a critical factor in the development of focal excitations.\(^5\)–\(^9\) These excitations can be caused by spontaneous Ca\(^{2+}\) release (SCR) in the form of intracellular Ca\(^{2+}\) waves. These waves are initiated when Ca\(^{2+}\) release from a few Ca\(^{2+}\) release units (CRUs) on the sarcoplasmic reticulum (SR) causes regenerative release in adjoining units via Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR), causing Ca\(^{2+}\) wave propagation. The resulting depolarizing inward current through the electrogenic Na\(^{+}\)-Ca\(^{2+}\) exchanger (NCX) depolarizes the cell membrane to threshold, producing a triggered beat.\(^10\)–\(^14\)

The relationship between subcellular Ca\(^{2+}\) waves and focal excitations in cardiac tissue is, however, still not completely understood. The basic unanswered question is how Ca\(^{2+}\) release within a population of cardiac cells can induce sufficient inward current to overcome the electrotonic load of the neighbouring cells. In this paper, we will discuss some key ideas that are essential in answering this question, focusing on the probabilistic nature of SCR and the importance of measuring the timing distribution of Ca\(^{2+}\) waves in multicellular populations in tissue. We will also discuss our recent results showing that the likelihood of a triggered beat is determined by the variance of the timing distribution, which is dictated by the time course of SR reloading.\(^15\),\(^16\) In addition, we will discuss our observations of a form of Ca\(^{2+}\) wave that is distinct from SCR. These Ca\(^{2+}\) waves occur only during rapid pacing and occur with a latency that is significantly shorter than the spontaneous waves observed following cessation of pacing. We argue that these Ca\(^{2+}\) waves are ‘triggered’ as opposed to ‘spontaneous’, since they must be initiated by L-type Ca\(^{2+}\) channel (LCC) openings rather than random ryanodine receptor (RyR) openings. Finally, we discuss the complex dynamics and timing of these waves and why they may be at least as—and possibly more—arrhythmogenic than spontaneous waves.

2. How do spontaneous Ca\(^{2+}\) waves cause triggered arrhythmias?

Spontaneous Ca\(^{2+}\) waves are typically observed under conditions of SR Ca\(^{2+}\) overload and are known to cause depolarization of the cardiac cell membrane that, under the right conditions, can achieve the threshold for activation for spontaneous electrical activity.\(^17\),\(^18\) This process occurs because the Ca\(^{2+}\) released during Ca\(^{2+}\) waves induces sufficient inward current via the activation of Ca\(^{2+}\)-sensitive currents, particularly forward-mode NCX current but possibly other Ca\(^{2+}\)-sensitive inward currents. The resulting depolarization, known as a delayed afterdepolarization (DAD), is regulated by the timing and the amount of Ca\(^{2+}\) released by the Ca\(^{2+}\) wave so that if the DAD is early enough and large enough, it can activate the rapid Na\(^{+}\) inward current, producing a triggered beat.\(^12\),\(^19\),\(^20\)

The bi-directional coupling between subcellular Ca\(^{2+}\) and voltage is thought to be the mechanism underlying a variety of triggered arrhythmias. However, a fundamental question regarding the arrhythmogenic potential for Ca\(^{2+}\) waves is how Ca\(^{2+}\) release in single cells becomes sufficiently synchronized in both time and space to produce enough depolarization to bring a critical mass of tissue to voltage threshold. To address this question, recent studies have applied confocal imaging of subcellular Ca\(^{2+}\) in individual cardiac myocytes of the whole rat heart.\(^15\),\(^21\) These studies show that the timing of SCR is determined by localized Ca\(^{2+}\) release events which propagate as Ca\(^{2+}\) waves. The precise origin of these localized release events is not known, but it is likely that they are due to localized, SCR events of sufficient magnitude to propagate as a fire-diffuse-fire wave. Based on this physical picture, the probability distribution of the time to the first propagating SCR event (first latency distribution) dictates how Ca\(^{2+}\) waves are coordinated among cells in tissue. To quantify this probability distribution, we measured the average and variance of the first latency distribution.\(^15\) These quantities both decreased with increasing pacing rate and external Ca\(^{2+}\) concentration, thus explaining the reduced latency and increased synchronization of SCR across large cell populations.

In order to understand the mechanisms responsible for reduced timing variability, we applied numerical simulations of subcellular...
Ca\(^{2+}\) release within cells and groups of cells.\(^{15,16}\) These simulations revealed that the decrease in variance of the first latency distribution can be explained by the faster recovery of the SR Ca\(^{2+}\) load. This occurs because the uptake of Ca\(^{2+}\) back into the SR is up-regulated due to increased diastolic Ca\(^{2+}\) concentrations during rapid pacing. Thus, the faster recovery kinetics of SR Ca\(^{2+}\) content are directly responsible for both reduced latency and decreased timing variability. Therefore, even though the timing of Ca\(^{2+}\) waves is statistically independent between cells, these events tend to occur at the same time because all cells in the population experience a faster SR Ca\(^{2+}\) recovery. Thus, the apparent synchronization of Ca\(^{2+}\) waves among cells can be explained by the intrinsic properties of Ca\(^{2+}\) cycling, without invoking Ca\(^{2+}\) diffusion between cells.

### 3. Initiation of Ca\(^{2+}\) waves: triggered vs. spontaneous

In order to initiate a Ca\(^{2+}\) wave, Ca\(^{2+}\) release must first occur within a single CRU and then diffuse to initiate Ca\(^{2+}\) release at the neighbouring CRUs via CICR. Hence, it is important to understand the mechanisms that initiate the first Ca\(^{2+}\) release event. There are two distinct possibilities: (i) an LCC transitions to the open state and allows a flux of Ca\(^{2+}\) into the cell, diffuse, and then trigger Ca\(^{2+}\) release from the local RyR cluster; (ii) an RyR channel in the cluster randomly transitions to the open state and allows Ca\(^{2+}\) to flow from the SR into the CRU, which induces Ca\(^{2+}\) release via CICR. Ca\(^{2+}\) waves induced by the above mechanisms will be referred to as `triggered Ca\(^{2+}\)` waves and `spontaneous Ca\(^{2+}\)` waves, respectively. Most experimental studies typically measure Ca\(^{2+}\) waves following the cessation of pacing. In this case, the first Ca\(^{2+}\) wave typically occurs during the resting membrane potential, when the open probability of LCCs is low and they are therefore more likely to be spontaneous rather than triggered. However, triggered Ca\(^{2+}\) waves can be observed under the appropriate conditions, such as in heart failure.\(^{22,23}\) Figure 1A shows a longitudinal recording of a line-scan confocal image of a single myocyte from the epicardial surface of an intact failing spontaneously hypertensive rat (SHR) heart. Despite small Ca\(^{2+}\) transients evoked during stimulation at a basic cycle length (BCL) of 400 ms, a series of large Ca\(^{2+}\) waves (indicated by arrows) occurred during stimulation but were absent following the cessation of pacing. The incidence of triggered waves is non-uniform in the failing heart as shown in the transverse recording (Figure 1B) of two neighbouring myocytes in which normal excitation–contraction (E–C) coupling is present in Cell 1 while triggered waves (arrows) occur only during pacing in Cell 2. Supplementary material online, Video S1, shows a site where there are small Ca\(^{2+}\) transients in all cells in the visual field, even though many of the cells are also showing propagated triggered Ca\(^{2+}\) waves which cease immediately when pacing stops, after which several spontaneous waves develop in some cells before pacing is re-initiated. Hence, we can conclude that these waves are not spontaneous and are most likely triggered by LCC openings during the action potential (AP).

### 4. Mechanism for triggered waves

Ca\(^{2+}\) waves propagate in cardiac cells due to a fire-diffuse-fire mechanism that allows local Ca\(^{2+}\) release events to spread throughout the cell. A crucial requirement for this to occur is that there must be a large enough population of CRUs in the cell that have not already been activated. If this condition is not met, then Ca\(^{2+}\) waves will abort since they will quickly encounter regions of the cell that have recently released Ca\(^{2+}\) and are refractory. Therefore, an essential condition for the formation of triggered waves is the presence of a large population of available CRUs shortly after the AP upstroke. Under normal pacing conditions, the number of available CRUs following the AP upstroke is low since most CRUs have been triggered by LCC openings. However, under conditions of Ca\(^{2+}\) overload or rapid pacing, it is likely that the number of available CRUs is much larger than normal. There are two distinct mechanisms for this to occur. First, the open probability of LCCs during the AP plateau may be much smaller than normal so that only a few LCCs open to trigger Ca\(^{2+}\) sparks, because high diastolic Ca\(^{2+}\) levels during rapid pacing will induce Ca\(^{2+}\)-induced inactivation of LCCs and reduce their open probability. Furthermore, elevated diastolic Ca\(^{2+}\) increases the likelihood of Ca\(^{2+}\) wave propagation, since RyR open probability increases with Ca\(^{2+}\) concentration. Secondly, the response of CRUs to LCC openings is a sharp threshold function of the SR load,\(^{24}\) so that the availability of CRUs depends on the time course of the SR reloading. Consequently, triggered waves can occur if the SR load is below some threshold immediately following an AP upstroke but then crosses that threshold during the ensuing AP. In this case, the timing of the triggered waves is dependent on the time course of the SR load immediately following the AP upstroke.

The mechanisms described above can explain the presence of triggered waves during heart failure. In these cells, E–C coupling is disrupted due to an abnormal t-tubule pattern, which leaves a large number of RyRs that can no longer be triggered by LCC channel

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**Figure 1** Triggered waves in congestive heart failure. (A) Longitudinal line-scan recording of a single cell on the epicardial surface of a failing intact SHR heart paced at BCL = 400 ms. Arrows indicate triggered waves. The top trace is the ECG and the bottom trace is the mean intensity profile. (B) Transverse recording of two neighbouring myocytes during pacing at BCL = 700 and 400 ms. Reproduced from Wasserstrom et al.\(^{15}\) with permission.
openings. Thus, LCC openings in a large number of CRUs are insufficient to induce Ca\(^{2+}\) release. This leaves a large population of CRUs that can support a Ca\(^{2+}\) wave, thus promoting triggered waves. Furthermore, elevated diastolic Ca\(^{2+}\) in heart failure ensures that the number of LCC openings is reduced due to Ca\(^{2+}\)-induced inactivation, while RyR channels are more prone to induce and support Ca\(^{2+}\) waves.

5. Triggered waves as a novel mechanism of early afterdepolarizations

An important and novel feature of triggered waves is that they occur during the AP, whereas spontaneous waves occur only during diastole. Thus, the timing of spontaneous Ca\(^{2+}\) waves suggests their involvement in DADs while triggered waves might also be involved in the induction of early afterdepolarizations (EADs), depending on the timing of the first triggered spark. This mechanism for EADs is novel as it is generally believed that EADs are due to a voltage-dependent mechanism involving Ca\(^{2+}\) and K\(^{+}\) currents, although there is some evidence that SR Ca\(^{2+}\) release, and perhaps even Ca\(^{2+}\) waves, may cause EADs. Certainly, the timing of triggered waves is consistent with this intriguing notion since these Ca\(^{2+}\) release events are both large and late during systole, so that the inward current generated late during the AP plateau could be sufficient in both timing and magnitude to produce enough inward current to produce a late Phase-2 or -3 depolarization. However, if it is in fact true that EADs might also occur as the result of Ca\(^{2+}\) cycling, possibly arising from triggered waves, then this behaviour should respond quite readily to pharmacological interventions which target intracellular Ca\(^{2+}\) cycling that is independent of traditional notions of SR Ca\(^{2+}\) overload, since this is not a requirement for triggered waves as it is for spontaneous Ca\(^{2+}\) waves.

6. Triggered waves and arrhythmias: role of ectopic foci

How might triggered waves contribute to cardiac arrhythmias? In order for an ectopic beat to occur due to triggered Ca\(^{2+}\) waves, it will be necessary that these events occur in large cell populations. For this to occur, a triggered wave should occur within a critical fraction of cells in tissue, at roughly the same time, in order to summate to overcome the electrotonic current drain to the surrounding cells. It is possible for this to occur if the frequency of triggered waves is high. This is precisely what we would expect in Ca\(^{2+}\) overload and heart failure where, as observed experimentally, the frequency of triggered waves increased dramatically. A particularly important feature of triggered waves is that they occur during systole at rapid rates. Thus, the timing variability of these events is naturally less than that of spontaneous Ca\(^{2+}\) waves, which can occur over a much longer time interval during diastole. Thus, it may be possible that during rapid pacing, triggered waves can occur nearly simultaneously in many cells. Also, it is important to consider that an ectopic excitation due to triggered waves may be more dangerous than that due to spontaneous Ca\(^{2+}\) waves, because triggered waves can occur at short time intervals after an AP upstroke. Therefore, the coupling interval between a triggered excitation and the previous paced beat can occur during or towards the end of the AP so that the ensuing wave front will closely follow the wave back of the preceding beat and will have a higher likelihood of propagation failure. Hence, we expect that ectopic foci due to triggered waves would have a higher probability of leading to wave break and fibrillation.

7. Triggered waves and arrhythmias: formation of a dynamic substrate

Wave break occurs when ectopic excitation propagates in cardiac tissue with a non-uniform AP duration (APD) distribution. In particular, if there are large gradients in APD, then a triggered excitation can propagate into a region where a short APD occurred, whereas block occurs in a refractory region following a long APD. Triggered waves can lead to a heterogeneous distribution of APD, since they occur during an AP and therefore significantly change the APD on a beat-to-beat basis. Thus, if a region of tissue exhibits a larger than average fraction of triggered waves on a certain beat, then the APD in this region may be significantly larger than in neighbouring regions. Another mechanism for the formation of a heterogeneous APD substrate is that since Ca\(^{2+}\) and voltage are bi-directionally coupled, triggered waves destabilize the periodic response of the APD. Thus, a triggered Ca\(^{2+}\) wave between beats will typically induce an increase in the APD. This effect will then feed back on Ca\(^{2+}\) on the next beat, since a larger APD in one beat will tend to lead to reduced LCC current in the next, owing to the shorter diastolic interval. Furthermore, a triggered Ca\(^{2+}\) wave will deplete the SR and prevent Ca\(^{2+}\) release on the next beat. Thus, we expect that a triggered wave will disrupt the dynamics of voltage and Ca\(^{2+}\) under rapid pacing conditions. In the tissue setting, this will lead to spatial gradients in APD, since different cells will respond differently to rapid pacing, preventing spatiotemporal synchronization of the voltage and Ca\(^{2+}\) response. This mechanism is similar to spatially discordant APD alternans where one region of tissue alternates in a long–short–long–short pattern, while a neighbouring region alternates out of phase (short–long–short–long). Here, we expect an even more complex dynamic pattern, as triggered waves will disrupt the non-linear response of both voltage and Ca\(^{2+}\) cycling.

8. Conclusions

Numerous studies have shown that various cardiac arrhythmias are initiated by triggered excitations that are caused by SCR. However, it is not well understood how Ca\(^{2+}\) release at the single-cell level can lead to triggered activity in tissue. In this manuscript, we described several ideas that are necessary to understand the precise connection between subcellular Ca\(^{2+}\) and the triggers for arrhythmias in the heart. In particular, the timing distribution of SCR within a large cell population in tissue is the key factor that determines whether SCR activity can summate to form ectopic foci. Moreover, it is the time course of SR calcium release that determines the variance of this distribution and that is the critical factor to determine the likelihood of triggered activity. Insights gained from these studies suggest novel gene-based or pharmacological treatments that seek to target the formation of dangerous ectopic beats. For example, our work has identified the rate of SR Ca\(^{2+}\) refilling as a potential therapeutic target since it controls the relative synchrony of SCR in a population of
cells. Furthermore, we have described ‘triggered waves’ which may be highly arrhythmogenic since they occur early in the AP and thus can induce propagating excitations that are more likely to undergo wave break. Also, owing to their early onset, triggered waves provide a putative mechanism for EADs that has not been described before. These findings should stimulate further exploration of these complex spatiotemporal features of Ca\(^{2+}\) and their connection with cardiac arrhythmias.

**Supplementary material**

Supplementary material is available at Cardiovascular Research online.

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