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Invited Editorial

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CALCIUM HANDLING, CONTRACTION, AND RYANODINE RECEPTORS: integration for cardiac “electricians”

This issue of Dialogues contains a feature article by Edward G. Lakatta, which develops the historical background and some of our present-day understanding of normal cardiac myocyte Ca²⁺ transport and how that may be involved in cardiac inotropy and pathophysiological triggered arrhythmias (via effects in ventricular myocytes) and also normal pacemaker activity (via affects in sinoatrial node [SAN] cells).

This lead article is accompanied by reviews that describe how enhanced ryanodine receptor (RyR) leak may occur in cardiac myocytes during heart failure (Stephan E. Lehnart and Gerd Hasenfuss), how local mechanical inhomogeneities may create initiation sites of triggered activity (Henk E. D. J. ter Keurs), and how local triggered activity can propagate to whole-heart arrhythmias (James N. Weiss). This suite of reviews by leaders in these fields paints an expert current picture of how cardiac myocyte Ca²⁺ transport and mechanics can mediate pacemaker activity and triggered arrhythmias.

We are now coming full circle, in the following way. We have for many years collectively pursued a dominant (and productive) reductionist approach in cardiac electrophysiology to understand which membrane ion channels and electrophysiological properties (and the molecular bases thereof) contribute to the observed cardiac electrophysiology. This is similarly true for foci on Ca²⁺ transport and myofilament properties. For a number of years cardiac “electricians” focused on membrane ion channels and cellular electrical coupling, without considering how Ca²⁺ handling and mechanics could influence electrophysiology. That is, we assumed that there was predominantly unidirectional control from membrane potential (Vₘ) to Ca²⁺ transient to contraction.

Keywords: calcium; sarcoplasmic reticulum; pacemaker; arrhythmia

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During recent years there has been an increasing awareness that there is interactive bidirectional coupling of each of these three Ca2+-electromechanical processes in the heart. It is now widely appreciated that alterations in myocyte Ca2+ handling can be a predominant initiator of arrhythmias and can alter the electrophysiological substrate. Thus, Ca2+ can dynamically influence \( V_m \), just the way \( V_m \) can influence [Ca2+]i (eg, due to numerous Ca2+-dependent ion channels and transporters). It is also now well appreciated that force and myofilament properties can influence [Ca2+]i (eg, when force or sarcomere length change, it can influence Ca2+ dissociation from the myofilaments). There are also stretch-activated channels in myocytes that connect mechanical stress to \( V_m \) changes. Thus, each of these subsystems can influence each other. While this makes full understanding of cardiac electrophysiology more challenging, it opens up new opportunities to understand fine control of the heart by multiple interacting pathways.

**Ca2+-INDUCED Ca2+-RELEASE AND Ca2+ RELEASE-INDUCED DEPOLARIZATION**

During the normal myocyte action potential (AP) and excitation-contraction coupling (ECC),5 depolarization activates Ca2+ current (\( I_{Ca} \)), which triggers sarcoplasmic reticulum (SR) Ca2+ release via the RyR via Ca2+-induced Ca2+-release. In addition to activating contraction, this SR Ca2+ release causes strong Ca2+-dependent \( I_{Ca} \) inactivation, providing feedback on \( V_m \) and Ca2+ influx. The elevated submembrane [Ca2+]i also stimulates Ca2+ extrusion via inward Na+/Ca2+ exchange current (\( I_{NCX} \)), which tends to prolong action potential duration (APD). There are other channels directly regulated by intracellular Ca2+ (chloride, monovalent cation and potassium channels), but \( I_{Ca} \) and \( I_{NCX} \) are probably the most important quantitatively. Some channels are also indirectly influenced by [Ca2+]i due to activation of Ca2+-calmodulin-dependent protein kinase (CaMKII; including Na+, Ca2+ and K+ channels6), which can have further major impact on Ca2+ handling, force, and electrophysiological properties.

RyRs exhibit stochastic openings, which are very rare during diastole, especially in early diastole. However, the leak rate gradually increases as SR [Ca2+] rises, and importantly as RyR recover from inactivation. This steady rate of SR Ca2+ leak is sometimes detectable as Ca2+ sparks (where a cluster of neighboring RyR coactivate at one site). Under normal ventricular myocyte conditions, these diastolic local Ca2+ sparks can activate \( I_{NCX} \) locally, but this creates so little inward current that in ventricular myocytes with robust inward rectifier K+ current (\( I_{K1} \)) it causes no perceptible depolarization. RyR opening and SR Ca2+ leak is increased as intra SR [Ca2+] \( \text{([Ca}^{2+}]_{SR}) \) rises.7,8 If [Ca2+]_{SR} is driven up (as in sympathetic stimulation or reduced Ca2+ extrusion by Na+/Ca2+ exchange upon Na/K-ATPase inhibition) Ca2+ spark probability is greatly increased. Once this exceeds a key threshold level, because of the higher Ca2+ release and greater RyR sensitivity to Ca2+, propagating waves of Ca2+-induced Ca2+-release occur that traverse the entire...
myocyte. In this case there is relatively synchronized activation of $I_{\text{NaCa}}$ and a measurable transient inward current or delayed afterdepolarization (DAD) occurs. Again, in a normal myocyte that has large $I_{K1}$ and is electrically coupled to its neighbors in the heart (which provide a current sink) these DADs may rarely reach threshold to trigger a propagating AP throughout the heart. However, in pathophysiological settings like heart failure where Na$^+/\text{Ca}^{2+}$ exchange is upregulated and $I_{K1}$ and cell-cell coupling are reduced, the possibility that a Ca$^{2+}$ wave and DAD can trigger an AP$^9$ and arrhythmia is enhanced. Furthermore, enhanced phosphorylation of the RyR by phosphokinase A (PKA) or CaMKII has been reported to additionally sensitize the diastolic SR Ca$^{2+}$ release process, reducing the above threshold and further facilitating arrhythmogenic propensity$^2,10,11$ In this scenario the normal ECC situation is reversed and SR Ca$^{2+}$ release can trigger depolarization and an action potential. It is also now clear that this mechanism is an important contributor to triggered arrhythmias$^4$.

The Ca$^{2+}$ processes at the heart of this mechanism are also working at the local cellular level and Ca$^{2+}$ waves generally do not cross cell borders (which contrasts strongly with the long electrical space constant in the heart). This may help to foster spatially discordant alternans and chaotic synchronization that Weiss discusses as critical steps with respect to transitions to ventricular tachycardia$^4$.

Another feature of these Ca$^{2+}$ waves in myocytes is that if Ca$^{2+}$ is not adequately removed from the system (eg, by $I_{\text{NaCa}}$), the system can produce relatively stable oscillations of SR Ca$^{2+}$ release, reuptake and re-release. Lakatta’s group$^1$ has championed the idea that in SAN cells this type of oscillatory cycle is highly tuned to work as a regulated SR “Ca$^{2+}$ clock” that is coupled to a “membrane current clock” (in which the hyperpolarization-activated cyclic nucleotide gated [HCN] current $I_f$ is important) and that the Ca$^{2+}$ clock is the major driver in heart rate regulation. While their emphasis on the Ca$^{2+}$ clock may be stronger than preferred by some colleagues whose work has shown importance of $I_f$ in SAN and heart rate control,$^12$ the case is now compelling that both of these systems exist and must be functionally coupled. Arguments about clear predominance of one system makes for healthy scientific debate that can push useful experimental tests,$^13$ but what is also clear is that these systems create a redundancy of control points that ensure that the heart has a regulated pacemaker, even if one system fails.$^{14}$

**SINOATRIAL NODE PACEMAKER CELLS AS A VARIANT OF VENTRICULAR MYOCYTES?**

The above discussion of RyR and oscillatory Ca$^{2+}$ waves and DADs in ventricular myocytes almost begs the question (also discussed by Lakatta)$^1$ how do SAN cells differ and is their Ca$^{2+}$ clock pacemaker activity mechanistically the same as for pathophysiological DADs in ventricular myocytes? Let’s think about SAN properties that differ from...
myocytes that may set the stage. First, SAN cells have low $I_{K1}$ levels, which causes both their relatively depolarized diastolic $V_m$, but also enhances the amount depolarization caused by a given Ca$^{2+}$ signal and $I_{NCX}$. Second, SAN cells have higher basal cAMP and PKA activity levels that enhance Ca$^{2+}$ influx via $I_{Ca}$, SR Ca$^{2+}$-pumping and keep the SR Ca$^{2+}$ load high enough to reach threshold for substantial Ca$^{2+}$ sparks or waves. Third, the relatively high basal RyR phosphorylation by PKA and/or CaMKII may diminish the $\left[Ca^{2+}\right]_{SR}$ threshold for RyR activation. Fourth, SAN cells have reduced cell-cell coupling, which limits the $V_m$-dissipating effect of the large current sink experienced by ventricular myocytes. Fifth, SAN cells exhibit T-type Ca$^{2+}$ channels plus a variant of L-type Ca$^{2+}$ channel (Ca$_{1.3}$) and possibly higher PKA-dependent activation of $I_{Ca}$. All of these shift the activation voltage-dependence of $I_{Ca}$ to more negative $V_m$ that enhances excitability. This may also create a smooth handoff of depolarization from T-type Ca$^{2+}$ current to PKA-shifted L-type $I_{Ca}$ and then unphosphorylated Ca$^{2+}$ channel current. Sixth, many of these steps can be modulated by changes in cAMP associated with sympathetic activation (eg, Ca$^{2+}$ current, and SR Ca$^{2+}$-pumping, load, and release) giving this system the ability to alter heart rate appropriately in response to physiological sympathetic and vagal stimulation. Seventh, this system can operate in SAN cells in parallel to, and coupled with, cAMP-regulated hyperpolarization-activated $I_f$ and acetylcholine activated K$^+$ channels.

At the risk of overdoing the analogy, SAN cells have behaviors like the ventricular myocyte, except they are specialized as above to optimize the Ca$^{2+}$ clock–like function that in healthy ventricular myocyte is importantly suppressed (to minimize triggered arrhythmias). However, many of the pathophysiological changes in ventricular myocyte (eg, in heart failure) alter their characteristics to be more like pacemaker cells (making them more proarrhythmic). So these cardiac cells seem to be on a phenotypical continuum rather than being totally distinct. Implicit in this is a kind of phenotypic plasticity that may also be important to keep in mind.

Cardiac electrophysiologists are used to thinking about heterogeneity with respect to altered excitability or action potential duration, and these days even local Ca$^{2+}$-dependent heterogeneities. However, ter Keurs' group has really opened our eyes to ways that contractile nonuniformity can be arrhythmogenic. Most of us can readily appreciate that the very high positive cooperativity observed in Ca$^{2+}$-dependent myofilament activation is beneficial in rapidly activating contraction once SR Ca$^{2+}$ release occurs.

This occurs because Ca$^{2+}$ binding to troponin C is enhanced by a neighboring Ca$^{2+}$-bound troponin C molecule, myosin cross-bridge binding, and sarcomere length/filament force. These factors are crucial also in explaining the Frank-Starling law of the heart that all physiology and medical students learn about. What is less widely appreciated is that these same properties can contribute to unique problems during relaxation.
where there is inhomogeneous contractile activation. If some cells in a region produce less force (eg, because of local acidosis or oxidative stress), the healthy cells nearby will stretch them during systole and they will shorten as the normally activated region relaxes. This sharp interface can result in rapid local Ca\textsuperscript{2+} release from the myofilaments that can initiate a wave of Ca\textsuperscript{2+}-induced SR Ca\textsuperscript{2+}-release that functions much as discussed above for DADs, including with respect to arrhythmic propensity. These local mechanical perturbations could also produce more direct local alterations in Ca\textsuperscript{2+} handling processes (eg, RyR) and ionic currents (eg, stretch-activated currents), but details of such potential pathways remain to be worked out.

**IN CONCLUSION**, these four thoughtful reviews show us how complementary perspectives on the interplay of cardiac myocyte Ca\textsuperscript{2+} handling, arrhythmias, and pacemaking enrich our overall understanding of these important processes. They also reinforce the notion that we must endeavor now to integrate our rich understanding of cardiac electrophysiology, Ca\textsuperscript{2+} signaling, and contractile processes, as highlighted here. Of course that will also need to broaden to take more full account of metabolism and mitochondrial pathways and also the myriad signaling networks that cross all of these systems via posttranslational modifications (phosphorylation, oxidation, nitrosylation ...) and the pathways that produce long-term changes in protein expression that alter these systems during physiological adaptation and pathophysiological changes.

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Spatial nonuniformity of excitation-contraction coupling causes arrhythmic Ca2+ waves in rat cardiac muscle.  
Intracellular Ca\textsuperscript{2+} cycling is a general regulator of how fast and how strong the heart beats

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Heart performance, in its most evident form, is gauged by how often the heart beats and the strength of each beat, i.e., cardiac chronotropy and inotropy, respectively. Only a fraction of the maximum cardiac performance is utilized in the basal state; but during stress, e.g., dynamic exercise, when the demand for cardiac output increases, the ability of the heart to acutely beat faster and stronger is central to our survival instinct. Different types of cells within the heart, that is, pacemaker cells within the sinoatrial node and ventricular myocytes, determine how fast and strong, respectively, the heart beats.

The basis of the generation of rhythmic heart beats is a conundrum that has intrigued inquisitive minds of philosophers and scientists for centuries, and current
The dogma of the initiation and regulation of rhythmic heart beats has evolved from the intertwining of theory and experimentation for almost 200 years. Advances in biophysics, immunohistochemistry, confocal imaging, genomics, and proteomics continue to provide crucial information regarding expression, localization, and functions of proteins within these two cardiac cell types.

In order to function, excitable cells within the heart, ie, ventricular, atrial, or sinoatrial nodal cells, maintain electrochemical gradients of ions, eg, Ca²⁺ or Na⁺, that are far removed from their thermodynamic equilibriums (Figure 1). Ca²⁺ flux into and out of the cell and cytosol is regulated or modulated by surface membrane molecules, eg, Ca²⁺, K⁺, and Na⁺ channels, Na/K-ATPase, Na⁺/Ca²⁺ exchanger (NCX), and Na⁺/H⁺ exchanger, and by molecules of organelles such as the sarcoplasmic reticulum (SR) that can pump Ca²⁺, ie, SERCA2 (sarco/endoplasmic reticulum Ca²⁺-ATPase) (Figure 3, page 256) and release Ca²⁺, ie, ryanodine receptors (RyR) (Figure 3). Moreover, the normal function of these molecules is regulated by ion concentration gradients or membrane potential. In other words, molecules that regulate intracellular and transcellular ion gradients and membrane potential are regulated by the very functions that they regulate, leading to a robust, dynamically entrained, complex molecular system. Other important modulators of excitability of cardiac cells include pH, temperature, receptor-initiated signaling, redox state of proteins, and energy supply. Each heartbeat results from acute shifts in ion gradients creating surface membrane voltage oscillations, ie, action potentials, Ca²⁺ oscillations, ie, intracellular Ca²⁺ transients, and myofilament length and force oscillations, ie, transient contractile protein interactions that cause myocardial contraction.

Early studies of the origin of the heart's function focused simultaneously on both excitability and contraction (Figure 2A). However, following development of quantitative membrane excitability theory in the 1950s, research into mechanisms of pacemaker cell function and cardiac myocyte contraction have proceeded along essentially separate paths (Figure 2: B-J and L-S), often in isolation of each other. The pacemaker research field became dominated by the idea that pacemaker cell function could solely be accounted for by an ensemble of sarcolemmal ion channels that produce rhythmic action potentials (AP), via reciprocal activation and inactivation of ion channels that cause change in membrane voltage, ie, rhythmic APs. Research on the duty cycle of ventricular myocytes, ie, contraction, in contrast, became dominated by studies of intracellular Ca²⁺ regulation by proteins that regulate Ca²⁺ flux into and out of the cytosol (Ca²⁺ cycling), particularly those of the SR, the major intracellular Ca²⁺ store within the cells, Ca²⁺ interactions with myofilament proteins, and with proteins that regulate flux Ca²⁺ into and out of the cell. While recognition of the importance of Ca²⁺ cycling in pacemaker function has been late to emerge, recent evidence that rhythmic, spontaneous Ca²⁺ releases from SR within cardiac pacemaker cells prompt the surface membrane to generate rhythmic APs provides the key that reunites pacemaker and ventricular cell research (Figure 2: K and T). Thus, perspectives gleaned from isolated reductionist achievements to elucidate how the ventricular myocytes regulate their strength of contraction and how pacemaker cells regulate the spontaneous beating rate have finally converged, leading to the emergence of a general theory to explain how often and how strong the heart beats, ie, cardiac chronotropy and inotropy, respectively: sequestration and release of Ca²⁺ to and from internal stores by surface membrane and intracellular molecules common to both ventricular myocytes and pacemaker cells regulates the duty cycle of both cell types, rhythmic excitation, and contraction, respectively.
EXECUTION OF THE HEARTBEAT BY VENTRICULAR MYOCYTES

The key role of Ca\(^{2+}\) in intrinsic excitation/contraction coupling in cardiac ventricular cells

Action potential–initiated intracellular Ca\(^{2+}\) release

Heart researchers had been “tuned in” to the crucial importance of extracellular Ca\(^{2+}\) in the cardiac muscle duty cycle since 1883, when Ringer’s London tap water fluke revelation illuminated the field.\(^1\) It was later inferred from early studies of contractile mechanics and energetics of skeletal muscle that the action potential initiated an “active state” within muscle cells that resulted in contraction (Figure 2L).\(^2\) Because studies in isolated myofilament preparations showed that Ca\(^{2+}\) caused myofilament interaction, it was further inferred that the active state in intact muscle cells likely included a transient rise in cytosolic [Ca\(^{2+}\)] that coupled surface membrane excitation to contraction.\(^3\) Cardiac muscle cells produce a broad AP, due to a slow

**Figure 2. Emergence of a general theory of the initiation and strength of the heartbeat.**

Research on membrane excitation and contractile function proceeded along separate paths (separated by a dashed line) until the critical importance of Ca\(^{2+}\) cycling became evident not only in cardiac muscle (ventricular or atrial cells), but also in normal pacemaker cells (see text for details). DAD, delayed afterdepolarization; EC, excitation-contraction; SANC, sinoatrial nodal cell; SCaRIE, spontaneous Ca\(^{2+}\) releases that ignite excitation.\(^105\)
inward Ca\(^{2+}\) current (discovered by Reuter in 1966,\(^4\) and later referred to as L-Type Ca\(^{2+}\) current \([I_{\text{CaL}}]\). Channels that carry this Ca\(^{2+}\) current were discovered via their binding of dihydropyridines (Ca\(^{2+}\) channel blockers) and are sometimes referred to as dihydropyridine receptors. Activation of these ion channels, also referred to as L-type Ca\(^{2+}\) channels (LCCs), during the AP results in influx of Ca\(^{2+}\) into the cell (Figure 3A). AP-induced Ca\(^{2+}\) influx leads to Ca\(^{2+}\) release from SR that evolves into a global rise in intracellular Ca\(^{2+}\) that activates myofilaments to produce a contraction (Figure 3B).

The increase in intracellular Ca\(^{2+}\) not only activates the myofilaments to effect a contraction, but also has potent modulatory effects on sarcolemmal ion channel currents, and affects the shape of the AP.\(^3\) This is one manifestation of the complex dynamic mutual interactions of intracellular Ca\(^{2+}\) cycling and surface membrane electrogenic molecules. Relaxation, ie, contractile filament lengthening and force dissipation, occurs as the intracellular Ca\(^{2+}\) concentration returns to preexcitation levels. The SR Ca\(^{2+}\) pump isoform, SERCA2A, is the dominant mechanism that removes Ca\(^{2+}\) from the cytosol following excitation/contraction (Figure 3A). In most mammalian ventricular myocytes, SERCA2A function accounts for about 60% to 75% of Ca\(^{2+}\) removal from the cytosol. About 25% to 40% of Ca\(^{2+}\) released into the cytosol is extruded from the cytosol via the sarcolemmal NCX (Figure 3A), insuring that the same quantity of Ca\(^{2+}\) that enters the cell during each cycle via L type channel activation leaves the cell,\(^6\) (otherwise the cardiac myocyte would turn into cement!). The discovery that the major source of the AP-triggered increase in intracellular Ca\(^{2+}\) in ventricular myocytes is due to Ca\(^{2+}\) release from the SR was soon followed by studies defining mechanisms of excitation-induced Ca\(^{2+}\) release. That a small Ca\(^{2+}\) trigger produced by Ca\(^{2+}\) influx via LCCs on the surface membrane that are activated by the AP induced large release of Ca\(^{2+}\) from the SR, ie, Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR), was discovered in cardiocytes in which the surface membrane had been peeled away.\(^7,18\) SR Ca\(^{2+}\)-release channels were later identified in cardiac ventricular cells on the basis of their ability to specifically bind the plant alkaloid ryanodine. Hence, these Ca\(^{2+}\)-release channels of the SR have been dubbed “ryanodine receptors” (Figures 3A and 4A).

When fluorescent Ca\(^{2+}\) probes that could easily be introduced into single cardiac cells became available, great strides were achieved in discovering and interpreting mechanisms of intracellular Ca\(^{2+}\) release.\(^8-11\) RyRs of SR and LCC channels within T-tubules of the surface membrane face each other across a 30-nm cleft, a geometrical relationship referred to as a “coupion” (Figure 4A). Detailed biophysical studies showed that graded LCC activation, achieved by graded membrane

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Figure 3. Ca\(^{2+}\) cycling regulates the duty cycle of ventricular myocytes.

Panel A. Action-potential (AP)-initiated Ca\(^{2+}\) influx via \(I_{\text{CaL}}\) initiates global Ca\(^{2+}\) release from sarcoplasmic reticulum (SR) (Ca\(^{2+}\) transient), and the Ca\(^{2+}\) transient activates myofilament contraction. NCX, Na\(^+[/Ca\(^{2+}\)]\) exchanger; PLB, phospholamban; RyR, ryanodine receptor; SERCA, sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase.\(^105\) Panel B. The temporal profile of an action potential (AP) cytosolic Ca\(^{2+}\) transient (\(C_{\text{ai}}\)) elicited by the AP, and the contraction elicited by the \(C_{\text{ai}}\) transient.
depolarizations, resulted in graded RyR Ca\(^{2+}\) releases and global cytosolic Ca\(^{2+}\) transients of graded amplitude. The amount of Ca\(^{2+}\) released from SR via RyRs in response to a Ca\(^{2+}\) trigger of a given magnitude, ie, the “gain of CICR,” is a general measure of excitation/contraction (EC) coupling efficiency. The gain of CICR is under firm local control\(^{12}\) by local Ca\(^{2+}\) fluxes of individual LCCs and RyRs within a couplon (Figure 4A).

“Nanoscopic” details of LCC-RyR crosstalk become manifest when the spread of local RyR Ca\(^{2+}\) release within individual couplons is curtailed by a slow Ca\(^{2+}\) buffer (eg, EGTA [ethylene glycol tetraacetic acid]) (Figure 4B). Several additional fundamental facets of EC-coupling, for example, interaction of RyRs and termination of RyR Ca\(^{2+}\) release, are comprehensively discussed in a recent review.\(^{13}\)

Early studies\(^{14}\) had also demonstrated a “memory” within cardiac cells that enables contractions of differing amplitudes in response to changes in the rate or pattern of stimulation. It was later inferred that this involved changes in the extent of SR Ca\(^{2+}\) loading,\(^{15}\) which occur over several beats, due to beat-dependent net changes in cell Ca\(^{2+}\).

To summarize, the AP of ventricular cells is caused by an electrical impulse generated within the sinoatrial node (SAN) and conducted to the ventricular myocardium. The resultant initial depolarization of the ventricular myocyte membrane activates Na\(^+\) channels, which produce a large depolarization that activates LCCs to generate \(I_{\text{CaL}}\), which triggers SR Ca\(^{2+}\) release to generate a global cytosolic Ca\(^{2+}\) transient. The amplitude of the AP-triggered global intracellular Ca\(^{2+}\) oscillation within a given heartbeat is determined by: (i) the amplitude of the \(I_{\text{CaL}}\) trigger that effects CICR; (ii) how much Ca\(^{2+}\) is released from the SR, determined in part by the SR Ca\(^{2+}\) load; and (iii) the extent of restitution of SR Ca\(^{2+}\) following the prior AP-induced SR Ca\(^{2+}\) depletion, by repumping Ca\(^{2+}\) back into the SR and removal of RyR inactivation that occurred as a result of the prior Ca\(^{2+}\) release.

**\(\beta\)-AR modulation of intrinsic ventricular cardiac myocyte excitation–Ca\(^{2+}\) release–contraction mechanisms makes the heart beat stronger**

Neither the SR Ca\(^{2+}\) load nor its Ca\(^{2+}\) cycling restitution kinetics are fixed, but change dramatically in response to heart-brain communication via the autonomic nerv-
ous system. A cascade of phosphorylation reactions initiated by β-adrenergic receptor (β-AR) stimulation of ventricular myocytes (Figure 5A) leads to an increase in amplitude and acceleration of the kinetics of LCC-gating mechanisms and to an increase in the amplitude of the global cytosolic Ca²⁺ transient and contraction (Figure 5B). β-AR stimulation increases the likelihood for RyRs within a given couplon to fire synchronously (ie, RyR recruitment) (Figure 6A), augmenting the local Ca²⁺ release amplitude (Figure 6C). β-AR stimulation also synchronizes the occurrence of RyR Ca²⁺ release to earlier times following excitation within a given couplon (Figure 6A). Thus, in addition to recruiting Ca²⁺ RyRs to augment Ca²⁺ release within a given couplon, β-AR stimulation appears to “front-load” the system to deliver most of the Ca²⁺ at the onset of depolarization, rather than spreading it out throughout depolarization. The fraction of couplons that fire in response to excitation is also increased by β-AR stimulation (Figure 6B). In other words, β-AR stimulation also synchronizes RyR firing among couplons. Thus, the enhanced augmentation of RyR Ca²⁺ release by β-AR stimulation results from synchronization of RyR gating (to the active state) both within and

![Figure 5. β-AR Signaling Cascade](image)

**Panel A.** The signaling cascade that transmits β-adrenergic receptor (β-AR) stimulation to charges in the amplitude and kinetics of Ca²⁺ and contraction. TnC, troponin C.β-AR stimulation increases the amplitude and kinetics of Ca²⁺ and contraction. TnC, troponin C.β-AR stimulation increases the amplitude and kinetics of Ca²⁺ and contraction. TnC, troponin C.β-AR stimulation increases the amplitude and kinetics of Ca²⁺ and contraction. TnC, troponin C.β-AR stimulation increases the amplitude and kinetics of Ca²⁺ and contraction. TnC, troponin C.β-AR stimulation increases the amplitude and kinetics of Ca²⁺ and contraction. TnC, troponin C.β-AR stimulation increases the amplitude and kinetics of Ca²⁺ and contraction. TnC, troponin C.β-AR signaling cascade transmits β-adrenergic receptor (β-AR) stimulation to charges in the amplitude and kinetics of Ca²⁺ and contraction.

![Figure 6. Stimulation of β-Adrenergic Receptors of a Cardiac Myocyte](image)

**Panel B.** Simultaneous recordings of membrane current, global cytosolic Ca²⁺, indexed by Indo fluorescence, and contraction (cell length) measured by a photodiode array in a representative single rat ventricular cell. β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.β-AR stimulation by norepinephrine (NE) (+ prazosin) increases the amplitude and kinetics of all three parameters.
Figure 6. Role of β-adrenergic receptor in resynchronizing local Ca\textsuperscript{2+} in ventricular myocytes.

Panel A. Confocal images show that β-adrenergic receptors activate more local calcium releases (LCRs), and these are ignited in a more synchronous fashion, and become brighter. NE, norepinephrine; Pra, prazosin.\textsuperscript{16} Panel B. The fraction of active transverse tubule–sarcoplasmic reticulum (T-SR) junctions (ε) increases as a function of membrane voltage and in response to β-AR stimulation.\textsuperscript{16} Panel C. Average β-AR stimulation effect on LCR amplitude as a function of membrane voltage.\textsuperscript{16} Panel D. Top: representative traces of rat single L-type Ca\textsuperscript{2+} channels (LCCs) activity measured in the same cell-attached patches prior to and following β\textsubscript{1}-adrenergic receptor (β\textsubscript{1}-AR) stimulation. Bottom: histograms of times to first opening of LCCs (lower), measured as time from onset of depolarization to the first channel opening. Note the shift to earlier times for LCC openings effected by β-ARs.\textsuperscript{17}
among couplons. Additionally, β-AR stimulation synchronizes the triggers for RyR Ca\(^{2+}\) release,\(^{17}\) ie, it synchronizes the activation of individual LCCs following depolarization to earlier times following depolarization (Figure 6D). Thus, the synchronization of individual RyR firing following excitation effected by β-AR stimulation is attributable to effects on both RyRs and LCCs, producing a larger \(I_{\text{CaL}}\) and a global Ca\(^{2+}\) transient of larger amplitude (Figure 5B). Enhanced synchronization of Ca\(^{2+}\) pumping by SERCA2 molecules, effected by cAMP and Ca\(^{2+}\)-dependent phospholamban phosphorylation, in the context of enhanced synchronization of RyR Ca\(^{2+}\) release, effects a more rapid decay of the global cytosolic Ca\(^{2+}\) following its release (Figure 5B). Synchronized RyR Ca\(^{2+}\) release among RyRs by β-AR stimulation also likely synchronizes Ca\(^{2+}\)-dependent RyR-dependent inactivation and termination of Ca\(^{2+}\) release.

Synchronous exposure of relaxed myofilaments to Ca\(^{2+}\) is required for their optimal integrated displacement and force production. By synchronizing LCC and RyR activation, β-AR stimulation facilitates the highly cooperative action of the released Ca\(^{2+}\) on the contractile apparatus, eliciting a greater contraction for a given total Ca\(^{2+}\) release. More synchronized termination of RyR Ca\(^{2+}\) release and SR Ca\(^{2+}\) pumping, combined with a cAMP-PKA (phosphokinase A)-dependent reduction in Ca\(^{2+}\) binding to troponin within the contractile filament lattice, accelerates the kinetics of contractile relaxation (Figure 5A,B).

The ability to spontaneously oscillate Ca\(^{2+}\) becomes unmasked when SR is disconnected from its AP entrainment

Although not usually articulated as such, it is apparent from the foregoing evidence, that the cardiac SR is “wired” to rhythmically oscillate Ca\(^{2+}\), and that the essence of the normal duty cycle of a cardiac ventricular myocyte is, in fact, an AP-initiated global intracellular Ca\(^{2+}\) release that generates a global oscillation in cytosolic Ca\(^{2+}\), ie, a global cytosolic Ca\(^{2+}\) transient (Figure 3B). In the normally functioning heart, the ultimate cause and determinant of the periodicity of SR Ca\(^{2+}\) oscillators within ventricular cells is the period of the rhythmic ventricular cell membrane excitations (APs), which, in turn depends upon the period at which APs emanate from the SAN and are conducted to the ventricular myocardium. During normal heart beating, when rhythmically occurring APs emanating from the SAN synchronize RyR activation to produce rhythmic global cytosolic Ca\(^{2+}\) transients (Figure 3B), concomitant rhythmic depletions of the SR Ca\(^{2+}\) load occur. This Ca\(^{2+}\) depletion resets the SR’s rhythmic clock, and a restitution process for SR Ca\(^{2+}\) cycling then ensues. The potential for spontaneous Ca\(^{2+}\) release is inherent in the design of this organelle. When the restitution process is complete prior to the arrival of a subsequent AP, the SR Ca\(^{2+}\) load continues to increase and when it exceeds the threshold for spontaneous RyR activation, the SR becomes a “free-running” Ca\(^{2+}\) oscillator that generates spontaneous, diastolic Ca\(^{2+}\) releases, even in the context of physiologic intracellular [Ca\(^{2+}\)].\(^{7,18-26}\) If a subsequent AP is delivered prior to the achievement of a Ca\(^{2+}\) load that reaches threshold for spontaneous RyR activation, spontaneous Ca\(^{2+}\) release does not occur. Thus, acutely slowing the rate of external electrical stimulation to elicit APs in ventricular myocytes permits the SR clock to continue to run for a longer time following the prior AP, and increases the likelihood for spontaneous SR Ca\(^{2+}\) release to occur in the form of diastolic local Ca\(^{2+}\) releases (LCRs).\(^{20,22,27,28}\)

Like the synchronized SR Ca\(^{2+}\) release triggered by an AP, the restitution time for successive spontaneous LCRs is not fixed, but varies with the Ca\(^{2+}\) available for the SR to pump, the speed at which it pumps Ca\(^{2+}\), and the activation status of RyRs. These factors are potentially regulated by cAMP-mediated, PKA-dependent phosphorylation, phospholamban, and RyRs.\(^{6,29-31}\)

A detailed report of mechanisms that govern spontaneous SR Ca\(^{2+}\) release is provided in the accompanying article in the following section of this issue of Dialogues in Cardiovascular Medicine, by Stephan E. Lehnart and Gerd Hasenfuss: “How can calcium leaks occur within heart cells?”

Spontaneous LCRs have been observed in all cardiac cell types,\(^{32}\) including ventricular myocytes and pacemaker cells.\(^{33-35}\) In contrast to AP-initiated global SR Ca\(^{2+}\) release, or the myofilament contraction driven by it, spontaneous Ca\(^{2+}\) release occurs locally and asynchronously within and among cells comprising the myocardium. Although initiated locally within cells, LCRs achieve varying degrees of synchronization and wave-like propagation, via spontaneous CICR.

Ca\(^{2+}\) sparks at rest, generated by a few RyRs,\(^{33}\) which occur in ventricular myocytes of species that maintain a high SR Ca\(^{2+}\) local in the resting state, eg, rat or mouse, are stochastic, ie, an unsynchronized form of spontaneous LCRs, and do not produce a measurable local contraction or surface membrane depolarization. More synchronized forms of spontaneous LCRs generated by “free running” SR, ie, Ca\(^{2+}\) waves are roughly periodic and affect myofilament interaction and the surface membrane potential.\(^{36,37}\)
Figure 7. Status of the entrained sarcoplasmic reticulum (SR) clock in rabbit and rat.

Panel A. Confocal line scan image of Ca\(^{2+}\) signals (Fluo-4) measured in rabbit ventricular myocyte during β-adrenergic receptor (β-AR) stimulation by isoproterenol. The red line is the time course of normalized Fluo-4 fluorescence averaged along the scanning line. AP, action potential. Panel B. Simultaneous recording of Ca\(^{2+}\) signals (Indo-1), cell shortening, and membrane potential in a rat ventricular myocyte when external stimulation ceases. Panel C. Relationship between interstimulus interval and the time to the first local calcium releases (LCR) (spontaneous wave) when regular elevated stimulation is discontinued in rat ventricular cells in control (blue circles) and during β-AR stimulation by isoproterenol (ISO, red circles). Panel D. Left: Spontaneous Ca\(^{2+}\) oscillations reported by oscillations in fluorescence of the Ca\(^{2+}\) indicator, Indo-1 (top), contraction (middle), and membrane potential (lower) traces in a single rat cardiac myocyte. Note that the increase in Ca\(^{2+}\) (measured as the whole cell average of Indo fluorescence) is greater during multifocal LCRs (right tracings) than in unifocal LCRs (left tracings), and that this is associated with a larger membrane depolarization and contraction. Right: Ca\(^{2+}\) and membrane potential phase-plane diagrams for events in panel A form a continuum.

During rhythmic AP-induced ventricular cell beating, the status of the entrained SR clock can be “interrogated” experimentally by discontinuing external electrical stimulation and measuring the time from the last AP-induced Ca\(^{2+}\) release to the first spontaneous Ca\(^{2+}\) release (Figure 7A-C, page 261). Regularly occurring APs entrain the SR Ca\(^{2+}\) clock periodically and the likelihood for partially synchronized LCRs to occur when external electrical stimulation ceases depends upon the Ca\(^{2+}\) and phosphorylation status of the cell. With time, following cessation of AP-induced entrainment of SR Ca\(^{2+}\) cycling, LCRs gradually become desynchronized, as multifocal LCRs give way to unifocal LCRs and Ca\(^{2+}\) sparks, and cessation of spontaneous Ca\(^{2+}\) releases ensues as the SR becomes Ca\(^{2+}\)-depleted (Figure 7A). In rat (Figure 7B, C) and mouse ventricular myocytes, spontaneous Ca\(^{2+}\) sparks persist in the absence of electrical stimulation, because the SR Ca\(^{2+}\) load in these species remains high in the absence of regularly occurring APs.

Maneuvers that increase the strength of the AP-induced contraction via increasing cell and SR Ca\(^{2+}\) loading increase the likelihood for spontaneous Ca\(^{2+}\) release to occur between AP induced SR Ca\(^{2+}\) release and contractions.\(^{38}\) β-AR stimulation, for example, notably “speeds up” the “SR Ca\(^{2+}\) clock” ticking speed, reducing its recovery time from a prior release. (This is a hint of why spontaneous Ca\(^{2+}\) release occurs between APs in pacemaker cells, as discussed below.) There is a striking correlation between the wide range of gradations in contraction amplitude affected by gradations in perturbations that increase cell and SR Ca\(^{2+}\) load and the reduction in recovery time of the SR Ca\(^{2+}\) clock (Figure 8).\(^{22,27,28,32,39}\) As such, inotropic perturbations increase the SR Ca\(^{2+}\) cycling kinetics and SR Ca\(^{2+}\) load, the SR Ca\(^{2+}\) cycling restitution time becomes reduced and the SR Ca\(^{2+}\) clock begins to run faster as noted above. When its restitution time becomes less than that of the surface membrane clock that produces regular APs (ie, the fired rate of external electrical stimulation) spontaneous, partially synchronized LCRs begin to occur between APs, and the inotropic state saturates.

When partially synchronized spontaneous SR Ca\(^{2+}\) releases (eg, Ca\(^{2+}\) waves) occur between AP-driven Ca\(^{2+}\) releases they interact with the myofilaments to generate “aftercontractions” and membrane depolarization. This is often accompanied by subtle contractile amplitude “alternans.”\(^{38}\) Increasing the rate at which APs occur overdrives the free running SR Ca\(^{2+}\) clock, prevents spontaneous diastolic Ca\(^{2+}\) release, abolishes contractile alternans, thus allowing the average contractile state of all beats to further increase.\(^{27}\)

**Figure 8.** Correlation between perturbations that increase cell and SR Ca\(^{2+}\) load and reduction in recovery time of the SR Ca\(^{2+}\) clock.

**Panel A.** Action potential (AP)-initiated contraction amplitude at varying bathing [Ca\(^{2+}\)] in rat myocytes continuously stimulated at 0.2 Hz (▲), at 1 Hz: (●; n = 5), in the presence of ISO at 1 Hz (◊; n = 5), or following a brief exposure to 1 µM ryanodine (○; n = 5). The maximum contraction amplitude is similar in the four groups, although it occurs at varying bathing [Ca\(^{2+}\)].\(^{38}\) Panel B. A unique relationship between bathing [Ca\(^{2+}\)] at which the contraction amplitude peaks and spontaneous local calcium releases (LCRs), manifest as spontaneous contractile waves, first appeared for all cells studied in four protocols for all myocytes from panel A. Regardless of the drug or stimulation frequency, the bathing [Ca\(^{2+}\)] at which the action potential (AP)-initiated contraction saturates varies linearly with the bathing [Ca\(^{2+}\)] at which LCRs first became manifest.\(^{27}\)
**Spontaneous- Ca\(^{2+}\) release-ignited- excitations of the cell surface membrane**

The occurrence of spontaneous Ca\(^{2+}\) release usually produces a small depolarization at rest or between successive APs, ie, a diastolic afterdepolarization or DAD (Figure 7B) of the cell membrane via activation of an inwardly directed \(I_{\text{NCX}}\). This does not usually initiate a spontaneous AP, due, in part, to the presence, in ventricular myocytes, of an outwardly directed current (\(I_{\text{K1}}\)), which stabilizes the diastolic membrane potential. When the SR clock ticks at a faster rate, the ensemble of LCRs within and among cells becomes more synchronized, and the likelihood for spontaneous Ca\(^{2+}\) release to become multifocal is increased (Figure 7D). Multifocal Ca\(^{2+}\) release beneath the surface membrane exposes a greater area of the surface membrane and the NCX molecules to high Ca\(^{2+}\). The increased NCX inward current that is ignited by multifocal spontaneous Ca\(^{2+}\) release can be sufficient to depolarize the surface membrane to the level required to activate Na\(^+\) channels, and to generate a spontaneous AP.\(^{21}\)

**So what is Ca\(^{2+}\) overload?**

There is a general misconception within the cardiac research field that spontaneous SR Ca\(^{2+}\) release is synonymous with “Ca\(^{2+}\) overload.” The aforementioned considerations indicate that this is surely not the case, and that over a broad range of cell Ca\(^{2+}\) loading that leads to a broad range of AP-induced Ca\(^{2+}\) transient amplitudes, whether spontaneous diastolic Ca\(^{2+}\) release occurs depends upon the period of externally arriving APs relative to the restitution period of the SR Ca\(^{2+}\) clock, determined in large part by the kinetics of SR Ca\(^{2+}\) loading and Ca\(^{2+}\) release processes. A true state of “Ca\(^{2+}\) overload” is present when partially synchronized spontaneous diastolic LCRs (often multiple in number) occur between APs and cannot be suppressed by a further increase in the external stimulation rate. In genuine Ca\(^{2+}\) overload states, increasing the stimulation rate further increases the SR Ca\(^{2+}\) load and further increases the number and synchronization of diastolic spontaneous LCRs between APs, rather than suppressing these. Contractile alternans become marked, the average diastolic Ca\(^{2+}\) rises, and the inotropic state deteriorates as discussed in detail in the accompanying article, by Henk E. D. J. ter Keurs: “Intracellular Ca\(^{2+}\) in the wrong place at the wrong time: a catastrophe for systolic and diastolic function?” The above perspective on the true nature of “Ca\(^{2+}\) overload” is most often not understood even by “experts” within the field of cardiac Ca\(^{2+}\) dynamics, and the term “Ca\(^{2+}\) overload” is often misapplied to a physiologically fast ticking SR clock, mismatched to a slower rate at which external APs are delivered by the experimenter, or, in nature, when the APs that emanate from the SAN with a period less than the restitution time of SR Ca\(^{2+}\) release mechanisms that generate spontaneous Ca\(^{2+}\) release in ventricular myocytes. In Ca\(^{2+}\) overload states, multifocal LCRs can initiate lethal ventricular arrhythmias. The complex interactions between diastolic Ca\(^{2+}\) release, membrane depolarization, AP duration, AP conduction within the myocardium, and local Ca\(^{2+}\) and membrane potential restitution heterogeneities within the myocardium that set the stage for preventive ventricular action potentials and lethal ventricular tachycardia and fibrillation are discussed in detail in the accompanying article, by James N. Weiss: “How does the falling out of phase of intracellular potentials and action potentials across the heart’s wall spell the beginning of chaos for the heart?”

**THE INITIATION OF HEARTBEAT WITHIN THE SINOATRIAL NODE**

Thus far, we’ve discussed how cardiac ventricular muscle cells in response to externally applied rhythmic APs that emanate from the SAN generate synchronized SR Ca\(^{2+}\) release via RyRs. The global increase in cytosolic Ca\(^{2+}\) leads to Ca\(^{2+}\)-contractile protein interactions that generate a mechanical force and displacement. Myocytes contracting coordinately within the ventricular myocardium allow it to pump blood to execute a heartbeat. We, as the “academic” cardiology community, also realized that the SR is wired to be a Ca\(^{2+}\) oscillator, and as noted above, when the period of this oscillator becomes less than the period of APs arriving from the SAN, depending upon the myocyte Ca\(^{2+}\) balance, spontaneous LCRs can occur during diastole. The next important question is to resolve what determines the occurrence and periodicity of rhythmic APs that emanate from SAN and are conducted to the ventricle to drive rhythmic APs in ventricular cells? In other words, what mechanism(s) within SAN cells initiate each heartbeat?

**Early explorations of excitability of cardiac pacemaker cells**

**Concept of slow diastolic depolarization**

The essence of cardiac pacemaker cell automaticity is the generation of spontaneous periodic oscillatory diastolic depolarizations (DD) that initiate APs (Figure 10A, page 267). A slow potential change preceding the discharge of cardiac impulses was first observed by
brane potential is the presence of the outward current, between ventricular myocyte and SANC diastolic membrane potential, which is in the range of 50 to 60 mV. This contrasts with the following repolarization of the prior AP, which in SANC cells, leading ultimately to depolarization of the ventricular cardiac myocytes. Sinoatrial nodal cells (SANC) are the dominant cardiac pacemaker cells because they exhibit shorter periods between spontaneous APs than do atrioventricular, nodal, or His-Purkinje cells. Spontaneous diastolic depolarization begins from the most negative diastolic membrane potential that occurs following repolarization of the prior AP, which in SANC is in the range of 50 to 60 mV. This contrasts with the diastolic membrane potential of ventricular myocytes, which is in the range of ≈80 mV. One major difference between ventricular myocyte and SANC diastolic membrane potential is the presence of the outward current, \( I_{K1} \), in the former and its absence in the latter cell type.

**Evolution of the dogma that spontaneous depolarization of cardiac pacemaker is dominated by a single specific ion channel current, “THE pacemaker current”**

In 1952, the Nobel Prize was awarded to Hodgkin and Huxley in 1952 for developing a quantitative membrane excitation theory in nerve (Figure 2B). Its application to heart, in 1960, shifted attention from the intracellular pacemaker mechanism, suggested earlier by Bozler, to exploration of the membrane-delimited pacemaker mechanisms. Although specific cardiac ion currents had not yet been measured in cardiac pacemaker tissues or cells at that time, AP shapes, known from microelectrode studies, were initially reproduced in numerical models by an interplay of hypothesized voltage-gated and time-dependent Na\(^+\) and K\(^+\) currents. By 1962, spontaneous DD was assigned to the decline of K\(^+\) channel conductance, which unmasked a background inward Na\(^+\) current (\( I_{\text{inhal}} \)), leading to membrane depolarization (termed “gK decay” mechanism). This theoretical membrane-delimited pacemaker mechanism became the subject of extensive experimentation, greatly assisted by successful application of the voltage clamp technique to various cardiac pacemaker preparations, in quest of the specific surface membrane ion current that underlies spontaneous DD. Numerous pacemaker specific ion channel currents were identified over the last several decades, suggesting a variety of pacemaker mechanisms and requiring frequent modifications of surface membrane delimited numerical models (see references 51-55 for reviews).

**An alternative pacemaker mechanism initiated by an intracellular Ca\(^{2+}\) oscillator**

But, could it be that not all of the crucial mechanisms that are implicated in spontaneous excitation of pacemaker cells are embodied in the ensemble of cell surface ion channels? Moreover, and very importantly, could it be that the formal cause of spontaneous rhythmic AP firing by SANC, i.e., the initiating step of spontaneous rhythmic AP generation, is an intracellular process? Rhythmic intracellular Ca\(^{2+}\) cycling, not directly dependent on surface membrane signals, for example, is an integral component of biological clocks that regulate diverse vital functions throughout nature. A capacity of cells to generate rhythmic changes in intracellular Ca\(^{2+}\) is imparted by organelles that can sequester and release Ca\(^{2+}\) at specific intervals. One type of intracellular Ca\(^{2+}\) cycling in a variety of cells emanates from endoplasmic reticulum, or, as discussed above in the context of ventricular myocytes, from SR. Intracellular Ca\(^{2+}\) storage compartments are endowed with a Ca\(^{2+}\) pump and release channels that permit rhythmic cycling of Ca\(^{2+}\) into and out of this compartment. The periodicity of Ca\(^{2+}\) cycling is regulated in such cells by the Ca\(^{2+}\) pump and Ca\(^{2+}\) release channel characteristics, the quantity of Ca\(^{2+}\) available for pumping, their acute modulation by various signaling pathways, and the rate at which SR is cycling depleted by Ca\(^{2+}\) by action potentials. Sound familiar? Yes! It’s very similar to the spontaneous SR Ca\(^{2+}\) release described above for ventricular myocytes, which does not usually occur when ventricular myocytes are driven by regularly occurring APs, but can occur when SR becomes a free-running Ca\(^{2+}\) oscillator, i.e., when the AP period is less than the period required for restitution of the SR Ca\(^{2+}\) release. So the plot thickens!

**Early studies of intracellular Ca\(^{2+}\) release in pacemaker tissue implicate intracellular Ca\(^{2+}\) release in “abnormal automaticity”**

Following the discovery of CICR from the cardiac SR by Fabiato in cardiac myocytes (Figure 2P) (in the early 1970s), ideas regarding intracellular Ca\(^{2+}\) be-
by Reuter64 has been a milestone not only with respect to indirect evidence (since intracellular Ca2+ had not yet been measured), that these spontaneous electrical events involved transient increases in intracellular Ca2+, because they were accompanied by transient contractions, which required a transient increase in cytosolic Ca2+.59,60 It was also initially suggested that DADs that occur during Na+/K+-inhibition–induced toxicity caused the concomitant spontaneous contractions. The application of voltage clamp, however, quickly reversed the prevalent mindset: spontaneous oscillatory currents observed when the membrane voltage is clamped, presumably generated by intracellular Ca2+ oscillations, caused both the spontaneous depolarization and contraction.51 That putative spontaneous intracellular Ca2+ oscillations could produce current oscillations that drive voltage oscillations in pacemaker tissue, led to the idea that pacemaker cells might have “dual oscillators”: the classic surface membrane oscillator, consisting of the ensemble ion channels that had been discovered by that time, and an intracellular Ca2+ oscillator.62 It was envisioned that during these experimentally devised arrhythmogenic Ca2+ overload states, elevated intracellular Ca2+ could affect membrane potential to drive dysrhythmic APs, and abnormal automaticity ensued.51,63

The discovery of the nature of a “slow inward current” (I\textsubscript{s}) that followed excitation in cardiac Purkinje tissue by Reuter64 has been a milestone not only with respect to our understanding of excitation Ca2+ release-contraction in cardiac ventricular cells, but also for a realization of the role for Ca2+ in pacemaker cell function. The slow inward current had more than a single component, indicating that it was due to a mixture of overlapping inward currents. The initial component turned out to report Ca2+ influx via LCCs, ie, I\textsubscript{CaL} as noted above. It is important to note that in most types of SANC, since these cells lack fast Na+ channels, the rapid AP upstroke is generated by activation of LCCs. This differs from ventricular cells, in which, as noted, the rapid AP upstroke is due to activation of fast Na channels. One idea regarding a later component of the slow inward current in pacemaker cells was that it was a Na+/Ca2+ exchange (NCX) current,51 since it had been discovered years earlier by Reuter and Seitz that NCX was an electrogenic mechanism, and could generate an inward current in response to an elevation in intracellular [Ca2+]65 One view was that the NCX current could be the mechanism that generates the aforementioned spontaneous oscillatory current that drives spontaneous depolarizations.51

Was it also possible that this intracellular Ca2+-dependent mechanism within pacemaker cells envisioned to cause “abnormal automaticity” was involved in normal automaticity? If this were indeed the case, it would necessitate somewhat of a “paradigm shift” for the heart’s pacemaker. To make a long story very, very short, the idea was tested in numerical pacemaker models available at the time. The dénouement of these model simulations was that abnormal intracellular ionic concentrations (generated by experimental Na+ and Ca2+ overload) were required to generate oscillatory currents of sufficient magnitude to generate spontaneous APs.51,63 Thus, intracellular Ca2+ oscillations were not awarded a place in the “starting lineup” of mechanisms that generated normal pacemaker automaticity, but were relegated to the realm of spontaneous depolarizations involved in “abnormal” automaticity, for example, as discovered in ventricular myocytes.21 The “book” on a role for intracellular Ca2+ in normal pacemaker automaticity became closed… but not for too long.

**Studies of intracellular Ca2+ involvement in SANC normal automaticity**

Numerous studies over the last two decades have investigated specific, detailed mechanisms of the role of intracellular Ca2+ cycling in normal automaticity of cardiac pacemaker cell function (Figure 2H).66–73 These studies make a compelling argument that Ca2+ cycling within SANC, the cells of primary pacemaker tissue, is crucial for their normal automaticity. Ca2+ cycling proteins, SERCA, RyR, NCX, previously identified in atrial or ventricular cells, were identified in SANC (Figure 9, page 266). As in ventricular myocytes, the SR in SANC is wired to oscillate Ca2+ via its Ca2+ pumps (SERCA-2) and Ca2+ release channels (RyR) (Figure 10A). The
idea that SR-generated Ca\textsuperscript{2+} release induction of activation of I\textsubscript{NCX} was related to SANC pacemaker function and was generated by an effect of ryanodine to slow the spontaneous AP firing rate in these cells (Figure 10B, C). The maximum height represents the brightest possible pixel in the source image. cRyR, cardiac ryanodine receptor; NCX, Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger.84 Panel E. The intracellular distribution of total and active anti-calmodulin-dependent protein kinase 2 (CaMKII) in sinoatrial node cells (SANC). The distribution of the total CaMKII immunolabeling is uniform (top); activated CaMKII is localized beneath the sarcolemmal membrane (middle); a negative control (bottom), ie, in the absence of the primary antibodies.75

Spontaneous local Ca\textsuperscript{2+} releases beneath the sarcolemma in SANC are crucial for their normal automaticity

Use of Ca\textsuperscript{2+} sensitive indicators, coupled with fluorescence microscopy or confocal imaging, and simultaneous measurement of membrane potential or current, has elucidated the role of spontaneous local LCRs during diastolic depolarization in normal cardiac pacemaker function.71-90 Subsarcolemmal LCRs emanate from subsarcolemmal RyRs in SANC as 4-to-10-\textmu M Ca\textsuperscript{2+} wavelets that emerge following the dissipation of
the global systolic transient effected by the prior AP (Figure 11A, page 268). LCRs crescendo during the DD, peaking during the late DD, as they merge into the global cytosolic Ca²⁺ transient triggered by the next AP (Figure 9A).⁸¹,⁸³ LCRs, or the integral of LCRs, ie, late diastolic Ca²⁺ elevations (LDCAEs), have now been documented in pacemaker cells in numerous species.⁶⁸,⁶⁹,⁸³,⁸⁵-⁸⁷ LCRs activate I_{NCX} to generate miniature current and voltage oscillations that confer the exponential increase to the late phase of DD (Figure 11B). This late DD acceleration by I_{NCX} is required for sufficient I_{CaL} activation to generate the subsequent timely rapid AP upstroke. In the acute absence of this LCR-activated I_{NCX} "prompt" to the surface membrane, timely rhythmic APs do not occur (Figure 11C) and in the chronic absence of I_{NCX}, effected by Na⁺-free bathing fluid, spontaneous rhythmic APs cease to occur (Figure 10E).

**The concept of a coupled clock system within pacemaker cells that regulates their normal automaticity**

Since the occurrence of Ca²⁺ oscillations generated by the SR of SANC in the absence of sarcolemmal function,⁸¹,⁸² (ie, in voltage-clamped or "detergent-skinned...
cells”) and in novel silico models80 is rhythmic, the SANC SR has been conceptualized as an intracellular “Ca2+ clock.” Similarly, the ensemble of surface membrane electrogenic molecules, formerly thought to be the exclusive regulator of SANC automaticity, as noted above, has been recently conceptualized as a surface membrane “M clock,” because when voltage- and time-dependent properties of each individual ion channel derived from voltage clamp studies are entered into numerical models, as noted above, the ensemble of ion channels in silico can generate rhythmic spontaneous APs.50

Figure 12 illustrates the specific voltage/time/Ca2+- dependent coupling within and between surface membrane and intracellular Ca2+ cycling during the SANC AP duty cycle M and Ca2+ clock events and coupling. Figure 12A presents a graphic scheme of the temporal profiles of sarcolemmal ion currents, membrane potential (V_m), and intracellular Ca2+ cycling during SANC duty cycles. Functional interactions that are critical for normal SANC automaticity occur between the Ca2+ and surface membrane clocks are illustrated in more detail in Figure 12B (reviews in 77 and 78). It is important to note that specific surface membrane proteins not only effect changes in membrane potential, but also directly or indirectly regulate intracellular Ca2+ cycling; and, conversely, intracellular Ca2+ cycling proteins also regulate surface membrane voltage via Ca2+ modulation of surface membrane electrogenic molecules. These numerous and complex interactions confer “fail-safe” robustness to the coupled pacemaker clock system. (The reader is urged to refer to Figure 12 while digesting the text).

The crucial role of Ca2+ and PKA-dependent phosphorylation of intracellular Ca2+ cycling and surface membrane proteins in coupled pacemaker clock basal function

Coupling factors, ie, Ca2+, protein phosphorylation by protein kinase A (PKA), or Ca2+-calmodulin-dependent protein kinase II (CaMKII), which affect function of proteins of both clocks (Figure 12B), are critical for regulation of normal automaticity by the coupled-clock system, even in the absence of β-AR stimulation. The
Figure 12. The coupled-clock pacemaker system.
Panel A. Schematic illustration of key phases of the functional interactions between M clock and Ca\(^{2+}\) clocks. DD, diastolic depolarization; CICR, Ca\(^{2+}\)-induced Ca\(^{2+}\) release; MDP, maximum diastolic potential; NCX, Na\(^{+}/Ca\(^{2+}\) exchanger; SR, sarcoplasmic reticulum.\(^{106}\) Panel B. Schematic illustration of interactions of molecules comprising the full coupled-pacemaker clock. Note that the same regulatory factors (red lettering) of the sarcoplasmic reticulum (SR) Ca\(^{2+}\) clock (gray intracellular area, black lettering) couple the Ca\(^{2+}\) clock to the M clock (blue membrane area, blue lettering). G-protein–coupled receptors (GPCRs, green lettering) regulate both the Ca\(^{2+}\) clock and membrane clock via those same factors (red lettering) and other coupling factors (green shapes). See text for numerous additional details. CaM, calmodulin; CaMK-II, calmodulin-dependent protein kinase 2; NCX, Na\(^{+}/Ca\(^{2+}\) exchanger; PLB, phospholamban; RyR, ryanodine receptor; SERCA2, sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase–2.\(^{106}\)
Intracellular calcium cycling in the heart’s chronotropy and inotropy - Lakatta

complex phosphorylation-dependent regulation of intracellular Ca\(^{2+}\) cycling and surface membrane proteins of the coupled-clock system has numerous functional redundancies (Figure 12B). SANC have a high constitutive activation of adenylyl cyclase (AC) that results in a high level of basal cAMP\(^{92}\) (Figure 9A). Although SANC, like ventricular myocytes, also express high levels of Ca\(^{2+}\)-inhibited AC types 5 and 6,\(^{91}\) the discoveries of Ca\(^{2+}\)-activated AC types, ie, AC1 and AC8, in rabbit and guinea-pig SANC,\(^{92-94}\) and localization of the basal Ca\(^{2+}\)-activated AC activity within lipid raft microdomains,\(^{94}\) link Ca\(^{2+}\) to localized cAMP production (Figure 12B). Ca\(^{2+}\) binds to calmodulin to activate AC, leading to a high basal level of cAMP-mediated, PKA-dependent phosphorylation of surface membrane and intracellular proteins involved in cell Ca\(^{2+}\) balance and SR Ca\(^{2+}\) cycling (Figure 10B).\(^{82,94}\)

Spontaneous, partially synchronized basal state activation of RyRs generates spontaneous local Ca\(^{2+}\) wavelets. This process occurs normally in SANC because of high constitutive levels of basal PKA- and CaMKII-dependent phospholamban phosphorylation that drive the kinetics of SR Ca\(^{2+}\) pumping and RyRs activation. Ca\(^{2+}\)-CaMKII–dependent phosphorylation of SERCA stimulates Ca\(^{2+}\) pumping,\(^{95}\) and phosphorylation of RyR at both PKA and CaMKII sites modulates Ca\(^{2+}\) release characteristics. This spontaneous SR Ca\(^{2+}\) cycling occurs in SANC at a Ca\(^{2+}\) load similar to that in ventricular myocytes in which spontaneous LCRs do not occur at normal AP rates (Figure 3C) because in ventricular myocytes cAMP and PKA-CaMKII Ca\(^{2+}\) signaling is low (Figure 3A). In addition to modulating, the SR clock component of the coupled pacemaker clock, PKA- and CaMKII-dependent mechanisms (Figure 12B, red) also regulate function of surface electrogenic proteins (Figure 12B, blue) and couple the M and Ca\(^{2+}\) clocks. PKA is target-ed to the LCCs through the membrane-associated anchoring protein AKAP15/18,\(^{96,97}\) and because specific PKA inhibitor peptide (PKI) suppresses \(I_{\text{cal}}\) by \(\approx 80\%\),\(^{98}\) there appears to be a high basal PKA-dependent phosphorylation of L-type Ca\(^{2+}\) channels. \(I_{\text{Ks}}\) is also modulated by cAMP-mediated, PKA-dependent phosphorylation (Figure 12B).\(^{99}\) Augmented Ca\(^{2+}\) influx and accelerated Ca\(^{2+}\) cycling via PKA-dependent protein phosphorylation enhances basal CaMKII activity,\(^{100}\) which further promotes Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) cycling. Activated CaMKII in SANC is localized beneath the cell membrane, whereas the total CaMKII is uniformly present throughout the cell (Figure 7A).\(^{75}\) This restricted localization of active CaMKII to the subsarcolemmal space of SANC is consistent with the idea that CaMKII targets sarcolemmal and subsarco-

Ca\(^{2+}\) signaling in SANC is “feed-forward” (Figure 12B), ie, Ca\(^{2+}\) release begets Ca\(^{2+}\) release, because Ca\(^{2+}\) activates signaling that generates its release that causes its effects on the surface membrane. This feed-forward Ca\(^{2+}\)-cAMP-PKA-Ca\(^{2+}\) signaling is kept in check by factors that regulate Ca\(^{2+}\) and protein kinase activities, so that basal state Ca\(^{2+}\) influx and Ca\(^{2+}\) cycling kinetics remain stable. High constitutive basa-phosphodiesterase (PDE) activity, which degrades cAMP, is one such control checkpoint within SANC (Figure 12B).\(^{89}\) Indeed, inhibition of basal PDE in SANC markedly elevates cAMP-mediated, PKA-dependent phosphorylation of SR proteins, and proteins of surface membrane ion channels (that generate \(I_{\text{cal}}\) and \(I_{\text{Ks}}\) (Figure 12B), resulting in an acceleration the spontaneous AP firing rate of rabbit SANC.\(^{89}\) Phosphoprotein phosphatase activity (Figure 12B)\(^{103}\) is likely another control point of phosphorylation-dependent Ca\(^{2+}\) cycling in SANC, but this has not yet been elucidated.

The robustness of coupled-clock pacemaker system (Figure 12B) is guaranteed by the mutual entrainment of the molecular function of both clocks, which results from numerous interactions between the M and Ca\(^{2+}\) clock subsystems.\(^{78,80}\) Indeed, by releasing Ca\(^{2+}\) into the subsarcolemmal space with precise timing during late DD (Figure 12A), the spontaneous Ca\(^{2+}\) clock interacts with membrane proteins to induce an inward \(I_{\text{NCX}}\) current that facilitates the “on-time” occurrence of the next AP. By “igniting” the membrane depolarization that facilitates the production of a timely AP, the Ca\(^{2+}\) clock guarantees its own existence during future cycles: the AP (that the Ca\(^{2+}\) clock prompts) resets the Ca\(^{2+}\) clock via CICR-mediated SR Ca\(^{2+}\) depletion, and refuels the Ca\(^{2+}\) clock with Ca\(^{2+}\), via Ca\(^{2+}\) influx through

}\)
the LCCs (Figure 12A). The M clock, in turn, not only generates the AP, the sine qua non of SANC function, but, by ensuring the continual function of the Ca\(^{2+}\) clock, the M clock also insures its future normal function to generate on-time rhythmic APs via surface membrane ignitions prompts by its partner, the Ca\(^{2+}\) clock.

**How the coupled pacemaker clock keeps time**

The delay between the onset of the global SR Ca\(^{2+}\) depletion triggered by an AP (via CICR) and the spontaneous emergence of an LCR during the subsequent DD corresponds to the LCR period (Figure 13A). The LCR period is the master integrated function of the

![Figure 13. How the coupled pacemaker keeps time.](image)

Panel A. Confocal line-scan image of local Ca\(^{2+}\) releases (LCRs) with superimposed spontaneous action potentials in rabbit sinoatrial node cells. White arrowheads show LCRs. The LCR period is defined as indicated.82

Panel B. Conceptual perspectives on how SR Ca\(^{2+}\) pumping and the cycle length relate to the local Ca\(^{2+}\) release LCR period. The concept that the rates of sarcoplasmic reticulum (SR) Ca\(^{2+}\) refilling (1-3) and Ca\(^{2+}\) release threshold determine the LCR period, and the timing (1-3) of the LCR induced diastolic depolarization are schematically illustrated.106
coupled-clock system, and determines the “ticking speed” of the coupled-clock system. While an occurrence of an AP-induced global SR Ca\textsuperscript{2+} depletion temporarily causes the spontaneous LCRs to stop, the Ca\textsuperscript{2+} clock does not stop: after being synchronized in a relatively Ca\textsuperscript{2+} depleted state, it continues to measure time from the onset of its Ca\textsuperscript{2+} depletion, and when threshold conditions for spontaneous Ca\textsuperscript{2+} release are achieved later during DD, ie, following the LCR restitution period, SR begins to generate LCRs again. The LCR period is determined by the duration of the restitution process required for a subsequent Ca\textsuperscript{2+} release. The schematic in Figure 13B illustrates the concept of how the restitution process that determines the LCR period is regulated: (i) by the kinetics of SR Ca\textsuperscript{2+} cycling, ie, by the rate of Ca\textsuperscript{2+} pumping into the SR, and (ii) by the threshold of SR Ca\textsuperscript{2+} load required for spontaneous RyR activation.

It’s important to note that the LCR period does not report Ca\textsuperscript{2+} clock function per se, but reports the function of the coupled-clock system, because of the intimate interactions of the electrogenic sarcolemmal molecules with the intracellular Ca\textsuperscript{2+} cycling apparatus described above and depicted in Figure 12B. Ca\textsuperscript{2+} and PKA- and CaMKII-dependent phosphorylation, by controlling cell Ca\textsuperscript{2+} balance and SR Ca\textsuperscript{2+} cycling (Figure 12), determine the LCR period. For example, Ca\textsuperscript{2+} available for SR pumping, which is regulated by PKA- and CaMKII-dependent phosphorylation of SR Ca\textsuperscript{2+} cycling proteins (Figure 12B), is critically depend-ent with regard to beat-to-beat Ca\textsuperscript{2+} influx via LCCs. Ca\textsuperscript{2+}, cAMP-mediated, PKA-dependent and CaMKII-dependent phosphorylation (Figure 12B, red) are crucial nodes within the coupled-clock system because their joint action on Ca\textsuperscript{2+} and M clock proteins determines the LCR period in a given steady state. The LCR period is linked to the phosphorylation state of Ca\textsuperscript{2+} cycling proteins illustrated for phospholamban (PLB) phosphorylation in Figure 14A. In turn, the LCR period predicts spontaneous AR cycling length over a range encompassing the physiologic range of heartbeats (Figure 14B).

**Modulation of the basal cell firing rate of the coupled pacemaker clock system by GPCR signaling**

In addition to robustness or “fail-safe,” stable basal operation, flexibility of the pacemaker clock’s “throttle” is required to permit the clock to tick over the wide range of frequencies that encompass the physiologic range of heart rates. G protein–coupled receptor (GPCR) modulation of the LCR period (Figure 12B, green) is achieved by modulation of the same factors, ie, Ca\textsuperscript{2+} and protein phosphorylation, that regulate the basal state function of the coupled system of membrane and Ca\textsuperscript{2+} clock (Figure 12B). Specifically, β-AR stimulation further increases—and cholinergic receptor (ChR) stimulation reduces—basal levels of phosphorylation of coupled-clock proteins, leading to an increase and a reduction, respectively, in LCR period (Figure 14A).

![Figure 14. How the AP cycle length relates to the LCR period via phosphorylation-dependent mechanisms.](image-url)

**Panel A.** The relative effects of a phosphokinase A (PKA) inhibitor (PKI), the cholinergic receptor agonist, carbachol (CCh), phosphodiesterase (PDE) inhibition (IBMX [3-isobutyl-1-methylxanthine], milrinone), or ISO (isoproterenol) to alter the LCR period are linked to their effects to alter phospholamban (PLB) phosphorylation. The dashed line is the best fit least squares logarithmic function through the points. Panel B. The relative effects of PKI, CCh, PDE inhibition, and ISO to alter the spontaneous cycle length over a wide range are predicted by their effects on the LCR period. The best fit least squares linear functions (dashed line) through the points is the line of identity: cycle length = 0.89 LCR period +11.43 ms.
It follows, therefore, that SERCA2 Ca\(^{2+}\) pumping and Ca\(^{2+}\) release within the coupled-clock system is a required "switchboard" that links a change in Ca\(^{2+}\) influx via M clock proteins in response to GPCR stimulation to a change in the SANC firing rate. The specific linking function of this switchboard is to generate variations in LCR signal mass that occur at an earlier or later time during DD. The LCR signal mass is an integral of the amplitude, width, duration of LCRs, and of the number of diastolic LCRs, and reflects the total numbers of RyRs activated in a given diastolic depolarization (DD). The signal mass of local Ca\(^{2+}\) releases during diastole in SANC, and its phase, determine the impact of this Ca\(^{2+}\) release with respect to \(I_{\text{NCX}}\) activation on membrane potential depolarization and beating rate.\(^{83,89,90}\) \(\beta\)-AR stimulation increases the number of local subsarcolemmal Ca\(^{2+}\) releases during a given DD, and also increases their amplitudes. Thus, synchronization of RyR activation states (RyR recruitment) by \(\beta\)-AR stimulation in SANC leads to an increased number of RyRs participating in local diastolic Ca\(^{2+}\) release in SANC. (Does this also sound familiar? It should, because this effect of \(\beta\)-AR stimulation to synchronize spontaneous activation of RyRs is essentially the same as that described above for AP-induced RyR Ca\(^{2+}\) release in ventricular myocytes.) This spatiotemporal synchronization of spontaneous LCR release in SANC due to synchronization of RyR gating by \(\beta\)-AR stimulation augments the inward current via NCX during the DD. In contrast to \(\beta\)-AR stimulation, ChR stimulation results in a reduction in LCR frequency and size and desynchronization of local RyR activation during DD.

Modulation of LCR period and spontaneous AP cycle length by experimental maneuvers that interrupt basal PKA-dependent signaling and those effected by GPCR stimulation form a continuum (Figure 14): graded changes in the steady-state phosphorylation status of proteins that regulate Ca\(^{2+}\) within the coupled-clock system by GPCR signaling lead to gradations in the LCR period (Figure 14A) that result in graded changes in the timing of the onset and amplitude of the late DD exponential increase, and thus cause concomitant gradations of the steady-state AP cycle length (Figure 14B).\(^{82,89,90,101}\) It is necessary to emphasize, however, that modulation of the LCR period and SANC beating rate by coupled-clock functions in response to GPCR stimulation depends, not only on the direct modulation of protein phosphorylation per se that is produced by GPCR stimulation (Figure 12B), but also depends upon the secondary modulation via the change in rate and rhythm of AP occurrence that results from GPCR stimulation: changes in the AP firing rate or AP shape, per se, due to any cause, indirectly effect changes in intracellular Ca\(^{2+}\) cycling characteristics via an effect on steady-state cell Ca\(^{2+}\) levels. This is another type of feed-forward signaling in SANC. The Bowditch treppe effect in ventricular myocytes noted above is produced by a similar effect caused by the rate at which external APs are delivered to these cells.\(^{104}\)

**SUMMARY: A GENERAL THEORY TO EXPLAIN BOTH HOW FAST AND HOW STRONG THE HEART BEATS**

A general theory for how fast and how strong the heart beats emanates from the realization that signals for both faster and stronger beating of the heart are transmitted via common cell effectors. Specifically, sequestration and release of Ca\(^{2+}\) to and from SR is an intracellular signal transduction mechanism common to both functional states of ventricular myocytes and pacemaker cells, and is required for their normal function. Graded synchronization of local Ca\(^{2+}\) releases from the SR is the basis of graded function within both cell types. An increase in the number of LCRs occurring within a given epoch, eg, during spontaneous diastolic depolarization in SANC, or due to triggered LCR activation via CICR induced by an AP in ventricular cells, involves local recruitment of additional RyRs to fire within an epoch in either cell type. This local recruitment is a form of synchronization of the kinetics gating and activation of individual RyRs. For example, an increase in the global cytosolic Ca\(^{2+}\) transient amplitude by an AP induced by CICR in ventricular cells indicates that more RyRs have been recruited to fire within that epoch. Ditto for an increase in the spontaneous LCR signal mass during AP in SANC. A crucial difference in Ca\(^{2+}\) cycling within the two cell types is that in SANC, a high level of basal cAMP-PKA-CaMKII signaling is present, which even in the absence of GPCR stimulation, allows partially synchronized, spontaneous LCRs to normally occur during the DD between successive APs. This LCR occurrence amplifies the rate of DD change, prompting surface membranes to generate an AP. Extension of the same basal transduction signaling pathways by GPCR receptor stimulation in SANC effects slower or faster heartbeats, and in ventricular myocytes, changes in synchronization of the functions of cycling molecules occurring in response to GPCR result in stronger or weaker heartbeats. It follows, therefore, that synchronization of RyR activation and Ca\(^{2+}\) release effected by a coupled complex system of cardiac intracellular and membrane molecules functions is a unified mechanism that links chronotropy in the heart’s pace-maker cells to inotropy in ventricular cells.
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Calcium Handling and Ryanodine

Expert Answers to Three Key Questions

1. How can calcium leaks occur within heart cells?
   
   S. E. Lehnart, G. Hasenfuss

2. Intracellular Ca$^{2+}$ in the wrong place at the wrong time: a catastrophe for systolic and diastolic function?
   
   H. E. D. J. ter Keurs

3. How does the falling out of phase of intracellular calcium and action potentials across the heart’s wall spell the beginning of chaos for the heart?
   
   J. N. Weiss
Heart failure (HF) is characterized by molecular and cellular defects that jointly contribute to arrhythmias and decreased cardiac pump function. Adaptive responses activate physiological countermeasures to overcome depressed cardiac function and maintain blood supply to vital organs. In chronic HF, these compensatory mechanisms are sustained for months and contribute to progressive maladaptive remodeling of the heart, which is associated with a worse outcome. HF-induced dysregulation of intracellular second messenger systems of calcium ($\text{Ca}^{2+}$) and/or cAMP directly contribute to adverse remodeling and depression of cardiac function. Here, we provide a perspective about the interplay of $\text{Ca}^{2+}$ and cAMP-dependent signaling changes in HF, how these changes affect cardiac function, and about novel therapeutic strategies related to these signaling defects.

**Keywords:** intracellular calcium; cardiomyocyte; heart failure; arrhythmia; cardiac remodeling; therapy

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Selected Abbreviations and Acronyms

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<tr>
<td>AP</td>
<td>action potential</td>
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<td>$\beta$-AR</td>
<td>$\beta$-adrenergic receptor</td>
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<tr>
<td>$\text{Ca}^{2+}$</td>
<td>free ionized calcium</td>
</tr>
<tr>
<td>CaMKII</td>
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</tr>
<tr>
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<td>3’-5’-cyclic adenosine monophosphate</td>
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<td>heart failure</td>
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<tr>
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<td>$\text{Ca}^{2+}$ influx current</td>
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</tr>
<tr>
<td>PLN</td>
<td>phospholamban</td>
</tr>
<tr>
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<td>cAMP-dependent protein kinase A</td>
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<td>phosphatase 1</td>
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<td>RyR2</td>
<td>cardiac ryanodine receptor</td>
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<tr>
<td>SERCA2</td>
<td>sarco/endoplasmic reticulum $\text{Ca}^{2+}$ pump</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum $\text{Ca}^{2+}$ storage organelle</td>
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derstanding of molecular and cellular disease processes to overcome the current limitations of HF therapy. This chapter focuses on intracellular calcium (Ca\(^{2+}\)) leak, which is both an important pathophysiological mechanism and a candidate pharmacological target in experimental HF therapy.

**HEART FAILURE: A DISEASE OF THE HEART MUSCLE**

Any intrinsic defect of the myocardium can be expected to become more severe through the effect of higher heart rates or other stressors, and this is also reflected in the functional classification of HF severity in patients (eg, New York Heart Association [NYHA] I-IV). Accordingly, in the human myocardium, the positive force-frequency relationship (FFR) or staircase phenomenon that characterizes the heart rate–dependent increase in contractile force can be regarded as a representative functional readout of healthy heart muscle function. In the failing human heart, the frequency-dependent increase of contractile force is significantly blunted, both in vivo in patients and in isolated muscle preparations. These alterations of the FFR in the failing myocardium result in inadequate cardiac output and diminished exercise capacity in symptomatic HF patients.

Fluorescence microscopy of intracellular Ca\(^{2+}\) transients during systolic contraction has evidenced a close relationship between FFR and Ca\(^{2+}\) changes, by showing an absence of adequate increases in force production at higher stimulation frequencies in the failing human myocardium. Experimental protocols that facilitate increased Ca\(^{2+}\) storage in diastole typically increase force production after a pause in pacing in healthy myocardium by post-rest potentiation (PRP). However, when applied to failing heart muscle preparations, PRP results in depressed force production and smaller Ca\(^{2+}\) transients.

Since peak intracellular Ca\(^{2+}\) concentration determines the extent of systolic force production, this suggests that intracellular Ca\(^{2+}\) storage by the sarcoplasmic reticulum (SR) as the driving force of SR Ca\(^{2+}\) release may be altered in HF.

**Figure 1. Ca\(^{2+}\) sparks after myocardial infarction.**

Panel A: Average line-scan image (left) of Ca\(^{2+}\) sparks and its 3-dimensional projection (right) (n = 141). Panel B: Averaged Ca\(^{2+}\) sparks from post-myocardial infarction (PMI) cells (n = 338) as in A. Panel C: Averaged Ca\(^{2+}\) sparks from PMI cells after ouabain exposure (100 nmol/L) as in A (n = 111). Panel D: Number of Ca\(^{2+}\) sparks/µm observed during 200-ms depolarizations plotted against voltage for control (C, n = 59) and PMI (v, n = 56) cells. Nifedipine (1 mmol/L) was used in these experiments to allow Ca\(^{2+}\)-spark resolution over a full range of membrane potentials. *P<0.05, **P<0.005.

higher heart rates increase SR Ca\textsuperscript{2+} content in the healthy heart, whereas failing human myocardium SR Ca\textsuperscript{2+} content is decreased. Which are the mechanisms that may compromise intracellular SR Ca\textsuperscript{2+} storage in HF?

**MECHANISMS THAT CONTROL SR Ca\textsuperscript{2+} LOAD AND LEAK**

Combined use of confocal microscopy and patch-clamp recordings in cardiac myocytes has shown that in failing hearts the plasma membrane Ca\textsuperscript{2+} inward current ($I_{Ca}$) activates less Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (SR). This is measured as a significantly reduced number of “Ca\textsuperscript{2+} sparks” for the $I_{Ca}$ voltage steps (Figure 1). Of note, the elementary Ca\textsuperscript{2+} release signals (sparks) are not changed in HF. Which mechanism(s) may depress the ability of $I_{Ca}$ to activate intracellular SR Ca\textsuperscript{2+} release in failing hearts?

Excitation-contraction (EC) coupling is initiated by membrane depolarization and the opening of voltage-dependent L-type Ca\textsuperscript{2+} channels (cardiac isoform Ca\textsubscript{v}1.2). The resulting Ca\textsuperscript{2+} influx current ($I_{Ca}$) next activates a much larger intracellular Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (SR). This is measured as a significantly reduced number of “Ca\textsuperscript{2+} sparks” for the $I_{Ca}$ voltage steps (Figure 1). Of note, the elementary Ca\textsuperscript{2+} release signals (sparks) are not changed in HF. Which mechanism(s) may depress the ability of $I_{Ca}$ to activate intracellular SR Ca\textsuperscript{2+} release in failing hearts?

The activity of SERCA2 is regulated by phospholamban (PLN). Unphosphorylated PLN inhibits SERCA2 activity, and phosphorylation by PKA (cAMP-dependent protein kinase A) and/or CaMKII (Ca\textsuperscript{2+} calmodulin–dependent protein kinase II) increases SR Ca\textsuperscript{2+} uptake. In parallel, RyR2 phosphorylation by PKA and/or CaMKII increases SR Ca\textsuperscript{2+} release (CICR). Loading of Ca\textsuperscript{2+} into the SR occurs via Ca\textsuperscript{2+} pumps (SERCA2, sarco/endoplasmic reticulum Ca\textsuperscript{2+} pump) and extrusion to the extracellular space by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX).

Figure 2. Activation of RyR2 by CaMKII phosphorylation.

Single-channel recordings of RyR2-WT and mutant RyR2, untreated or treated with PKA in the absence or presence of inhibitor PKI5-24 or with CaMKII. CaMKII phosphorylation of RyR2 increases open probability ($P_o$); PKA phosphorylation also increases $P_o$ and induces subconductance states. Parameters $P_o$, $T_o$ (mean open time), and $T_c$ (mean closed time) are presented for the shown single-channel experiments. All channel traces shown were recorded at 150 nmol/L [Ca\textsuperscript{2+}]\textsubscript{cis}.

**Abbreviations:** CaMKII, Ca\textsuperscript{2+} calmodulin kinase II; PKA, phosphokinase A; RyR2, ryanodine receptor; WT, wild type.

CARDIAC REMODELING PRECIPITATES IMPORTANT INTRACELLULAR DEFECTS IN HF

In HF patients, a critical transition occurs when the heart can no longer provide adequate blood flow and/or pressure to meet the body’s demands. Increased neurohormonal outflow may initially offset reduced cardiac performance. However, in HF, continuing stimulation of the heart by extracellular stress signals contributes to maladaptive remodeling. A broad range of molecular pathways are involved in the development of HF, and there is likely to be substantial overlap between these pathways. Among these, sympathetic stimulation contributes to a hyperadrenergic state that chronically activates intracellular PKA pathways despite desensitization of β-adrenergic receptors (β-ARs) and increased inhibitory Gi-protein expression.11-13 In addition, catecholamines may exert toxic effects, as suggested by isolated cardiomyocytes that undergo apoptosis following β1-AR selective stimulation.14,15 An additional maladaptive pathway in HF appears to be β1-AR-dependent cross-activation of CaMK, which may contribute to apoptotic cell death.16 Are these signaling mechanisms also connected to changes in intracellular Ca2+?

INTRACELLULAR CALCIUM CHANGES IN HEART FAILURE

Muscles from failing hearts have a reduced capacity to restore low nanomolar resting Ca2+ levels during diastole.17 In addition, failing heart muscle shows a reduced amplitude of the intracellular Ca2+ transient in systole.3 Consistent with data from multicellular preparations, single cardiomyocytes from failing human hearts display prolonged relaxation, depressed systolic contraction, and elevated diastolic tension.18 The contractile dysfunction of failing cardiomyocytes occurs in conjunction with reduced SR Ca2+ release and elevated resting Ca2+ concentration.19,20 Similar changes of intracellular Ca2+ transients have been documented in dilated5,21 and hypertrophic cardiomyopathy.22 HF after myocardial infarction,6 coxsackievirus B3 myocarditis,23 and following genetically engineered changes of enzymatic activities in PKA24 and CaMKII.25 Therefore, despite very different causes, HF results in similar changes in intracellular Ca2+ transient and associated contractile dysfunction.

INTRACELLULAR CALCIUM LEAK IN HEART FAILURE

Current evidence unanimously supports that changes in SR Ca2+ cycling contribute to depressed performance of the failing heart.17,26 While cardiac contraction is controlled by the cell-wide Ca2+ transient, intracellular Ca2+ release occurs within subcellular Ca2+ release units, as evidenced by elementary Ca2+ release events called “sparks.”9,27 Important advances in the understanding of how contractile dysfunction depends on changes in intracellular Ca2+ cycling in HF has been provided by means of combined voltage-clamp and Ca2+ spark experiments, which evidenced decreased sensitivity for SR Ca2+ release activation in a model of hypertension-induced HF.22 Among potential causes, structural subcellular changes are likely to contribute to defective EC coupling by altering the geometry of the nanometer-sized Ca2+ release unit, leading to reduced CICR.28,29 In addition, decrease in
intracellular Ca\textsuperscript{2+} stores may lead to a Ca\textsuperscript{2+} depleted state, as has been experimentally confirmed in cardiomyocytes from dogs and humans with HF.\textsuperscript{5,30} Decreased SR Ca\textsuperscript{2+} load may result in a decreased amplitude and slower upstroke of the intracellular Ca\textsuperscript{2+} transient. However, SR Ca\textsuperscript{2+} store depletion in HF does not necessarily prevent an increase in Ca\textsuperscript{2+} spark frequency,\textsuperscript{25} possibly due to chronically increased RyR2 function.\textsuperscript{31}

In samples from patients and animals with HF, RyR2 has been found to be PKA hyperphosphorylated, and may thus directly contribute to abnormally increased channel activity and intracellular Ca\textsuperscript{2+} leak.\textsuperscript{8,32} If a hyperadrenergic state in HF results in downregulation of β-AR signaling, why are RyR2 chronically PKA hyperphosphorylated? One mechanism may be reduced phosphatase activity (PP1 and PP2A) in the RyR2 channel complex and a

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**Figure 4.** A mutant FKBP12.6-D37S restores normal single-channel function to channels from exercised FKBP12.6\textsuperscript{-/-} mice and phosphokinase A (PKA)-phosphorylated CPVT (catecholaminergic polymorphic ventricular tachycardia) mutant channels. For detailed explanation refer to reference 34. Modified after reference 34: Wehrens et al. Cell. 2003;113:829-840. © 2003, Cell Press.
reduced rate of RyR2 dephosphorylation.\textsuperscript{8,33} In addition, activity of the cAMP-specific phosphodiesterase isoform PDE4D3 is decreased in the RyR2 channel complex (Figure 3, page 284).\textsuperscript{31} Single RyR2 channels from human failing hearts showed a significantly increased open probability consistent with intracellular Ca\textsuperscript{2+} leak.\textsuperscript{8} In addition, sustained PKA hyperphosphorylation promotes depletion of the stabilizing subunit calstabin2 (FKBP12.6) from the RyR2 channel, which contributes to instable channel closure and increased SR Ca\textsuperscript{2+} leak (Figure 4, page 285).\textsuperscript{8,31,32,34}

In addition, chronic sympathetic stimulation in HF may cross-activate CaMK and intracellular Ca\textsuperscript{2+} leak through a cAMP-binding protein called EPAC.\textsuperscript{35} A specific role of CaMK during decompensation to HF by increased RyR2 phosphorylation has been suggested.\textsuperscript{36} Using mutagenesis of the full-length RyR2, distinct CaMK and PKA phosphorylation sites have been identified.\textsuperscript{7} The rate-dependent increase in RyR2 phosphorylation by CaMK was compromised in rat hearts with post-myocardial infarction HF\textsuperscript{7} While the exact pathophysiological roles of PKA and CaMK phosphorylation are debated, there is general agreement that RyR2 hyperphosphorylation increases SR Ca\textsuperscript{2+} leak in HF, likely through increased channel activity and consistent with increased SR Ca\textsuperscript{2+} leak occurring even from Ca\textsuperscript{2+} depleted SR stores in HF.\textsuperscript{25} Recent studies that have specifically addressed the role of CaMK and PKA phosphorylation sites in the RyR2 channel in HF confirmed that PKA phosphorylation is a major mechanism leading to RyR2 Ca\textsuperscript{2+} leak.\textsuperscript{37,38}

### Therapeutic Inhibition of Calcium Leak in Heart Failure

Several rationales exist that aim to correct molecular Ca\textsuperscript{2+} signaling abnormalities in HF (Table I). One of these strategies, stabilization of the RyR2 physiological closed state

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Advantage</th>
<th>Disadvantage</th>
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<tbody>
<tr>
<td>RyR2 phosphorylation</td>
<td>β-Blockers</td>
<td>Improved survival, slows HF progression</td>
<td>Indirect mechanism</td>
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<td>ACE inhibitors</td>
<td></td>
<td>Off-target activity</td>
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<td>Tetracaine</td>
<td>Unclear</td>
<td>Potentially negative inotropic activity</td>
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<tr>
<td>RyR2 stabilization of physiological closed state</td>
<td>JTV519</td>
<td>RyR2-specific effects inhibit SR Ca\textsuperscript{2+} leak</td>
<td>Off-target activity</td>
</tr>
<tr>
<td></td>
<td>S107</td>
<td></td>
<td>No off-target activity</td>
</tr>
</tbody>
</table>

**Table I. Therapeutic rationales targeting RyR2 Ca\textsuperscript{2+} leak.**

Abbreviations: ACE, angiotensin-converting enzyme; HF, heart failure; RyR2, ryanodine receptor; SR, sarcoplasmic reticulum.

In addition, chronic sympathetic stimulation in HF may cross-activate CaMK and intracellular Ca\textsuperscript{2+} leak through a cAMP-binding protein called EPAC.\textsuperscript{35} A specific role of CaMK during decompensation to HF by increased RyR2 phosphorylation has been suggested.\textsuperscript{36} Using mutagenesis of the full-length RyR2, distinct CaMK and PKA phosphorylation sites have been identified.\textsuperscript{7} The rate-dependent increase in RyR2 phosphorylation by CaMK was compromised in rat hearts with post-myocardial infarction HF\textsuperscript{7} While the exact pathophysiological roles of PKA and CaMK phosphorylation are debated, there is general agreement that RyR2 hyperphosphorylation increases SR Ca\textsuperscript{2+} leak in HF, likely through increased channel activity and consistent with increased SR Ca\textsuperscript{2+} leak occurring even from Ca\textsuperscript{2+} depleted SR stores in HF.\textsuperscript{25} Recent studies that have specifically addressed the role of CaMK and PKA phosphorylation sites in the RyR2 channel in HF confirmed that PKA phosphorylation is a major mechanism leading to RyR2 Ca\textsuperscript{2+} leak.\textsuperscript{37,38}

**Figure 5. Improvement in contractile dysfunction with K201 (JTV519) following changes in extracellular [Ca\textsuperscript{2+}]\textsubscript{e} in myocardial preparations from terminally failing human hearts.**

Original recordings from two experiments during the increase of [Ca\textsuperscript{2+}]\textsubscript{e} from 3.0 to 5.0 mmol/L from control (A) or with 0.3 µM K201 (B) muscles strip experiments. (C) Effect of [Ca\textsuperscript{2+}]\textsubscript{e} on the diastolic (open symbols) and developed tension (filled symbols) of human failing muscle strips treated with either 0.3 µM K201 (dotted line, n=7) or control (solid line, n=7). Tension is expressed relative to tension at [Ca\textsuperscript{2+}]\textsubscript{e} of 1 mmol/L. \*P<0.05.

to inhibit SR Ca\textsuperscript{2+} leak and the dependent remodeling in HF, is unique since it does not block the ion channel pore as is the case with existing ion channel blocking drugs. Ion channel blockers that affect RyR2 include tetracaine (Table I), which inhibits ion permeation through RyR2 as well as other ion channels, and due to this lack of specificity may lead to contractile dysfunction, thereby limiting therapeutic applicability in HF.\textsuperscript{39,40} Therefore, the recent discovery of non pore-blocking RyR2 channel stabilizing drugs originally derived from 1,4-benzo-thiazepine JTV519 (K201) has been a breakthrough in terms of therapeutic perspectives (Table I).

In animal models in which there is HF progression, structural remodeling and cardiac dysfunction are inhibited by JTV519 treatment.\textsuperscript{31,41,42} In addition, novel RyR2 stabilizing compounds with improved channel specificity have shown in vivo efficacy as potential HF therapy.\textsuperscript{43,44} Additionally, β-AR and angiotensin II (AT-II) receptor blockers have been associated with beneficial effects on RyR2 channel dysfunction in HF through mechanisms preventing excess posttranslational modification of RyR2, eg, by PKA hyperphosphorylation or nitrosylation.\textsuperscript{45-47} Recently, JTV519 has been found to improve contractile dysfunction in myocardial preparations from terminally failing human hearts (Figure 5).\textsuperscript{48} Of note, novel derivatives of 1,4-benzo-thiazepines are currently investigated in stage I/II safety trials in patients with heart failure.

**SUMMARY AND PERSPECTIVES**

HF is characterized by progressive maladaptive remodeling and a poor prognosis despite modern therapy. Chronic activation of sympathetic and other signaling pathways mediates structural and functional remodeling of the failing heart. EC coupling is affected at several levels of intracellular Ca\textsuperscript{2+} signaling and contributes directly to contractile dysfunction and triggered arrhythmias. Abnormally increased SR Ca\textsuperscript{2+} leak through defective RyR2 channels represents a major mechanism contributing to HF pathology. Translational approaches have identified specific molecular mechanisms that underlie defective RyR2 closure both as a novel disease mechanism and as a therapeutic rationale. Indeed, pharmacological stabilization of the RyR2 closed state inhibits SR Ca\textsuperscript{2+} leak and cardiac remodeling. We anticipate that ongoing clinical trials with RyR2 stabilizing agents will lead to the development of a Ca\textsuperscript{2+} leak–targeted pharmacotherapy in HF.

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K201 improves aspects of the contractile performance of human failing myocardium via reduction in Ca^{2+} leak from the sarcoplasmic reticulum.
The calcium ion (Ca\(^{2+}\)) plays a crucial role in normal myocardium contraction. Ca\(^{2+}\) trafficking may go astray and jeopardize the exquisite mechanism of systole and diastole, triggering arrhythmias. Several factors, including gene mutations, acute ischemia, and unfavorable cardiac remodeling, modify Ca\(^{2+}\) transport at various loci in cardiac myocytes and may weaken the cells. The interplay between weakened and strong segments in nonuniform cardiac muscle leads to mechanoelectric feedback mediating an early diastolic Ca\(^{2+}\) transient caused by the rapid force decrease during the relaxation phase. These rapid force changes in nonuniform muscle may cause arrhythmogenic Ca\(^{2+}\) waves to propagate by activation of neighboring sarcoplasmic reticulum by diffusing Ca\(^{2+}\) ions. Drugs stabilizing the Ca\(^{2+}\) handling system are being actively developed.

**SELECTED ABBREVIATIONS AND ACRONYMS**

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<th>Description</th>
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<tr>
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<td>store overload induced Ca(^{2+}) release</td>
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<td>sarcoplasmic reticulum</td>
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**NORMAL MYOCARDIUM**

The words in the title of this chapter “the wrong place at the wrong time” suggest that we know where in the cardiac myocyte Ca\(^{2+}\) ions should be at the right time. In fact, researchers in this field are still debating the answer to this question. For the purpose of the current discussion, we will defend the simplest scenario that allows a heart to contract and put the maximal amount of energy into the circulation during systole, while it completely relaxes and receives venous blood during diastole as a superbly compliant structure.

How this might work is a useful introduction into the mainstay of the discussion of what might happen when Ca\(^{2+}\) trafficking goes astray. The lead chapter in this series describes a model of excitation-contraction coupling in the cardiac cell, which also forms the backbone of this discussion. *Figure 1* shows that a cardiac myocyte is completely packed with myofibrils enveloped in a network of Ca\(^{2+}\) storing intracellular tubules (the sarcoplasmic reticulum [SR]) and mitochondria that accompany virtually every sarcocome in the myofibril. The cell membrane conveys information...
to the latter trio of organelles by tubules, which invaginate the surface at every Z-disk.

The transverse tubules branch into longitudinal branches forming a network that extends cell membrane and extracellular space throughout the core of the cell and reduces the distance between extracellular events and the center of the sarcomere to less than 1 \( \mu \)m. The model illustrated in Figure 2 allows the heart to cycle between diastole and systole by shuttling Ca\(^{2+}\) ions between the extracellular milieu and the interior of the myocytes, and equally importantly by shuttling Ca\(^{2+}\) ions between the SR and the cytosol. The released Ca\(^{2+}\) diffuses into the cytosol, starting two simultaneous events. The mechanically important event is that Ca\(^{2+}\) binds to the protein troponin C on the actin filaments in sarcomeres of the myocardium. Binding of Ca\(^{2+}\) to troponin C starts a chain reaction that results in the movement of the inhibitory protein tropomyosin away from the binding sites for myosin cross-bridges, thus enabling the cross-bridges to convert chemical energy of ATP into force development and sliding of the filaments, and thus sarcomere shortening. This process is amazingly close to synchronous in the hundred million billion sarcomeres of the left ventricle and allows pressure development and ejection of blood.

An important asset of striated muscle like the heart is that when the transverse tubules pass the SR, a specialized structure, the dyad or triad, is formed by the terminal cisternae of the SR, which embrace the transverse tubular membrane (Figure 2).\(^{1,2}\) At the interface between the terminal cisternae transverse tubular proteins, especially L-type Ca\(^{2+}\) channels make close contact with Ca\(^{2+}\) channels of the SR (named after ryanodine, the insecticidal alkaloid produced by the plant *Ryania speciosa* [Figure 2]). Some of the terminal cisternae remain free.

Figure 2 shows that the membrane action potential, which invades the myocyte along its transverse tubules, drives a Ca\(^{2+}\) current through L-type Ca\(^{2+}\) channels into the clefts of the dyads and triads between the transverse tubules and the terminal cisternae of the SR.\(^{3,4}\) The Ca\(^{2+}\) ions in the clefts bind to receptor sites on the ryanodine receptors; this triggers a large and rapid flux of Ca\(^{2+}\) ions through these channels from the SR into the cytosol. The magnitude of the Ca\(^{2+}\) flux depends on the degree of filling of the SR with Ca\(^{2+}\) ions and the interval between the action potentials because the ryanodine receptors need to recover (\(\approx 3\) seconds) before they are ready for the next release.

The released Ca\(^{2+}\) diffuses into the cytosol, starting two simultaneous events. The mechanically important event is that Ca\(^{2+}\) binds to the protein troponin C on the actin filaments in sarcomeres of the myofibril. Binding of Ca\(^{2+}\) to troponin C starts a chain reaction that results in the movement of the inhibitory protein tropomyosin away from the binding sites for myosin cross-bridges, thus enabling the cross-bridges to convert chemical energy of ATP into force development and sliding of the filaments, and thus sarcomere shortening. This process is amazingly close to synchronous in the hundred million billion sarcomeres of the left ventricle and allows pressure development and ejection of blood.

The event that is important to relaxation is that released Ca\(^{2+}\) also reaches a system of Ca\(^{2+}\) pumps (the sarcoendoplasmic Ca\(^{2+}\) pump...
proteins (SERCA2) that responds to a slight rise in the concentration of Ca\textsuperscript{2+} by immediately sequestering Ca\textsuperscript{2+} back into the SR. A more modest amount (≈30%) of the released Ca\textsuperscript{2+} is simultaneously extruded from the cell by ion-exchange proteins that borrow the energy from the gradient of Na\textsuperscript{+} ions across the cell membrane. This Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX) is important because 3 to 4 Na\textsuperscript{+} ions enter the cell during transport of a single Ca\textsuperscript{2+} ion. As a consequence, the cell tends to become more positive during extrusion of Ca\textsuperscript{2+}. During the normal action potential this Ca\textsuperscript{2+} extrusion by NCX only slows the repolarization somewhat, but we will see the consequences of this depolarization when spontaneous Ca\textsuperscript{2+} release occurs later in this chapter. We know that the transverse tubules carry more than half of the NCX molecules of the membrane and also a substantial fraction of the L-type Ca\textsuperscript{2+} channels. Ca\textsuperscript{2+} ions enter through both proteins during the action potential and thus contribute to the Ca\textsuperscript{2+} load of the SR. The presence of these molecules in the transverse tubular membrane is responsible for an important property of heart muscle: the amount of Ca\textsuperscript{2+} that enters the cell depends on the heart rate. Hence, increasing the heart rate increases the Ca\textsuperscript{2+} load of the SR and thereby increases Ca\textsuperscript{2+} release following the action potential. This phenomenon is known as the inotropic effect of increased heart rate.

An interesting puzzle is whether the Ca\textsuperscript{2+} transport shows any activity during diastole. The answer to this puzzle is complex. It has long been known that ion channels may open spontaneously, although this at rest (of the channel) is a rare event. It is, therefore, not unexpected that when a L-type Ca\textsuperscript{2+} channel opens in the transverse tubule, the resulting Ca\textsuperscript{2+} influx may trigger Ca\textsuperscript{2+} release by ryanodine receptors or clusters thereof in the terminal cisternae: these events are now known as Ca\textsuperscript{2+} sparks. Furthermore, the ryanodine receptors respond to Ca\textsuperscript{2+}; so some may respond to Ca\textsuperscript{2+} release by neighboring terminal cisternae by Ca\textsuperscript{2+} release. This phenomenon is noticeable in the form of Ca\textsuperscript{2+}-waves in cardiac myocytes.

The puzzle regarding spontaneous activity of the Ca\textsuperscript{2+} system during diastole thus reduces to the question: do Ca\textsuperscript{2+} sparks and Ca\textsuperscript{2+} waves occur during diastole in normal myocardium? Again, we propose a simple scenario. We do so for simplicity, but also in part on the basis of many years of work on isolated cardiac fascicles or trabeculae that can be found on the endocardial side of the ventricle.

We have used trabeculae from many species, including ferret, cat, pig, and rabbit, but the main source over the years has been the rat right ventricle (RV). Their behavior is strikingly similar. Loiselle and collaborators showed that these muscles were virtually identical to the fascicles that comprise the wall of the heart. The attraction of using these

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**Figure 3. Characteristics of action potential–induced [Ca\textsuperscript{2+}] transient.**

Trabeculae, endocardially running fascicles of myocardium respond to an action potential by uniform calcium release, uniform sarcomere length changes followed by quiescence during the diastolic interval.

of the muscle with the equipment. After relaxation the sarcomere length remains constant without perceptible fluctuations and the microscopic image of the muscle is crystal clear and completely quiescent. Studies using confocal microscopy after loading of the muscle with the ester of the Ca²⁺ probe Fluo 4 (AM) confirm that the cells neither show Ca²⁺ sparks, nor Ca²⁺ waves. These observations support the notion that cells in intact normal myocardium are completely quiescent during diastole. Other studies have suggested that a small amount of spontaneous contractile activity can be found in normal cells or normal muscle, but in our hands spontaneous activity was usually caused by us, for example by raising the [Ca²⁺] in the medium surrounding the muscle.

**INTERACTION BETWEEN [Ca²⁺] AND FORCE DEVELOPMENT**

The release of Ca²⁺ by the SR amounts to ≈40 µM per heart beat and brings the free [Ca²⁺] from ≈0.1 µM to ≈1 µM. It is obvious that 98% of the released Ca²⁺ is bound to ligand proteins in the myocyte. The most important ligand was mentioned in the above troponin C (75 µM in the cell), which has one binding site for Ca²⁺ ions at this range of [Ca²⁺]. The presence of a single binding site predicts that it would require at least 50 µM of free [Ca²⁺], before 98% of the troponin C would be occupied. It is obvious that other mechanisms must enhance the binding of Ca²⁺ ions by the troponin C molecule. In fact, three cooperative mechanisms have been proposed and are actively studied: (i) troponin C bound to Ca²⁺ enhances binding of Ca²⁺ ions by neighboring troponin C; (ii) cross-bridge attachment enhances binding of Ca²⁺ ions to troponin complex; and (iii) force exerted by the cross-bridges on the actin filament deforms actin and allosterically enhances binding of Ca²⁺ ions to troponin-C on the filament by slowing the rate at which the Ca²⁺ ion leaves the troponin C-calcium complex (the off-rate of Ca²⁺). These feedback mechanisms are of outstanding importance in the heart because they permit a highly effective coupling between the Ca²⁺ release process and the mechanical response of the sarcomere to the released Ca²⁺ ions.

In fact, we have shown that the feedback of cross-bridge force to Ca²⁺ binding predicts fundamental properties known for cardiac muscle (Figure 4). The molecular mechanism that couples actin deformation to the troponin C-Ca²⁺ off rate is unknown, but in view of the realistic reproduction of the steady state Force-[Ca²⁺] relationships and Force-Sarcomere Length relationships at varied activation levels for [Ca²⁺], this feedback is quite plausible. This interaction between force and Ca²⁺ predicts that the muscle behaves during the heart beat as if it were in a steady state and shows only little effect of shortening against a load, thereby explaining that the end-systolic force sarcomere length relationship is fixed as has been observed experimentally. Hence, this interaction between force and Ca²⁺ binding to troponin C explains the well-known End Systolic Pressure Volume Relationship of the left ventricle as well as Starling’s Law of the heart. The combination of these predictions is no proof for the postulated force–Ca troponin C feedback, but makes it a useful working model for cardiac muscle and should stimulate further studies of the dynamics of the interaction between troponin C and Ca²⁺. The time course of [Ca²⁺] and force during contraction is predicted by this model and is similar to those in experiments on trabeculae. We will see that an important consequence of this behavior is that a force-decrease accelerates dissociation of Ca²⁺ from Ca²⁺-triton C. This phenomenon is experimentally well known, therefore we propose that the latter mechanism plays an important role in nonuniform muscle and may be arrhythmogenic.
DISEASED MYOCARDIUM

Too little Ca2+ although at the right time

Myocardial infarction (MI) leads to ventricular dilatation and dysfunction and ultimately to congestive heart failure (CHF). There is considerable evidence that failure of Ca2+-transport in myocytes is a central cause of contractile dysfunction in CHF. Left ventricular myocardial infarction (MI) causes remodeling of infarct area into a scar as well as remodeling of viable myocardium to enable it to generate the increased workload.

With time, though, a substantial infarct may be followed by CHF. One of the hallmarks of CHF has been shown to be that muscle strips from LV of human hearts in CHF develop less force than those from normal LV, especially at increased heart rate. The same is found in experimental studies of CHF. The important consequence of the loss of inotropism of an increased heart rate is that the heart loses a powerful feedback mechanism that normally allows it to respond to and increased workload. Hence, the heart will rely more on other feedback mechanisms, such as increased filling pressure and increased β-adrenergic drive, which may enhance further pathophysiological remodeling. What are the causes of the reduced force output?

Several hypotheses have been put forward to account for the observed reduction in contractile force in CHF. We will focus here on the abnormalities that pertain to abnormal Ca2+ transport. Reduced SR Ca2+ pump activity described in both human CHF and experimental models of CHF is accompanied by increased expression and activity of the Na+/Ca2+ exchanger. Such changes could be responsible for slower relaxation of muscles in CHF, reduced SR Ca2+ load and force, particularly at increased heart rate. Both dysregulation of SR Ca2+ channels and inadequate coupling between T-tubular L type Ca2+ channels and the corresponding SR Ca2+ channels have been proposed. Both could lead to reduced force in CHF; however, such changes would be frequency independent.

The action potential duration (APD) of myocytes from hearts in CHF is increased as it is in LVH. Such APD prolongation, with little change in ICaL, is expected to permit an increased Ca2+ influx, providing an increased trigger for Ca2+ release as well as an increased SR Ca2+ load and would counter the effects of changes in SERCA2 and NCX on force in CHF. On the other hand, there is mounting evidence that maintenance of intact transverse tubules is at fault in CHF. The tubules are not rigorously connected to the surface membrane anymore and their regular structure is lost. We know that the transverse tubules carry more than half of the NCX molecules of the membrane and also a substantial fraction of the L-type Ca2+ channels. Ca2+ ions enter through both proteins during the action potential and thus contribute to the Ca2+ load of the SR. Loss of this source of Ca2+ would contribute to reduced inotropism of increased heart rate in CHF. Furthermore, regions of the cell that lose their tight coupling to the action potential owing to loss of the transverse tubular proximity will now rely more on Ca2+ diffusion and propagated Ca2+ release and the rate of development of force will decrease. The loss of the proximity of NCX predicts a shift of Ca2+ removal toward SERCA2. Therefore, relaxation in CHF muscle is slowed even more.

Ca2+ at the wrong time: sparks

Current data on Ca2+ sparks in myocytes from diseased tissues are not uniform, probably because of variability of disease scenarios. Multiple populations of Ca2+ sparks have been identified in normal rat trabeculae, as well as in guinea pig myocytes. Myocytes from SHR rats with left ventricular hypertrophy (LVH) exhibit an increased global Ca2+ transient as well as a redistribution of Ca2+ sparks to those with larger amplitudes without any changes in ryanodine receptors proteins. “Big” sparks in hypertrophied cells occurred without an alteration in trigger Ca2+ or overall SR content. On the other hand, Ca2+ sparks appeared lower and slower in human myocytes from failing hearts. Gomez et al18 showed in models ranging from LVH to CHF no changes of sparks when compared with control and suggested that abnormalities in excitation-contraction (EC) coupling in these CHF cells may be due to changes in the spatial organization of the dyad. Some ryanodine receptors in transverse tubuli are coupled to L-type Ca2+ channels, but other ryanodine receptors may be “orphaned.” That is, ryanodine receptors are still able to function, but are “physically” isolated from Ca2+ influx channels, predicting reduced global cellular Ca2+ transients.

Ca2+ at the wrong time: waves

Lakatta’s group observed that light-scatter by papillary muscles of normal rat fluctuated spontaneously. These fluctuations appeared related to spontaneous random sarcomere contractions in cells. Spontaneous light intensity fluctuations appeared to be coupled to asynchronous Ca2+ oscillations and caused increased...
force of the muscle during diastole as well as reduced active force development. These findings differ quantitatively from the behavior of sarcomeres in nonfailing trabeculae, which are typically quiescent during diastole at physiological \([\text{Ca}^{2+}]_0\).

Figure 5A illustrates both the quiescence during diastole and reproducible shortening during the twitch (Figure 5A and Figure 3) as well as rapid uniform lengthening during relaxation. Figure 5 shows dramatically that this uniform behavior is lost in trabeculae from the heart of animals with CHF, where spontaneous sarcomere motion occurs at low \([\text{Ca}^{2+}]\) and increases steeply with further increase of \([\text{Ca}^{2+}]_0\).

This increase was sevenfold larger in the muscles from CHF compared with controls. Microscopic inspection of these muscles confirmed that the spontaneous sarcomere length variations were in fact caused by \(\text{Ca}^{2+}\) waves causing contractions that propagate within individual cells. The spontaneous diastolic activity caused the shortening of sarcomeres following an action potential to become highly variable and reduced force of the twitch by as much as 30%. Spontaneous diastolic activity also increased following stimulation at a high rate and during catecholamine stimulation in cardiac muscle from CHF, and probably further contributes to the inability of failing cardiac muscle to increase its force in response to increased heart rate and sympathetic stimulation.

The spontaneous diastolic contractile activity in CHF appears to be due to a greater sensitivity of SR-\(\text{Ca}^{2+}\) channels to the SR \(\text{Ca}^{2+}\) load in muscles from animals with CHF—for example by a small amount of ryanodine—eliminated the spontaneous contractions and restored force development. The failure of membrane L-type \(\text{Ca}^{2+}\) channel blockers (eg, D 600), to do the same reinforces the notion that the malfunction underlying spontaneous activity in CHF resides in the SR-\(\text{Ca}^{2+}\) channel. It is not yet known what the molecular mechanism of this abnormality is, although several mechanisms have been proposed, including channel oxidation or nitrosylation.

Furthermore, excessive phosphorylation of the channel owing to the high \(\beta_1\)-adrenergic drive of the heart in chronic CHF has been postulated as a cause of spontaneous \(\text{Ca}^{2+}\) leak from the SR. Intuitively, this is an attractive proposition because \(\beta\)-blockers, especially carvedilol, form the backbone of treatment of CHF CHF in dog and human heart is thought to be accompanied by increased phosphorylation of ryanodine receptors by protein kinase A (PKA) causing the protein FK506-binding protein (FKBP)12.6 to dissociate from ryanodine receptors, which increases the open probability of ryanodine receptors. However, the question whether ryanodine receptors are excessively phospho-rylated in CHF is debated and the effect of increased phosphorylation of ryanodine receptors on dissociation of FKBP12.6 from ryanodine receptors is controversial.

The lack of a significant increase in ryanodine receptor phosphorylation in the RV in the rat model of chronic CHF makes it unlikely that ryanodine receptor destabilization is caused by phosphorylation alone. Further studies are required to reveal the other factors that are involved in ryanodine receptor desta-
bilation in chronic CHF, but is interesting to note that carvedilol is the only β-blocker which directly inhibits SR-Ca\(^{2+}\) induced Ca\(^{2+}\) release (Chen, personal communication).

The behavior of the SR-Ca\(^{2+}\) channels has been explored in detail since the discovery that mutations of the SR-Ca\(^{2+}\) channel protein are involved in malignant arrhythmias. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a malignant clinical entity that causes stress-related syncope and sudden death in children occurs without structural heart disease and can be treated by suitable β-blockers. CPVT is similar to familial polymorphic ventricular tachycardia (FPVT), but occurs without a long QT interval and is not accompanied by the pattern of torsades de pointes. The ECG pattern seen during exercise with induced bidirectional arrhythmias preceding VT is reminiscent of triggered arrhythmias occurring with digitalis intoxication. Genetic analysis has now shown that these patients exhibit a multitude of mutations in the gene for the ryanodine receptor. One of the (at least 6) forms of arrhythmogenic right ventricular dysplasia, ARVD (type 2), a catecholamine-sensitive autosomal dominant cardiomyopathy, is also mapped to the human ryanodine receptor type 2 gene. Recent work also shows that variants of the cardiac ryanodine receptor may even associate (the A allele of the rs3766871 variant) with the incidence of lethal cardiac arrhythmias in acquired CHF or (the A allele of the rs790896 variant) may act as a protective factor against sudden cardiac death. 28

Tests of the isolated single SR-Ca\(^{2+}\) channel with a CPVT mutation in planar lipid bilayer experiments suggest that the threshold for opening of the mutated channel in response to an increase of the [Ca\(^{2+}\)] on the SR lumen side of the channel is substantially lower than that of the wild type channel. Since these studies, the term store overload induced Ca\(^{2+}\) release (SOICR) has replaced Fabiato’s less specific term SR-Ca\(^{2+}\) overload induced Ca\(^{2+}\) release. 29 Semantics aside, the low threshold of the mutated ryanodine receptor for the SR Ca\(^{2+}\) load may explain that when the Ca\(^{2+}\) content of the SR is increased by a sudden surge of β-adrenergic activity and/or an increased heart rate the Ca\(^{2+}\) channels may open spontaneously and release a large amount of Ca\(^{2+}\), which would require the usual removal by SERCA2 and importantly the electrogenic NCX. The latter is accompanied by a depolarization known as DAD (delayed afterdepolarization, see the next section), which may initiate a new action potential and perpetuate in the form of a catastrophic tachycardia.

**Ca\(^{2+}\) coming from the “wrong” source: troponin C**

Nonuniform muscle

Electrical nonuniformity plays a major role in the reentry mechanism of arrhythmia and has been well investigated. 30 Much less is known about arrhythmogenicity of nonuniform segmental wall motion, with areas of hypokinesis, akinesis, and dyskinesis, which are well-known signs of ischemic heart disease. 31,32 Differences in the motion pattern around the LV may result from changes in the intrinsic force-generating capacity of the myocytes in the fascicles and may cause reduced systolic shortening or even lead to segment lengthening during contraction of surrounding healthy tissue. Thus, acute ischemia causes acute loss of force-generating capacity of the cardiac cell by impairment of EC coupling and may lead to nonuniform systolic strain distribution in the LV wall. We have seen in the above that spontaneous random diastolic Ca\(^{2+}\) release weakens the segment in which this occurs and therefore leads to weak segments contracting in series with more normal strong segments. Although nonuniform EC coupling...
has been linked to a variety of arrhythmogenic heart diseases, the role of nonuniform contraction in arrhythmogenesis has received far less attention.

Several studies have shown that nonuniform contraction itself causes the generation of Ca\(^{2+}\) waves. The increased free [Ca\(^{2+}\)] during these waves activates NCX-mediated Ca\(^{2+}\) extrusion and Ca\(^{2+}\)-sensitive nonspecific channels in the sarcolemma, and therefore induces DADs that trigger action potentials. This arrhythmogenic phenomenon in nonuniform myocardium has been coined reverse EC coupling. Reverse EC coupling may be clinically relevant as it has been shown that when patients receive resynchronization pacing that can improve the mechanical uniformity, both the incidence of sudden death and the degree of heart failure are reduced.

**Ca\(^{2+}\) waves in nonuniform muscle**

We will briefly scrutinize these studies here. Force (F) measurement by a silicon strain gauge and sarcomere length (SL) detection by laser diffraction techniques together with intracellular [Ca\(^{2+}\)] measurement by fluorescent dyes allow study of the effects of nonuniformity illustrated in Figure 6.

The reference scenario is uniform sarcomere behavior in the muscle when superfused by one solution at a physiological [Ca\(^{2+}\)]\(_{o}\) (≈1.2 mM). Arrhythmogenic effects of nonuniform contraction can be studied by exposing a restricted region of the muscle to a small “jet” of solution applied from a glass pipette (≈100-µm tip) perpendicular to the trabecula (Figure 6). The jet solution can be composed of standard saline solution containing either caffeine (CF) to deplete Ca\(^{2+}\) content in the SR or 2,3-butanedione monoxime (BDM), a poison that suppresses the activation of cross-bridges, or either low [Ca\(^{2+}\)]\(_{o}\) or high [Ca\(^{2+}\)]\(_{o}\), all of which weaken the jet-exposed segment. Consequently, twitch force decreases (by ≈40-50%) and the sarcomeres in the segment exposed to the jet are stretched by shortening of the regions outside the jet (Figure 7). The behavior of the weak and strong sarcomeres is consistent with a force balance owing to their force sarcomere length relationship and force velocity relationships. Intriguingly, the force decline during relaxation is accompanied by a Ca\(^{2+}\) surge that triggers Ca\(^{2+}\) waves starting from the border of the jet-exposed segment (Figure 7). These Ca\(^{2+}\)-waves always start during force relaxation of the last stimulated twitch and propagate into segments both inside and outside of the jet exposed segment.

The same happens when jet segment is exposed to high [Ca\(^{2+}\)]\(_{o}\), which causes SR-Ca\(^{2+}\) overload witnessed by diastolic contractile waves inside individual myocytes and renders this segment weak despite the fact that the [Ca\(^{2+}\)] transient during the electrically stimulated twitch is increased. This scenario leads to the generation of Ca\(^{2+}\)-waves that start inside the weak segment. It appears unlikely that SR-Ca\(^{2+}\) overload itself induces Ca\(^{2+}\) release in the segment exposed to the high [Ca\(^{2+}\)]\(_{o}\) jet accounts for the initiation of Ca\(^{2+}\) waves, because inhibition of cross-bridge activity
by high $[\text{Ca}^{2+}]_o + \text{BDM}$ in the jet shifted the site of origin of the $\text{Ca}^{2+}$ waves to the border zone of the jet.48

The local $[\text{Ca}^{2+}]_i$ surge occurs always at the same moment during relaxation as in muscle independent of the method by which the jet exposed segment had been weakened (CF, BDM, low $[\text{Ca}^{2+}]_o$, or high $[\text{Ca}^{2+}]_o$). This observation suggests that the $\text{Ca}^{2+}$ surge has the same cause in each scenario. We have proposed that this cause is probably $\text{Ca}^{2+}$ that dissociates from troponin C. It is important to repeat here that troponin C–$\text{Ca}^{2+}$ is a huge reservoir of $\text{Ca}^{2+}$ ions during contraction. This $\text{Ca}^{2+}$ is normally transported back to the SR and extruded from the cell.

Arrhythmias in nonuniform muscle

Figure 8 shows that this phenomenon is highly arrhythmogenic. Arrhythmias in the form of nondriven rhythmic activity were consistently triggered when the amplitude of the $\text{Ca}^{2+}$-wave is increased, eg, by raising $[\text{Ca}^{2+}]_o$. These arrhythmias disappeared when the muscle uniformity was restored by turning the jet off (Figure 8).37 The highest incidence of arrhythmias was found in muscles exposed to the jet of high $[\text{Ca}^{2+}]_o$.

Effect of force development and relaxation on $\text{Ca}^{2+}$ waves

We propose that the molecular mechanism underlying force dependence of $\text{Ca}^{2+}$ binding to troponin C is probably that cross-bridge force exerted on the actin filament deforms the troponin C molecule, thus retarding the dissociation of $\text{Ca}^{2+}$ from troponin C (Figure 9).47

The $\text{Ca}^{2+}$ surge and ensuing $\text{Ca}^{2+}$ waves start during the rapid decline of twitch-force when the sarcomeres in the strong segments relax and lengthen while rapidly releasing the sarcomeres in the weak segment.48,49 The initial $\text{Ca}^{2+}$ surge following rapid force relaxation can be explained by force feedback to $\text{Ca}^{2+}$ binding by troponin C, because the rapid force decline is expected to accel-
erate Ca\textsuperscript{2+} dissociation form troponin C. Other feedback mechanisms, including feedback between troponin C units and feedback of the number of cross-bridges, were not able to explain the Ca\textsuperscript{2+} surge.\textsuperscript{9} This model\textsuperscript{9,48} predicts that the initial Ca\textsuperscript{2+} surge is proportional to the amount of Ca\textsuperscript{2+} bound to troponin C and to the rate of force decline during relaxation. (Figure 3A). Indeed, the magnitude of the initial Ca\textsuperscript{2+} surge and the ensuing Ca\textsuperscript{2+} waves correlates clearly with force of the preceding contraction and the rate of decline of force during relaxation FT and -dF/dt\textsubscript{max}.

These observations show that arrhythmogenic Ca\textsuperscript{2+} waves may arise in regions of the myocardium with a Ca\textsuperscript{2+}-loaded SR, where the contractile apparatus exhibits active contraction owing to Ca\textsuperscript{2+}-dependent troponin C activation of cross-bridges, albeit weaker than that of the adjacent regions.

**DISCUSSION AND OUTLOOK**

Cardiac fascicles, made nonuniform so that a segment becomes weaker than the adjacent muscle segments, show three patterns of response in or near the weakened segment: (i) a local Ca\textsuperscript{2+} surge late during relaxation; (ii) the surge triggers propagated Ca\textsuperscript{2+} waves; (iii) the Ca\textsuperscript{2+} wave may trigger arrhythmia. SR loading facilitates the generation of Ca\textsuperscript{2+} waves. Figure 8 clearly shows that these arrhythmias are completely reversible and disappear when uniformity in the muscle is restored.\textsuperscript{37} The requirements for the initiation of arrhythmogenic Ca\textsuperscript{2+} waves are that the region from where the Ca\textsuperscript{2+} waves start has a Ca\textsuperscript{2+}-loaded SR and exhibits active contraction, albeit weaker than that of the adjacent muscle. These requirements are met in the border of the weak segment if the paradigm to weaken the segment lowers intracellular [Ca\textsuperscript{2+}] or SR-Ca\textsuperscript{2+}-load or reduces cross-bridge activity. The same requirements are met inside the weak segment following exposure of the muscle to [Ca\textsuperscript{2+}]\textsubscript{o} levels, which reduces contractile force in proportion to an increase of spontaneous Ca\textsuperscript{2+} release by the overlaid SR, as in failing heart. The mechanism of propagation of the Ca\textsuperscript{2+} waves needs further study in order to fully explain the high propagation velocity of the waves and test the hypothesis of Ca\textsuperscript{2+} diffusion coupled by Ca\textsuperscript{2+} diffusion, which permits a high propagation velocity of the Ca\textsuperscript{2+} ligands in the cell to be still partially occupied.

**IN SUMMARY**

We have seen that several factors, including gene mutations, acute ischemia, and unfavorable cardiac remodeling, modify Ca\textsuperscript{2+} transport at various loci in cardiac myocytes and may weaken the cells. In this scenario, the interplay between weakened and strong segments in nonuniform cardiac muscle leads to mechanoelectric feedback mediating an early diastolic Ca\textsuperscript{2+} transient caused by the rapid force decrease during the relaxation phase.\textsuperscript{30} These rapid force changes in non-uniform muscle may cause arrhythmogenic Ca\textsuperscript{2+} waves to propagate by activation of neighboring SR by diffusing Ca\textsuperscript{2+} ions. Facing the problem of treatment and prevention of arrhythmias, it is reassuring that the development of drugs that stabilize the Ca\textsuperscript{2+} handling system has started.\textsuperscript{51}

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The heartbeat is governed by the cardiac action potential (AP), via a rise in intracellular Ca (Ca$_i$). The AP shapes, and is shaped by, the Ca$_i$ transient, activating contraction. This bidirectional coupling between AP and Ca$_i$ transient produces a rich variety of dynamical behaviors, e.g., spatially discordant action potential duration (APD) alternans at fast heart rates and early afterdepolarization (EAD) islands at slow heart rates, both of which enhance the vulnerability of the tissue substrate to reentry. These interactions also generate triggers (delayed afterdepolarizations [DADs] and EADs), which enhance the vulnerability of the tissue substrate to initiation of lethal cardiac arrhythmias. Understanding these processes may lead to novel approaches to prevent sudden cardiac death.

Keywords: calcium; arrhythmia; heart failure; excitation-contraction coupling; early afterdepolarization; delayed afterdepolarization; ryanodine receptor

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In this article, we review the basic features of the bidirectional interaction between the AP and Ca$_i$ transient as it relates to cardiac arrhythmias. We begin by discussing a key physiological property of the AP called AP duration (APD) restitution, and describe how it is shaped by both AP-Ca$_i$ coupling and Ca$_i$-AP coupling. We then describe the role of APD restitution and Ca$_i$ cycling dynamics in the development of APD alternans, in which APD alternates from beat-to-beat in a long-short pattern. Moving from the cell to the tissue level, we next discuss the factors that cause APD alternans to dynamically exacerbate dispersion of refractoriness and markedly amplify tissue vulnerability to arrhythmias, as detected clinically by T-wave alternans. We also discuss why antiarrhythmic drugs may often increase the risk of lethal ventricular arrhythmias despite effectively suppressing premature ventricular complexes (PVCs). Next, we turn to the role of Ca$_i$ cycling in the generation of PVCs, mediated by afterdepolarizations. We show how Ca$_i$-AP coupling in this setting not only...
generates PVCs, but also concomitantly increases the vulnerability to ventricular arrhythmias at both slow and fast heart rates. Finally, we discuss some implications for novel therapy.

**APD RESTITUTION**

At rest, your heart rate typically averages 60 beats per min (bpm). During the 1000 ms between heart beats, systole (corresponding to APD) lasts ≈300 ms, leaving ≈700 ms for diastole, during which the ventricles refill and coronary flow oxygenates the myocardium before the next contraction. At maximal exercise, however, heart rate can reach 200 bpm, in which case the full cardiac cycle lasts only 300 ms. For the heart to function under these conditions, it is essential for the APD to shorten, to preserve diastole for ventricular filling and coronary flow (Figure 1A). This rate-dependent shortening of APD is called APD restitution, and is a critical physiological property of the heart. APD restitution can be measured by plotting APD against the preceding diastolic interval (DI), as shown in Figure 1B.

It has been recognized since the classic study of Nolasco and Dahlen 1968 that the steepness of APD restitution is related to APD alternans, in which APD alternates from long to short on alternate beats. Generally, if the slope of APD restitution exceeds 1, a dynamic instability (bifurcation) occurs, such that APD is short on one beat, long on the next, and so forth in a repeating short-long pattern (Figure 1B).

What causes steep APD restitution? APD is controlled by the balance of inward and outward ionic currents generated by a variety of ion channels and electrogenic ion transporters active during the cardiac AP plateau. As the heart rate increases and the DI shortens, inward currents carried mainly by voltage-dependent Na+ and Ca2+ channels have less time to recover from inactivation, and therefore fewer channels are available to open during the next AP, shortening its duration. Meanwhile, outward currents carried by voltage-dependent K+ channels have less time to deactivate as DI shortness, and so are quicker to reactivate during the next AP, also shortening APD. The net effect is less inward current and more outward current, causing APD to shorten progressively as the DI shortens.

How does the Ca transient fit into this scenario? APD-Ca coupling is usually positive, such that a longer APD results in a long-lasting Ca2+ current and therefore a larger Ca transient (Figure 2A, page 304). Therefore, when steep APD restitution drives APD alternans, the long APD is associated with the large Ca transient, and the short APD with a small Ca transient (electromechanically in-phase alternans). However, the Ca transient continuously feeds back on the AP. When the Ca transient is suppressed by depleting sarcoplasmic reticulum (SR) Ca...
stores, APD restitution slope becomes much flatter and APD alternans is prevented. Thus, the feedback of the Cai transient on the AP (Cai-AP coupling) plays a major role in regulating the steepness of APD restitution, and hence the propensity for APD alternans.

Cai ALTERNANS AS A CAUSE OF APD ALTERNANS

As noted above, when APD alternates due to steep APD restitution, the Cai transient also alternates secondarily due to AP-Cai coupling. However, the Cai transient is also capable of alternating on its own. This can be demonstrated by voltage clamping a myocyte with an AP waveform, so that APD cannot vary from beat to beat. When a myocyte is paced with an AP clamp, the Cai transient begins to alternate at a critical heart rate (Figure 2B).

This raises the important question: when the myocyte is paced without an AP clamp, so that the APD can vary freely, is the onset of APD alternans due to electrophysiological properties such as steep APD restitution, or due to Cai alternans, causing the APD to alternate secondarily? As mentioned above, the Cai transient affects a number of ionic currents that influence APD. Cai causes the voltage-dependent L-type Ca current to inactivate more rapidly, which tends to shorten APD. However, Cai entering through the L-type Ca current is also extruded by electrogenic Na+-Ca2+ exchange, which exchanges one Ca2+ ion for 3 Na+ ions. Thus, when Na+-Ca2+ exchange is removing Ca2+ from the cytoplasm, it generates an inward current, which tends to prolong APD. Cai also affects several other ionic currents, such that the net effect of the Cai transient on APD is complex. Depending on specific conditions, the net changes in these currents can either prolong or shorten the APD, referred to as positive and negative Cai-APD coupling, respectively (Figure 2C). When Cai-APD coupling is positive during APD alternans, the large Cai transient is associated with the long APD (electromechanically in-phase alternans). When Cai-APD coupling is negative, the large Cai transient is associated with the short APD (electromechanically out-of-phase alternans). Thus, the combination of APD-Cai coupling with positive or negative Cai-APD coupling can influence both the type of alternans (electromechanically in-phase, out-of-phase, or more complex patterns such as quasi-periodicity) and the heart rate threshold at which alternans develops.

Experimental evidence supports the conjecture that APD alternans is usually secondary to Cai alternans, since at the onset of APD alternans, the APD restitution slope is typically <1. However, because the AP and Cai transient are bidirectionally coupled, both factors together influence the threshold and amplitude of APD alternans.
WHY APD ALTERNANS IS ARRHYTHMOGENIC

APD alternans is equivalent to repolarization alternans, which can be detected clinically as electrocardiographic T-wave alternans. In patients with heart disease, the presence of subtle microvolt T-wave alternans, which develops at heart rates less than 110 bpm, confers an increased risk of sudden cardiac death. The reason why repolarization alternans is arrhythmogenic has been demonstrated both experimentally and theoretically. Since the refractory period of ventricular tissue closely parallels APD under most conditions, when APD alternans occurs, so does the tissue refractoriness. This is not a particularly arrhythmogenic as long as APD alternans remains spatially uniform throughout the tissue (Figure 3A).

However, when the APD alternates, so does the DI. At very short DI, conduction velocity (CV) slows because Na+ channels have not had adequate time to recover fully from inactivation (called CV restitution, analogous to APD restitution). Thus, during APD alternans, as the DI following the long APD progressively shortens, the next AP will conduct more slowly, causing the DI, and hence APD, to oscillate over space. At this point, spatially concordant APD alternans transitions to spatially discordant APD alternans (Figure 3B), in which APD of the same heart beat is long in one region of the tissue, and short in an adjacent region. On the next beat, the pattern reverses. The border separating these out-of-phase regions has a constant APD from beat-to-beat, and is called a nodal line. Spatially discordant APD alternans can also be induced by PVCs, heterogeneous tissue properties, and negative Ca2+-APD coupling. Spatially discordant APD alternans is a highly arrhythmogenic state, as illustrated in Figure 3C. Suppose that during spatially discordant alternans, a PVC occurs in the short APD region. As the PVC propagates towards the nodal line, it encounters a steepening APD gradient. If this APD gradient is too steep, the impulse will block. Meanwhile, the impulse can propagate parallel to the nodal line, waiting for the long APD region to repolarize. The impulse then enters the long APD area from the sides, and initiates figure-eight reentry. Computer simulations have documented this scenario, even in completely homogeneous tissue. If the tissue is heterogeneous with respect to the development of APD alternans, a PVC is not even required. Localized conduction block can occur in the region with increased susceptibility of APD alternans, while propagation is maintained in the adjacent regions to initiate reentry.

Figure 3. APD alternans and arrhythmogenicity. A. Spatially concordant action potential duration (APD) alternans. APD alternates, with the long-short pattern remaining spatially uniform throughout the tissue, so that the dispersion of repolarization in space is minimal, although the T wave alternates. B. Spatially discordant APD alternans. APD alternates, but due to CV restitution, the long-short pattern becomes spatially nonuniform, markedly increasing dispersion of repolarization. Both T wave and QRS complex alternate. C. Induction of reentry during spatially discordant APD alternans. A PVC originating from the short APD region (asterisk) blocks as it propagates upwards towards the long APD region, but propagates laterally until the long APD region recovers, initiating figure-eight reentry. Reproduced from reference 5: Weiss et al. Circ Res. 2006;98:1244-1253. © 2006, American Heart Association.
Spatially discordant APD alternans is believed to be the major mechanism by which rapid pacing induces ventricular fibrillation (VF). During pacing at rapid rates inducing spatially discordant APD alternans (typically >300 bpm), VF is induced in even normal human hearts. In failing hearts, the heart rate threshold for alternans becomes significantly lower, as reflected clinically by pulsus alternans and microvolt T-wave alternans. Abnormal Cai cycling due to excitation-contraction coupling remodeling, in combination with electrical remodeling, are both involved in reducing this threshold. This mechanism may play an important role in exercise-induced arrhythmias in the setting of heart failure, particularly since rapid heart rates also promote afterdepolarizations, causing PVCs (see below).

**WHERE DID CAST GO WRONG?**

The vast majority of PVCs, even in the setting of heart disease, are benign. Two PVCs per minute is a level of ventricular ectopy common in patients with cardiomyopathy, and amounts to one million PVCs per year. However, ventricular tachycardia (VT) and VF episodes occur over a time scale of months or years, not minutes, indicating that only one in a million PVCs encounters sufficiently heterogeneous refractoriness to initiate reentry leading to VT/VF. This is borne out by clinical studies using programmed electrical stimulation, in which up to triple premature extra stimuli have a low incidence of inducing VT/VF, especially in the setting of nonischemic cardiomyopathy. The dynamic appearance of spatially discordant APD alternans as heart rate increases provides one scenario to explain how PVCs occasionally become lethal.

In the Cardiac Arrhythmia Suppression Trial (CAST) clinical study, patients who had suffered a myocardial infarction more than 6 months prior were treated with Na⁺ channel blocking drugs (encainide, flecainide, or moricizine). If one of these drugs was effective at suppressing PVCs by >80% and nonsustained VT by >90%, then patients were randomized to the drug or a placebo. The surprising finding was that patients on the drug had nearly double the mortality rate compared to the placebo group. The implications were inescapable—although the number of potential triggered events (PVCs) had been reduced by more than 5-fold, the drugs must have increased the vulnerability to those PVCs by approximately 10-fold.

What could do this? This question has been debated extensively, and we may never completely know the answer. However, one possibility relates to the propensity of Na⁺ channel blockers to promote spatially discordant APD alternans. Na⁺ channel blocking drugs not only block the Na⁺ current, which slows CV, but they also delay Na⁺ channel recovery from inactivation, especially in chronically ischemic tissue. Thus, a longer DI is required for full Na⁺ current recovery, which broadens the range of heart rates over which CV restitution occurs. Since CV restitution plays a key role in the conversion of spatially concordant to spatially discordant APD alternans, this factor may have significantly enhanced the vulnerability of cardiac tissue to PVCs, despite their reduced frequency.

**TRIGGER-SUBSTRATE INTERACTIONS—Ca²⁺ OVERLOAD AND DADS**

The rapid heart rates that promote spatially discordant APD and Ca²⁺ transient alternans also increase the frequency of PVCs by promoting delayed afterdepolarizations DADs. DADs are a class of triggered activity that can generate PVCs, singly or in runs (Figure 4A). They are caused by spontaneous Ca²⁺-induced Ca²⁺ release from the SR during diastole, which propagates as a Ca²⁺ wave through the cytoplasm. The DAD results from the activation of inward Na⁺-Ca²⁺ exchange current (and/or a Ca²⁺-activated nonselective cation current) by the Ca²⁺ wave. If the depolarization is sufficient to reach the Na⁺ current threshold, then the DAD can trigger an AP (another manifestation of Ca²⁺-AP coupling). DADs occur in the setting of SR Ca²⁺ overload, and so are potentiated by fast heart rates or drugs that enhance cellular Ca²⁺ loading, such as digitals and catecholamines. As discussed above, if the fast heart rates that increase the probability of DAD-induced PVCs also induce spatially discordant APD alternans at the same time, the result can be the highly arrhythmogenic trigger-substrate combination shown in Figure 3C.

For Ca²⁺ waves to cause DADs in tissue requires a large number of myocytes to develop Ca²⁺ waves synchronously. In ventricular muscle, each myocyte is coupled to an average of 11 other myocytes by gap junctions. If a Ca²⁺ wave activates inward Na⁺-Ca²⁺ exchange current in only a single myocyte, the source-sink mismatch is too great to cause a DAD. That is, once the affected myocyte tries to depolarize in response to the inward Na⁺-Ca²⁺ exchange current, the resulting voltage difference with the surrounding myocytes will cause current to flow through gap junctions into the affected myocyte, suppressing the depolarization. Only when the majority of myocytes in a region have synchronous Ca²⁺ waves will their summated Na⁺-Ca²⁺ exchange current be sufficient to generate a DAD.
This has been demonstrated in intact tissue using confocal microscopy. After termination of rapid pacing, synchronous Ca waves occurring in multiple adjacent myocytes were associated with DADs. In contrast, during slow pacing, sporadic Ca waves in single myocytes did not elicit detectable DADs. DADs play a major role in catecholaminergic polymorphic ventricular tachycardia (CPVT). In this syndrome, mutations in ryanodine receptors or associated regulatory proteins such as calsequestrin lead to leakiness and accelerated recovery of SR Ca release units, promoting spontaneous Ca waves as SR Ca load increases. High catecholamine states promote CPVT due to defective Na and Ca current inactivation, respectively. A variety of other genetic defects affecting ion channel properties and trafficking or associated scaffolding and signaling proteins can also produce a spectrum of other CPVT subtypes.

Recent evidence suggests that the irregularity of EADs is due to dynamical chaos, rather than randomness. This is significant, because if the mechanism of chaos can be identified, then the irregular behavior may be controllable (see below). In the case of EADs, the formal mechanism of chaos has been identified as a Hopf bifurcation causing oscillations in membrane potential, subsequently destroyed by a homoclinic bifurcation inducing repolarization. The L-type Ca current plays a critical role in this mechanism, as discussed below.

Since EADs are typically irregular, a major question arises as to how they generate PVCs in well-coupled tissue. Similar to the situation...
cussed above for DADs, when a single myocyte is primed to develop an EAD, the source-sink mismatch is too great for the EAD to become manifest if the surrounding myocytes are not likewise primed to develop an EAD. That is, once the primed myocyte tries to reverse repolarization, the resulting voltage difference with the surrounding myocytes will cause current to flow through gap junctions into the primed myocyte, forcing it to repolarize along with the other myocytes. Only when the majority of the myocytes in a region are primed to have an EAD on the same beat can the EAD become manifest, and potentially trigger a PVC in the tissue. Democracy at work! For example, if 90% of the myocytes in a region are primed to have an EAD, then 100% will synchronize to manifest an EAD, whereas if only 89% are primed to have an EAD, 100% will synchronously repolarize. The exact cutoff percentage varies, depending on the strength of the EAD, gap junction coupling, etc, but the important point is that a sharp threshold exists, below which all the myocytes in a region will synchronously repolarize, and above which all will synchronously develop an EAD.23

If all of the myocytes in a region repolarize, then no arrhythmia occurs. Conversely, if all of the myocytes develop an EAD, then a PVC may be triggered. How can this cause a lethal arrhythmia? The chaotically irregular behavior of EADs is the key, since it causes the exact percentage of myocytes primed for EADs to vary from region to region. This leads to the formation of islands of tissue exhibiting EADs (regions in which the percentage of myocytes primed for EADs happens to exceed the threshold) coexisting next to regions without EADs (in which the percentage of myocytes primed for EADs happens to remain below the threshold) (Figure 5A). This creates marked dispersion of refractoriness in the tissue as a whole. If EADs in these EAD islands are capable of triggering APs, then the triggered AP can propagate into the adjacent recovered regions without EADs (Figure 5B). As the impulse then continues to propagate beyond the recovered region into another EAD island, localized conduction block can occur, initiating reentry. The triggered beats also generate new EAD islands, which can trigger APs at new locations. The result is a polymorphic VF with mixed focal and reentrant features, as has been observed experimentally in optical mapping experiments reproducing drug-induced LQTS.
The spacing between EAD islands (and hence the number of triggered foci) decreases as the violence of the EAD chaos increases and the strength of gap junction coupling is reduced.

Thus, regional synchronization of EAD chaos is a powerful arrhythmogenic mechanism, producing triggers (EAD-mediated PVCs) while simultaneously generating marked dispersion of repolarization (EAD islands next to repolarized tissue). This vulnerable substrate develops covertly during slow or normal heart rates, until the sudden appearance of an EAD-mediated PVC initiates polymorphic VT and VF. In computer simulations, this mechanism can also nicely reproduce the undulating QRS morphology of torsades de pointes.

**THERAPEUTIC IMPLICATIONS**

We have discussed several scenarios demonstrating that when Ca2+ and action potentials across the heart’s wall fall out-of-phase, the heart’s chaos begins, in both the colloquial and dynamical sense. We have discussed how these phenomena are promoted by the bidirectional coupling between membrane ionic currents controlling the cardiac AP (AP-Ca2+ coupling), and intracellular Ca2+ cycling regulating contractile force (Ca2+-AP coupling). Their interactions produce a rich variety of dynamical behaviors, including spatially discordant APD alternans at fast heart rates and EAD islands at slow heart rates, both of which markedly amplify dispersion of refractoriness to enhance the vulnerability of the tissue substrate to reentry. Concurrently, these interactions also generate triggers, in the form of DADs and EADs, capable of initiating lethal cardiac arrhythmias. If the dynamical mechanisms underlying these behaviors at the tissue level can be understood, and related to specific biological targets at the cellular and molecular levels, then novel therapies to prevent the one-in-a-million PVC from initiating VT/VF and sudden cardiac death may be achievable. Proteins involved in Ca2+ cycling, including ryanodine receptors and the L-type Ca2+ channels, as well as their associated signaling and regulatory proteins, are likely to figure importantly in these efforts. For example, drugs such as flecanide, which modulate SR Ca2+ release by ryanodine receptors,25 are being newly explored in this context. The L-type Ca2+ current represents a potential target for EAD suppression, since EADs are critically dependent on the reactivation of the L-type Ca2+ current in the “window” voltage range. If such targets can be successfully manipulated without adversely affecting normal excitation-contraction coupling, then novel approaches may come to light, as adjuvants, or ideally replacements, for current device therapy to prevent sudden cardiac death.

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I grew up in a relatively small town in Northeastern Pennsylvania and became interested in the general aspects of science at an early age. Interestingly, I learned later in my career that two of my contemporary cardiovascular colleagues, Eduardo Marban and Ed Lakatta, both grew up in the same general area of Northeastern Pennsylvania at the same time as I did. In fact, I was in the same class in grade school as Ed Lakatta’s wife, Loretta Cantwell. I remember that one Christmas my parents gave me a “working” model of the heart in which there was a plastic heart in the middle and red and blue “vessels” around a frame pumping “blood.” In high school, I found Chemistry fascinating and Physics boring. I had no Biology courses in high school. When I entered Stonehill College in Massachusetts in 1965, I began a major in Chemistry. However, I decided to move to the College of Notre Dame in Baltimore for my sophomore year and, in order to complete the entrance requirements, it was necessary for me to take a course in Biology during the summer. I loved it! So, when I arrived at the College of Notre Dame in 1966, I met with the director of the Biology department (Sister Mary Alma) and asked if I could change my major to Biology. She allowed me to do that and I continued loving each Biology course I took there. When it came time to decide what to do after college, Sister Alma recommended that I enter a graduate program. She had been involved in research during the summers at the University of Illinois Medical School in Chicago with Harold Feinberg who was a cardiovascular pharmacologist. While my intentions were to remain on the east coast of the US, I was awarded a fellowship by the Department of Pharmacology at the University of Illinois in Chicago and urged by my mentor to attend there.
I began my research there in 1969 with Richard Green (now my husband for over 30 years) working on the autonomic control of the embryonic chick heart. We were interested in how innervation of the heart by the sympathetic nervous system affected the sensitivity of the β-adrenergic receptors in the heart. Through my PhD dissertation I became interested in second messengers and protein phosphorylation, and afterwards pursued postdoctoral studies with Mariano Tao in the Biochemistry Department at the University of Illinois on the subject of protein phosphorylation in red blood cells. When I accepted my first academic position at the Chicago Medical School in 1978, I decided that my research goal was to understand the molecular events involved in the regulation of calcium channels in the heart by cyclic AMP–dependent protein kinase. I followed this theme for my entire academic career using pharmacological, biochemical, and molecular biological approaches. In addition, my PhD dissertation also led me to an interest in desensitization of receptors, and therefore the second theme that I followed during my career was the molecular basis of the desensitization of cardiac muscarinic cholinergic receptors. In particular, I was very interested

**SELECT BIBLIOGRAPHY**

in how agonist-dependent phosphorylation of the muscarinic receptors could participate in the desensitization of the receptors.

WHY AND HOW DID YOU START PAINTING?

I took one painting class in college, and pottery classes later, but I did not start to paint seriously until 1995, while I was working at Northwestern University. I needed a diversion to relieve the stress associated with my work (writing grant proposals, etc), and painting turned out to be such a diversion. I found it to be very relaxing and a great diversion from academia.

Are you a complete amateur, or did you get any artistic training?

When I started painting in 1995, I took classes in watercolor painting at night at a neighborhood art center in Chicago. I continued taking classes there for several years until I retired in 2001 and moved to Maine and Mexico. In Maine, I took several workshops with different painters and started painting in “plein air” with a group of friends. I have also taken workshops in Puerto Vallarta.

What inspires your painting: persons? events? your scientific profession?

My travels, my life in Mexico and Maine, are the major influences on my painting. My style is constantly evolving and I am influenced to some extent by the people that I paint with, but also by the desire to continue to evolve personally. My scientific profession is not a source of inspiration, or only very indirectly, inasmuch as my painting is an escape from it. But, in a sense, that is a source of inspiration, to want to be creative in a different “medium!”

What artistic styles influence your painting? What is your artistic creed?

Color is paramount. My paintings are very colorful and I do not like to use a lot of detail. I try to keep the paintings loose and fresh. I suppose expressionist painting would be an influence. As to my artistic creed, two words can sum it up: have fun!

Is painting a hobby, or more? Are you an occasional or prolific painter?

For me, painting is part of my life. I consider it more than a hobby and most times, I paint quite frequently. However, I do not feel compelled to paint every day. I want to continue to paint to enjoy the process, so I paint when I feel like it. If I have other things that I need to do, it is OK. Learning how to do this comes from living in Mexico. “Mañana” is not necessarily “tomorrow,” it is just not “today.” One learns to be more patient and laid-back living in Mexico. No one is in a hurry as they are in other places I have lived, and everyone is happy.
Do you see any special relationship between your profession and your art?
Being a scientist requires a lot of creativity. Every day in the lab is different and every day presents new challenges. And what goes for the bench also goes for the easel: each painting is different, even if the subject is similar to another painting. In both instances, that is, science and art, the outcome is never predictable! And, in both cases, the outcome is often surprising!

Is medicine an “art”? Why is it unlike other “life sciences?”
Medicine is truly an art that is governed by underlying principles that can be quite old, but that are enriched and expanded daily by new discoveries. As mentioned above, each day in one’s life as a research scientist is different. New discoveries are made almost daily, and even newer ones will be made as a result of the day’s discoveries. Not all “discoveries” are major, but more often, like little pieces of a puzzle that eventually fit together to create a new story.

Why is it that so many physicians and scientists are artists, or are artistically inclined?

Probably, I would think, it is because it requires creativity on a daily basis to be a physician or a research scientist. One is constantly searching for keys to unlock pieces of a puzzle, not unlike creating new pieces of art.

Do you engage in other forms of art? (sculpture, music, writing?)
There’s been the piano, which I played for 12 years while in grade school and high school, and while I was a post-doctoral fellow, I made pottery. I enjoyed that, but had to give it up as my career was more demanding at the time.

Do you keep your paintings, give them away, sell them?
I have sold quite a few paintings in Maine and in Mexico, and I have also given paintings away to family and friends. I like to know that they have found a home. It makes me happy to know that they are hanging somewhere and are enjoyed.
Did you ever think of making painting a full-time occupation when you retired?

Since 2001 when I retired, I have had no interest in having another “full-time” career. When I retired, I had more time to paint, which was great, but I do not want painting to be stressful, since I started to paint to relieve stress. I am sure if I had to make it a full-time career, I could do it, but I do not have to at this time, so I just enjoy it, along with other things that I do, like play tennis and bridge.

Have your paintings been exhibited? where? Do you have a Web site?

I have exhibited my paintings in Mexico, at the Galeria Arte Latino-Americana in Puerto Vallarta, where I had three solo exhibitions in 2003-2005. I have also exhibited my paintings in Maine at the Coveside Marina and Restaurant in South Bristol for the past 7 years and will continue to do. I tend to be “low-key” about exhibiting my paintings and have no interest in being represented in many galleries, because that requires a lot of work. I also participate by donating paintings for several charitable events in both Mexico and Maine yearly. As to your question about a Web site: no, I don’t!

Describe the paintings you’ve selected for this article

The paintings are colorful and represent work that I have done in Maine and Mexico for the past several years. I started purely as a watercolor artist working on paper, and now have expanded to include acrylics on paper and canvas, and, to a much more limited extent, some oils on canvas. Much of my work in Maine is painted outdoors on site while painting with a group of friends. In Mexico, I tend to work more at home.
The plethora of discrete discoveries that have become integrated to the field of calcium and heart function cannot be adequately encompassed by a few pieces in the literature: the usual selection of 10 seminal papers seemed like a straitjacket that would not even begin to do justice to the topic. It was therefore decided to depart from the usual format and offer, by means of telegraphic “mini-summaries,” a quick tantalizing glimpse into the contents of 10 “historic” seminal papers, which blazed the trail of our understanding of the heart’s handling of calcium, before analyzing in greater detail 10 “hot topic” seminal papers focusing on three aspects of special current interest (Inotropy and Excitation-Contraction Coupling; Chronotropy and Pacemakers; and the Convergence of Inotropy and Chronotropy). But even doubling the list of selected seminal papers remained a painful and frustrating exercise in self-restraint. We hope the readers’ appetite will have been whetted enough to read or reread in toto some of the publications cited here as well as in the Bibliography of One Hundred Key Papers given at the end of this issue of Dialogues.

"Superimposed pacemaker action potentials showing progressive effect of 10−6 M ryanodine on the late phase of diastolic depolarization (D2). Maximum diastolic potential was used as reference point. C represents control; numbers 20 through 169 indicate consecutive spontaneous beats. Dashed lines were drawn tangent with D2 for each beat to illustrate changes in slope. This figure illustrates in more detail the specific effect of ryanodine on the slope of D2. In this figure, every 20th action potential was traced and superimposed for the first 169 beats after exposure to ryanodine. Since there were no significant changes in maximum diastolic potential over this period (approximately 2 minutes), this portion of each action potential was superimposed. It is apparent that the predominant effect of ryanodine was a specific and progressive decrease in the slope of D2, with little effect on the slope of the early phase of diastolic depolarization (D1). At the 169th beat, the membrane potential failed to reach threshold. It should also be noted that ryanodine caused a brief initial shortening of the cycle length before lengthening occurred. This can be seen at the 20th (No. 20) action potential.” (See page 329 in this issue of Dialogues for detailed comments). Figure and comments reproduced from: Rubenstein DS, Lipsius SL. Mechanisms of automaticity in subsidiary pacemakers from cat right atrium. Circ Res. 1989;64:648-657. © 1989, American Heart Association.

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Mini-Summaries of Ten Historic Seminal Papers


2. S. Ringer (1883). A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J Physiol. 4:29-42.

Serendipitous discovery of the role of calcium in the heart function. In isolated heart preparations Ringer—famously known for inventing the isotonic solution used to prolong the life of tissues outside the body—showed that in order to maintain normal beating, heart muscle, unlike skeletal muscle, requires small amounts of calcium in perfusing solution. He made this discovery after realizing that instead of distilled water, in which the heart failed to exhibit sustained contractions, his technician was actually using tap water, which, in London, contained calcium at nearly the same concentration as the blood.


For decades research into skeletal muscle had been more advanced than that into cardiac muscle. In the 1960s, a series of seminal papers by Sonnenblick translated many important findings of skeletal muscle mechanics, including a concept of Hill’s “active state,” to the heart muscle. In 1962, Sonnenblick described that the force-velocity relation can be shifted after following the addition of calcium or norepinephrine. These studies served as a “jump start” for cardiac muscle mechanics research.


The seminal 1967 paper by Reuter discovered the Ca2+ current in cardiac cells. This discovery substantially advanced the field of cardiac physiology, including both cardiac electrophysiology and excitation-contraction coupling. It explained the nature of long action potential as the inward Ca2+ current supports the action potential plateau. Later it was discovered that Ca2+ influx via L-type Ca2+ current during the action potential induces Ca2+ release from sarcoplasmic reticulum and triggers cardiac cell contraction.


In 1963, Albrecht Fleckenstein was asked to investigate a substance called “iproveratril” (later verapamil) and “prenylamine.” In the 1967 paper, Fleckenstein et al. found that these substances affect myocardial contractility as well as smooth muscle tone and this effect was compared with that of calcium-free solution. When calcium was added to the solution, contractility recovered. In 1971, Fleckenstein was the first to postulate the principle of calcium antagonist action. The pharmacological capability of selective blockade of the Ca2+ current to modulate muscle contractility proved to be an invaluable tool in basic heart research and clinical practice.


The 1969 paper by Blaustein and Hodgkin disclosed that in squid axons the calcium efflux is coupled to sodium entry. The results of this paper were first reported by the authors as an abstract in 1967. At about this time a similar result was also reported in heart preparations by Reuter and Seitz (1967 abstract and the 1968 full paper). The re-
sults of these studies were crucial for establishing the role of Ca\(^{2+}\) in heart function because they provided the basis for the Na\(^+\)/Ca\(^{2+}\) exchange concept, a central mechanism of Ca\(^{2+}\) efflux in cardiac muscle cells, as well as an electrogenic mechanism of inward current for diastolic depolarization in cardiac pacemaker function. In 1990, the Na\(^+\)/Ca\(^{2+}\) exchanger was cloned in Philipson’s laboratory.


The paper by Wood et al suggested the importance of Ca\(^{2+}\) stored in the SR at the moment of excitation (“rapid release”) in heart contraction. The rapid release was found to be the major determinant of the tension level attained during the action potential. The data indicated that the amount of Ca\(^{2+}\) available for the release depends on intersystolic intervals and the duration and magnitude of the plateau phases of prior action potentials. This fundamental study, using the sucrose gap technique, set the stage for the subsequent understanding of the EC coupling process in cardiac muscle as a dynamic interaction process between membrane voltage changes during action potential and sarcoplasmic reticulum Ca\(^{2+}\) load/release characteristics.


Sarcoplasmic reticulum ATPase (SERCA) is a fundamentally important molecule of heart function as its Ca\(^{2+}\) pumping refills sarcoplasmic reticulum with Ca\(^{2+}\) at each contraction cycle. In 1970, MacLennan purified and characterized SERCA from sarcoplasmic reticulum of skeletal muscle of rabbit. The properties of the purified enzyme were the same as those of the enzyme in sarcoplasmic reticulum. In both cases the ATPase activity required Mg\(^{2+}\) and was stimulated by Ca\(^{2+}\), but inhibited by Ca\(^{2+}\) buffering with EGTA. In 1985 (Nature), MacLennan et al established the amino acid sequence of SERCA, and, in 1990, Zarain-Herzberg, MacLennan, and Periasamy characterized the cardiac SERCA gene.


The paper by Tada et al showed that cyclic AMP or protein kinase A can increase the rate of calcium transport by the cardiac sarcoplasmic reticulum without altering the efficiency of the calcium pump, thus suggesting the presence of a complementary protein that regulates SERCA pumping activity. The next year, Tada et al characterized this key regulatory protein as a 22 000-dalton component of the cardiac sarcoplasmic reticulum. After consultation with Arnold Katz’s wife, a Greek scholar, the protein was named phospholamban (in Greek to “receive”), meaning “phosphate receptor.” Phospholamban is absent in skeletal muscle, but is a key regulatory molecule mediating autonomic receptor modulation to increase or decrease sarcoplasmic reticulum Ca\(^{2+}\) pumping that provides adequate sarcoplasmic reticulum Ca\(^{2+}\) loads for the demands of both cardiac inotropy and chronotropy (as formulated in Lakatta’s recent general theory of cardiac inotropy and chronotropy).


This paper describes the isolation of the ryanodine receptor (RyR), a Ca\(^{2+}\) release channel, from the cardiac sarcoplasmic reticulum. The Ca\(^{2+}\) release channel, like the L-type Ca\(^{2+}\) channel, is of fundamental importance for cardiac cell function in health and disease. It senses Ca\(^{2+}\) influx of L-type Ca\(^{2+}\) current during the action potential and releases Ca\(^{2+}\) to activate contraction, a major paradigm of cardiac muscle function known as Ca\(^{2+}\)-induced Ca\(^{2+}\) release or CICR. The primary structure of the RyR was established in 1989 by Takeshima et al.
Summaries of Ten Hot-Topic Seminal Papers

Inotropy and Excitation-Contraction Coupling

1. Excitation-contraction coupling of isolated cardiac fibers with disrupted or closed sarcolemmas. Calcium-dependent cyclic and tonic contractions

2. Theory of excitation-contraction coupling in cardiac muscle
   M. D. Stern. Biophys J. 1992

3. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle

4. Local calcium transients triggered by single L-type calcium channel currents in cardiac cells

5. Ca$^{2+}$ scraps: local depletions of free [Ca$^{2+}$] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca$^{2+}$ reserve

6. Action potential duration restitution and alternans in rabbit ventricular myocytes: the key role of intracellular calcium cycling
   J. I. Goldhaber and others. Circ Res. 2005

7. Tonus changes in cardiac muscle and their significance for the initiation of impulses
   E. Bozler. Am J Physiol. 1943

8. Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres

9. Mechanisms of automaticity in subsidiary pacemakers from cat right atrium

Convergence of Inotropy and Chronotropy

10. Beyond Bowditch: the convergence of cardiac chronotropy and inotropy
    E. G. Lakatta. Cell Calcium. 2004
Excitation-contraction coupling of isolated cardiac fibers with disrupted or closed sarcolemmas. Calcium-dependent cyclic and tonic contractions

A. Fabiato, F. Fabiato

Circ Res. 1972;31:293-307

Discovery of Ca²⁺-Induced Ca²⁺ Release (CICR)

The fundamental paradigm of muscle duty cycle as electric excitation (E) initiating contraction (C), i.e., EC-coupling, was discovered in 1872 by Bernstein who observed that an action potential occurs before the onset of contraction. EC-coupling mechanisms have been extensively studied since that time and the seminal study of Alexandre and Françoise Fabiato on EC-coupling in cardiac muscle was published 100 years later after Bernstein’s discovery. At that time, Reuter had discovered the Ca²⁺ current in cardiac muscle, and Wood, Heppner, and Weidmann described the inotropic effects of current pulses. Data in skeletal muscle at that time indicated that Ca²⁺ release is mainly induced by voltage. Ca²⁺-induced Ca²⁺ release (CICR) could not be demonstrated in skeletal muscle under normal conditions. Thus, it remained unknown whether cardiac contraction is triggered directly by sarcolemmal Ca²⁺ influx during the action potential, or CICR, or voltage, as in skeletal muscle.

The key to success in the Fabiatos’ discovery of CICR in cardiac muscle was their development of suitable preparation, “skinned cells.” Heart muscle (of rat, rabbit, or cat) was blended by a homogenizer into cell pieces of 10 to 100 µm in size. This preparation allowed the measurement of contraction vs intracellular [Ca²⁺], set by bathing [Ca²⁺]. Interestingly, since that time in the early 1970s, this invaluable technique has been refined and greatly improved. The authors, Alexandre and Françoise Fabiato, were a married couple, and in subsequent studies Françoise Fabiato developed a unique skill to skin each cardiac cell manually by microsurgery under the microscope! More recently, however, cardiac cell have been skinned with the aid of chemical detergents, such as saponin or triton.

With strong Ca²⁺ buffering by EGTA, the function of sarcoplasmic reticulum (SR), as a dynamic Ca²⁺ store, was obviously disabled, and skinned cells were essentially a myofilament preparation. Their relationship of tonic contractions vs bathing [Ca²⁺] showed a rather high threshold of [Ca²⁺] (pCa of ≈ 6.5) for myofilament activation. With mild Ca²⁺ buffering, however, SR proteins competed for free Ca²⁺ and SR release could be measured in response to applied trigger [Ca²⁺]. In this case, the skinned cells were a functional SR preparation, which exhibited SR-generated oscillations in a wide range of bathing Ca²⁺. The Fabiatos reasoned that if a small amount of applied Ca²⁺ (pCa of ≈7.5) induces a contraction requiring a higher Ca²⁺ (pCa of ≈6.5 found under higher EGTA), then the extra Ca²⁺ must be released from SR by the small Ca²⁺ trigger. This sort of high-gain CICR was indeed observed and suggested by the Fabiatos as a mechanism for EC coupling: “a small Ca²⁺ flux across the membrane during the plateau phase of the action potential would permit activation.” Finally, observation of the propagation of the contractile oscillations (“localized cyclic contractions”) led to the idea of the importance of local Ca²⁺ interactions that “the Ca²⁺ released from a storage site not only triggers the contraction of nearby myofilaments, but also releases Ca²⁺ from other storage sites.”

This 1972 paper was one of the long series of seminal studies by the Fabiatos. Using skinned cells, they discovered the regulation of SR Ca²⁺ cycling by cyclic AMP (published in 1975 in Nature). CICR was further characterized in detail using extremely sophisticated experimental techniques developed by Alexandre Fabiato: for example, his 1985 setup of a six manipulator- and microprocessor-controlled microinjection system simulated a Ca²⁺ increase produced by the Ca²⁺ current. The current understanding of the role of Ca²⁺ in heart function is based in a large part upon the seminal discovery by the Fabiatos and detailed characterization of CICR in cardiac muscle.

1972

Bobby Fischer becomes the first American World chess champion defeating Boris Spassky; eleven Israeli athletes are murdered at the 1972 Summer Olympics in Munich; and Philippine President Ferdinand Marcos places the entire country under martial law
Theory of excitation-contraction coupling in cardiac muscle

M. D. Stern

Biophys J. 1992;63:497-517

Spark Predictions. Importance of Local Ca\textsuperscript{2+} Signaling

While many experimental results (e.g., A. and F. Fabiato's studies in the 1970s, described above) supported the idea that excitation-contraction (EC) coupling occurs in heart muscle cells via Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR) mechanism, it had remained puzzling how the intrinsically unstable mechanism of CICR (Ca\textsuperscript{2+} release begets more release) could reproduce the presence of smoothly graded sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} release and thereby graded contractions controlled by the sarcolemmal Ca\textsuperscript{2+} current over a wide range of its amplitudes. A solution to this problem was achieved by Michael Stern in his 1992 seminal theoretical paper. A dominant idea of the time regarding CICR was that the trigger Ca\textsuperscript{2+} reaches the SR via the same cytosolic Ca\textsuperscript{2+} pool into which SR Ca\textsuperscript{2+} is released. Stern dubbed this type of thinking as a "common-pool" model. Despite numerous mathematical tricks, such models had been inherently explosive (i.e., generated all-or-none release) and could not simulate the physiologic graded CICR without the inclusion of a direct voltage-dependent activation, which was in conflict with the experimental evidence. This indicated to Stern that something fundamentally important was missing in the current thought process, and he took a different philosophical approach: he split the one big "common pool" (where SR and Ca\textsuperscript{2+} influx interact) into a number of smaller pools. Each "small pool" was then mathematically solved, and finally the ensemble cell function was achieved by integration of the set of solutions for small pools.

Since each "partial model" was physically small, it operated on the "nanoscale" of individual Ca\textsuperscript{2+} channels (both L-type Ca\textsuperscript{2+} channels in the sarcolemma and release channels in the SR). Therefore, the paper had to deal with stochastic openings of individual channels, which would seem to complicate solving the problem. Based upon his background in theoretical physics (at Princeton and MIT), Stern employed methods of mathematical physics that at first glance are bulky and frightening, but ultimately provided the clean and elegant solutions described in his seminal paper. Surprisingly, and quite counterintuitively, it turned out that it was the "noisy" channel stochastics and local interactions that caused the graded release! More specifically, the paper suggested that one L-type Ca\textsuperscript{2+} channel triggers a regenerative cluster of several SR channels (Stern's "cluster bomb" model). Statistical recruitment of clusters resulted in graded response with high amplification. The essence of Stern's local control concept for cardiac EC coupling was that the Ca\textsuperscript{2+} release channel clusters are functionally independent, and are recruited only by Ca\textsuperscript{2+} that entered through closely associated L-type Ca\textsuperscript{2+} channels. In this case, the L-type Ca\textsuperscript{2+} current can control SR release, because the Ca\textsuperscript{2+} released from one cluster does not activate or influence Ca\textsuperscript{2+} flux from another. Importantly, the recruitment of release cluster to fire Ca\textsuperscript{2+} happens with some probability, i.e., not for every L-type channel opening. This creates a graded overall release response to L-type Ca\textsuperscript{2+} current, as the probability of the recruitment varies for different amplitudes and durations (kinetics) of a single L-type Ca\textsuperscript{2+} current over the range of membrane voltages. The model also explained the existence of propagating Ca\textsuperscript{2+} waves in the context of Ca\textsuperscript{2+} overload.

It is important to note that local Ca\textsuperscript{2+} dynamics of cardiac myocytes had not been measured at that time, so that the local control model was developed by Stern in the absence of direct experimental data about the local release quanta in the form of what was later called "local Ca\textsuperscript{2+} transients" or "Ca\textsuperscript{2+} sparks." Thus, Stern's theory was a rare case in cardiac physiology when a theory led experimental research and significantly advanced our knowledge.

Avant-garde American composer John Cage dies; the UN Security Council authorizes the use of force to ensure the delivery of humanitarian aid to Bosnia-Herzegovina; and archaeologists claim to have found the bones of Caiaphas, the high priest who handed Jesus over to the Romans.
A central thrust of the current dogma of excitation-contraction (EC) coupling mechanism of cardiac muscle is that Ca2+ release channels (RyR, ryanodine receptors) can produce local Ca2+ transients or Ca2+ sparks, with their activation being locally controlled by Ca2+ influx via L-type Ca2+ channels, a process known as Ca2+-induced-Ca2+ release (CICR). While the existence of Ca2+ sparks had been predicted in 1992 by Stern’s local control theory, it had remained unconfirmed experimentally until 1993, when Cheng et al reported the first experimental observation of Ca2+ sparks in isolated single ventricular cells using confocal microscopy and a new Ca2+ indicator, fluo-3. The indicator, developed in 1989 by Roger Tsien’s laboratory, had a visible excitation wavelength (as opposed to prior indicators fura-2 and indo-1 requiring UV excitation) and provided a better signal resolution: binding of Ca2+ increased its fluorescence by up to 40-fold.

Interestingly, as formulated in the introduction, the paper’s main quest was to establish a new method to record the activity of RyR channels directly in the native environment, rather than to explore the local mechanism of EC coupling per se. The authors reasoned that this new method would help to study CICR in the context of a "complex microarchitecture of the muscle cell, which precludes direct examination of intracellular events at the level of single channels."

Local Ca2+ signals were measured in 2-D confocal sections of nonstimulated rat ventricular myocytes. The authors noted that "the fluorescence is generally uniform, although there are discrete regions of increased fluorescence (‘sparks’) that vary in position between images." The sparks occupied small areas with a radius of about 1.5 µm. In line-scan mode (a single line across the cell was repeatedly scanned), the spark reached a peak of ~300 nM in ~10 ms and then declined in ~20 ms, i.e., much shorter time than the global Ca2+ transient evoked by electrical stimulation, indicating that the transient is likely produced by many sparks synchronized by an action potential. The RyR origin of sparks was confirmed by the fact that the alkaloid ryanodine (acting selectively on RyR) inhibited sparks in most cells exposed to ryanodine >1 µM. At a lower ryanodine concentration, some sparks had relatively long durations (consistent with the idea that ryanodine can lock RyR in a subconductance state). Observing single channel–like activity (switching between open and closed states) at low ryanodine concentration, the authors were excited to report the possibility of in situ measurements of the activity of an intracellular channel by an optical method. Assuming that a spark is generated by a single RyR channel, they even estimated its single channel amplitude (~4 pA). Further studies, however, revealed that a Ca2+ spark of cardiac cells is likely generated by a cluster of several RyRs with a cooperative gating (as predicted by Stern’s local control theory). Finally, the paper explored the emergence of waves of Ca2+ that propagate along the length of the cell in high bathing [Ca2+]i. While the waves were clearly initiated by sparks, the spark frequency was only slightly increased under these conditions. But the elevation of spark frequency with ryanodine did not produce waves in normal bathing [Ca2+]i. These observations suggested that wave emergence is not linked to the spark rate per se, but instead to the possibility that "the sensitivity of the sarcoplasmic reticulum (SR) Ca2+-release channel for activation by cytosolic Ca2+ may increase as a consequence of the greater amount of Ca2+ stored in the SR." Further studies confirmed a critical importance of SR Ca2+ load for emergence of spontaneous Ca2+ release in cardiac cells.

US novelist Toni Morrison wins the Nobel Prize for Literature; Nelson Mandela and F. W. de Klerk are jointly awarded the Nobel Peace Prize; and Andreas Papandreou begins his second term as Prime Minister of Greece
Local calcium transients triggered by single L-type calcium channel currents in cardiac cells


Science. 1995;268:1042-1045

Voltage-Current-[Ca^{2+}]_i Relations. EC Coupling Dissected at the Bench

Alexandre and Françoise Fabiato’s discovery of Ca^{2+}-induced-Ca^{2+} release (CICR), Stern’s “local control” theory, and observation of Ca^{2+} sparks by Cheng et al (all described above) pointed to the crucial importance of local interactions of sarcoplasmic reticulum (SR) release channels (RyRs, ryanodine receptors) and L-type Ca^{2+} channels (LCCs) (the Ca^{2+} current in cardiac cells was discovered by Reuter in 1967). However, it had remained unknown how specifically sarcolemmal LCC currents recruit RyR to generate graded Ca^{2+} transients and hence control cell contractions. This problem was “dissected at the bench” by Lopez-Lopez et al in single guinea pig ventricular myocytes using a voltage-clamp technique combined with confocal microscopy. The dissection involved one important experimental trick: the probability (but not amplitude) of LCC was reduced by verapamil, an agent blocking LCCs. Under these conditions of low probability of LCC openings, the probability of occurrence of sparks (termed “local Ca^{2+} transients” in this paper) became so low that each spark (that would comprise the global Ca^{2+} transient) could be distinguished unequivocally.

Firstly the paper showed that Ca^{2+} sparks are uniform in size and amplitude, ie, independent of voltage. Then the authors measured the latencies of Ca^{2+} spark occurrences and found that the probability of spark occurrence declined with time during the voltage pulse and was approximated by a single exponential fit (τ_{LCT}). The spark activity could not be compared directly with the single LCC activity because of the current noise in whole cell patch clamp configuration. However, the plot of the dependence on potential of τ_{LCT} was U-shaped, ie, similar to that of the slow component (τ_s) of the inactivation of the L-type Ca^{2+} current that had been reported for cardiac myocytes. Then the authors compared the calculated probability of evoking sparks with the reported open probability of LCC at each voltage, and finally plotted a ratio of the two. The ratio, in fact, evaluated a probability of evoking a spark by a single LCC. While, as expected from Stern’s local control theory, not each LCC evoked a spark, the probability of spark recruitment was found higher at lower voltages. The authors explained this phenomenon by a larger single LCC current at low voltages that would boost its recruiting capability.

The paper concluded that the Ca^{2+} transient in the whole cell can be explained in terms of the recruitment of single, stereotyped unitary events (local Ca^{2+} transients), controlled locally by single L-type Ca^{2+} channels in the plasma membrane.
Ca²⁺ scraps: local depletions of free [Ca²⁺] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca²⁺ reserve

Cir Res. 2003;93:40-45

The amount of Ca²⁺ stored in the sarcoplasmic reticulum (SR) at the moment of excitation (SR load) determines how much Ca²⁺ is available for the release. As caffeine lowers the threshold of Ca²⁺ release channel (ryanodine receptor, RyR) activation by Ca²⁺, the SR load can be assessed by the amplitude of the caffeine-induced Ca²⁺ transient. Using this technique, extensive studies in the 1990s (laboratories of Bers and Eisner) discovered a nonlinear, extremely steep dependence of release vs SR load. However, it was crucial to study local dynamics of free [Ca²⁺] within cardiac SR ([Ca²⁺]SR) rather than the total Ca²⁺ (assessed by caffeine), because [Ca²⁺]SR modulates RyR and determines the driving force for SR Ca²⁺ release.

Shannon et al adopted, for rabbit ventricular myocytes, a new technique of [Ca²⁺]SR measurement, suggested earlier by Kabbara and Allen in skeletal muscle fibers. Their method used a low-affinity Ca²⁺ indicator, Fluo-5N AM, with a Ca²⁺ binding constant in situ of about 400 µM. The indicator must travel through two membranes, of the sarcolemma and the SR, and the “AM” ester must be then cleaved to release the functional indicator.

The confocal imaging of fluorescence of the Fluo-5N-loaded ventricular cells nicely portrayed the anatomy of the SR as it wraps around the myofilaments (observed as dark regions within the sarcomere between wispy areas of free SR) with the functional SR located at the Z lines. Caffeine decreased the whole-cell fluorescence only partially, to only about 50%. Electrically paced cells exhibited synchronized contractions (termed twitches in the paper), with fluorescence decline to only 25% of the caffeine-sensitive component. Then the authors explored local Ca²⁺ depletions at a single SR junction. The depletions were dubbed “Ca²⁺ scraps,” since they are intra-SR correlate of evoked Ca²⁺ sparks. It turned out that the scraps exhibit amplitudes of depletions similar to that of the global SR signal (just as Kort and Lakatta reported about 20 years ago with respect to local vs global Ca²⁺ release). Furthermore, depletions in junctional and network [Ca²⁺]SR showed similar kinetics, indicating that Ca²⁺ diffusion within SR does not appreciably limit SR Ca²⁺ availability for release. As pacing frequency increased, diastolic [Ca²⁺]SR also increased, as does the extent of [Ca²⁺]SR depletion and the contraction amplitude. β-Adrenergic receptor (β-AR) activation produced a further increase in diastolic [Ca²⁺]SR and extent of [Ca²⁺]SR depletion and faster [Ca²⁺]SR recovery, consistent with the expected acceleration of [Ca²⁺]SR ATPase by β-AR activation via phospholamban phosphorylation. Calibration of SR signals revealed that minimum [Ca²⁺]SR (following an action potential-triggered Ca²⁺ release) remained rather high, varying from 0.36 to 0.61 mM, even during β-AR stimulation.

Thus, the study provided invaluable quantitative information about [Ca²⁺]SR dynamics with subsarcomere spatial resolution that is at the very heart of cardiac excitation-contraction (EC) coupling. Exhausition of available Ca²⁺ with the SR had been considered as a simple mechanism of Ca²⁺ release termination. Shannon et al, however, showed that this is not the case, as the release terminates at some particular level of [Ca²⁺]SR of about 0.4 mM, ie, when a lot of Ca²⁺ is still available for release. Why the release stops at this level of [Ca²⁺]SR remains a mystery and an area of active research. Another fundamental question of EC coupling is about a mechanism of restitution of the release, ie, a time delay, when the RyR can fire again after a release. This study showed that [Ca²⁺]SR is restored rapidly during the twitch, indicating that inter-SR Ca²⁺ diffusion is not a limitation factor, but the delay probably involves other factors, such as recovery of RyRs.

Sparks Counterpart. Role of SR Ca²⁺ in Release Regulation

Martina Navratilova equals Billie Jean King’s record of 20 Wimbledon titles; the World Meteorological Organization announces that recent extreme weather conditions around the world may be related to global warming; and Mexico declares a state of emergency following to an outbreak of West Nile virus.
Our understanding of cardiac muscle function mechanisms has been achieved, so far, largely by using a reductionist approach, ie, the full cell system was dissected into smaller and “simpler” pieces, such as sarcolemmal ion channels, Ca²⁺ release channels, ion pumps and exchangers, contractile proteins, etc. On the other hand, following ideas of systems biology, the cell system is obviously not a simple sum of its components: its behavior (often counterintuitive) emerges from numerous interactions of components within the system. Thus, it is important that someone should integrate the extensive knowledge and methods of the reductionist approach to understand the system function. This seminal paper by Goldhaber et al is a dazzling example of such integration.

The paper explores mechanisms of action potential duration (APD) alternans, ie, an abnormal behavior of cardiac cells when APDs alternate every other cycle. APD alternans is an important arrhythmogenic factor and its mechanisms remain an open fundamental problem of cardiology. Since ion channels generate action potentials, a common sense approach would be to blame an abnormal ion channel behavior in everything related to impaired electrophysiology, including the APD alternans resulting from an abnormal APD restitution. However prior studies showed that Ca²⁺ cycling can also manifest alternans. These can drive APD to alternate secondarily, because APD is formed by Ca²⁺-sensitive ion currents, such as L-type Ca²⁺ current and Na⁺/Ca²⁺ exchanger. The authors further reasoned that since the Ca²⁺ transient is shaped by the action potential, the action potential and Ca²⁺ cycling are bidirectionally coupled.

APD restitution was measured by two methods. For the standard extrastimulus (S1-S2) method, the myocyte was steadily paced and then an extrastimulus (S2) was delivered at progressively shorter S1-S2 coupling intervals until loss of capture. For the dynamic rapid pacing method, the myocyte was also steadily paced, but the cycle length was progressively decreased until 2:1 block occurred. APD restitution curves were constructed by plotting APD versus diastolic interval (DI).

Prior studies showed that the slope of the restitution curve is a crucial parameter of the system (Nolasco and Dahlen, 1968): a steep (>1) slope, ie, when APD increases more than DI, promotes APD alternans and also breakup of electrical waves into a fibrillation-like state (Karma 1993). The study by Goldhaber et al showed that the maximum APD restitution slope exceeded 1 by both methods, but was more shallow with the dynamic method due to greater Ca²⁺ accumulation during dynamic pacing. However, the onset of APD alternans occurred at diastolic intervals at which the APD restitution slope was significantly <1. The authors showed the crucial moment when action potential duration is still shorter than the cycle length, but the Ca²⁺ transient reaches only about its half decay during the cycle. The resultant APD and Ca²⁺ transient alternans were abolished by suppressing Ca²⁺ cycling. Thapsigargin and ryanodine (disabling sarcoplasmic reticulum [SR] function) flattened APD restitution slope to <1 when measured by the dynamic method, but not by the S1-S2 method. In turn, intracellular Ca²⁺ buffering with BAPTA failed to flatten APD restitution by either method.

The authors astutely concluded that "APD alternans requires intact Ca²⁺ cycling and is not reliably predicted by APD restitution slope when Ca²⁺ cycling is suppressed. Ca²⁺ cycling may contribute to differences between APD restitution curves measured by S1-S2 versus dynamic pacing protocols by inducing short-term memory effects related to pacing-dependent Ca²⁺ accumulation."

The President of Uruguay, Dr Tabaré Vázquez, restores diplomatic relations with Cuba; the last statue in Madrid of Francisco Franco is removed; and Iceland’s parliament votes to grant US chess champion Bobby Fischer Icelandic citizenship.
Tonus changes in cardiac muscle and their significance for the initiation of impulses

E. Bozler

Am J Physiol. 1943;139:477-480

A 1943 Hypothesis Heralds the Modern Concept of Cardiac Automaticity

Bozler published this paper in 1943, long before Ca\textsuperscript{2+} measurements and microelectrode techniques. He discovered an intracellular “chemical” oscillator of cardiac cells (Ca\textsuperscript{2+}-cycling in present terms) by recording tension of strips of turtle ventricle muscle by a sensitive isometric lever on a smoked drum or photographically. Muscle excitation and contraction were elicited by induction shocks. Bozler noticed that after treatment “with a large excess of calcium ions” the muscle tonus became elevated and relaxation slowed, particularly at high initial tensions. But the most striking effect of this treatment, however, was rhythmic variations of muscle tension that decreased and disappeared after 1 to 5 waves of contraction, exactly like the oscillatory afterpotentials that Bozler described in his prior studies. Similar oscillatory tonus changes in intact ventricles indicated that the observed new phenomenon was not an artifact of the isolated muscle strip preparation. In simultaneous recordings, the muscle tension and membrane potential changes ran in close parallel. Some of these oscillations were so strong that they initiated all-or-none response with a full-scale synchronous contraction and action potential (delayed afterdepolarization [DAD]-triggered excitation in present terminology) in the absence of an externally applied shock.

While the paper is very short, with only 3 figures with single examples, and no statistical comparisons, its discussion is a masterpiece of vision and deep thinking. The major question was about the nature of the intrinsic cardiac oscillator. Since cell membrane potential oscillations had been reported in neuronal cells, one possibility was that the cardiac oscillator could also originate on the cell membrane. Bozler reasoned, however, “That the tonus changes involved in the production of mechanical energy are shown most strikingly in the tonus oscillations. It must be assumed, therefore, that tonus changes are caused by changes of metabolism.” Since the oscillations were of low frequency, several hundred times smaller than in nerve fibers, Bozler further speculated that the oscillations “do not involve merely the cell surface. The phenomenon is perhaps comparable to chemical oscillations.”

Bozler had previously reported oscillatory afterpotentials of the sinus venosus and had linked them to initiation of cardiac impulses. In this study he further linked the primary “chemical” oscillator to the afterpotentials and therefore to cardiac automaticity. Specifically he said: “The tonus changes and the local potentials are probably manifestations of a more fundamental process, a fluctuation in resting metabolism. The mechanical changes are weak and hardly play any role as such. Their chief interest lies in their relation to the automaticity and rhythmicity of the muscle. It may be assumed that an increase in metabolism causes a rise in tonus and a decreased surface polarization. The decrease in polarization in turn may be considered as the last link in the chain of processes leading to the discharge of an impulse.”

While experimental data were obtained by Bozler “with a large excess of calcium” (Ca\textsuperscript{2+} overload in current terminology), more recent studies showed that the intracellular oscillator, manifested by diastolic local Ca\textsuperscript{2+} releases, can also operate under normal conditions when its activity is boosted by intrinsic phosphorylation activity in pacemaker cells. Thus, Bozler’s 1943 hypothesis is actually at the heart of the modern concept of cardiac automaticity (as recently formulated by Lakatta et al) driven by a system of two coupled oscillators, the membrane voltage oscillator and the Ca\textsuperscript{2+} oscillator of the sarcoplasmic reticulum, ie, a “grandson” of Bozler’s “chemical” or “metabolic” oscillator.
Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres

R. S. Kass, W. J. Lederer, R. W. Tsien, R. Weingart


A substantial part of the history of establishment of the role of Ca$^{2+}$ in heart function is related to numerous studies of abnormal excitations provoked by pharmacological interventions, such as cardiac glycosides, producing Ca$^{2+}$ overload. These studies can be traced to the 19th century, when Cushny (1897) reported “irregularity” of heart contractions induced experimentally by digitalis in dogs. As noted above, in 1943 Bozler suggested that both aftercontractions and afterpotentials (delayed afterdepolarizations, or DADs, in current terminology) are generated by an intracellular “chemical” oscillator (Ca$^{2+}$ cycling in current terms). Afterpotentials, however, preceded aftercontractions, were enhanced by Ca$^{2+}$, but were abolished in the presence of manganese ions or verapamil, inhibiting sarcolemmal slow Ca$^{2+}$ current, $I_{si}$ (L-type Ca$^{2+}$ current in current terms), pointing to a membrane voltage-driven mechanism. On the other hand, in 1976, Lederer and Tsien, discovered a strophanthidin-induced oscillatory current of unknown nature (dubbed “transient inward” current or shortly $I_{TI}$) that might cause the afterpotentials. Using voltage clamp in cardiac Purkinje fibers, Kass et al explored the modulation of $I_{TI}$ by Ca$^{2+}$. The oscillatory $I_{TI}$ and the aftercontractions were closely associated and modulated by extracellular [Ca$^{2+}$]. While manganese ions inhibited $I_{TI}$, the development and removal of the inhibition lagged far behind the inhibition of $I_{si}$. The authors caught two critical moments: (i) when $I_{si}$ was almost completely blocked, but $I_{TI}$ remained almost unchanged, and (ii) during washout, when $I_{si}$ was already present, but $I_{TI}$ had not developed yet. The paper also showed that $I_{TI}$ reversed near −5 mV, far negative to the Ca$^{2+}$ equilibrium potential. Thus, the authors excluded a possibility that $I_{TI}$ could be carried by a Ca$^{2+}$-specific pathway across the surface membrane. Furthermore, based on the results of simultaneous oscillatory changes of the current and contraction under voltage clamp, they suggested another, fundamentally different mechanism: “an oscillatory release of Ca$^{2+}$ from an intracellular store is the primary event underlying both the aftercontraction and the conductance change which generates $I_{TI}$.” The authors thought that $I_{TI}$ might be due to a nonspecific conductance analogous to the acetylcholine-induced conductance reported in the motor end-plate (neuromuscular junction). Further detailed studies showed, however, that $I_{TI}$ is more likely caused by activation of electrogenic Na$^{+}$/Ca$^{2+}$ exchange. The idea of the Ca$^{2+}$ oscillator as primary cause for both the oscillatory current (via a Ca$^{2+}$-dependent conductance or Na$^{+}$/Ca$^{2+}$ exchange) and oscillatory contractions was in line with prior experimental results by A. and F. Fabiato in skinned cardiac cells (ie, cells having no surface membrane), which often showed cyclic contractile activity in the presence of light EGTA buffering (thus enabling sarcoplasmic reticulum to cycle Ca$^{2+}$). While the primary event seemed to be the intracellular Ca$^{2+}$ release, the authors also speculated about possible interactions of the Ca$^{2+}$ oscillator and surface membrane potential: “…surface membrane potential may have an important influence on the oscillatory behavior. A sudden change in membrane potential seems to synchronize the events which take place spontaneously during the current fluctuations.” The idea of a coupled oscillator (intracellular Ca$^{2+}$ oscillator and electric membrane oscillator) as a mechanism of cardiac automaticity both normal (ie, pacemaker) and abnormal (delayed afterdepolarization [DADs]) was elegantly devised by Tsien, Kass, and Weingart in their seminal review of 1979, ie, shortly after this original publication, and developed further experimentally in 1982 study by Kass and Tsien. While the idea was mainly speculative at that time, it is now linked to a major modern concept of cardiac automaticity.

**1978**

Pope Paul VI dies of a heart attack at his summer residence, aged 80 years; Odie makes his first appearance in the cartoon strip Garfield; and Kenya’s founding father Jomo Kenyatta dies.
Mechanisms of automaticity in subsidiary pacemakers from cat right atrium

D. S. Rubenstein, S. L. Lipsius

Circ Res. 1989;64:648-657

Importance of Ca$^{2+}$ for Normal Pacemaking

This revolutionary paper provided the first experimental evidence for Ca$^{2+}$ release as a mechanism for normal cardiac pacemaker function, basically contrary to the consensus of that time. Indeed, since diastolic depolarization is an electrical process of the cell surface membrane, it seemed natural to assume that it is determined entirely (or almost entirely) by the ion channels, which, according to the Hodgkin-Huxley theory of membrane excitation, fully determines cell excitability. This dogma, in fact, had gripped the pacemaker field since 1960 when Noble applied the Hodgkin-Huxley theory to the heart. In the 1970s, Irisawa and Kass et al speculated about an intracellular Ca$^{2+}$ oscillator contributing to pacemaker function. But this idea was abandoned because the existing membrane theory could apparently explain pacemaker potentials, and the Ca$^{2+}$ oscillator seemed to be rather weak and clearly manifested only under the conditions of Ca$^{2+}$ overload.

Rubenstein and Lipsius measured pacemaker potentials in cat atrium cells exhibiting prominent subsarcolemmal cisternae (i.e., Ca$^{2+}$ stores) that might be related to pacemaker function. While both cesium (I$\text{f}$ inhibitor) and verapamil (Ca$^{2+}$ current inhibitor) increased spontaneous cycle length (SCL), they affected different phases of diastolic depolarization (DD), its early and late slopes, respectively. Both norepinephrine and Bay K (Ca$^{2+}$ channel opener) elicited a significantly greater increase in late than in early DD, resulting in a decrease in SCL. The most interesting result was obtained with ryanodine, which disables Ca$^{2+}$ cycling by locking release channels (ryanodine receptors) in a subconductance state, leading to a gradual depletion of sarcoplasmic reticulum (SR) Ca$^{2+}$ stores. A small concentration of ryanodine (1 µM) caused a small, but significant, initial decrease (~3.7%) followed by a progressive strong increase in SCL (+172%) and abolished tension. Ryanodine decreased the late DD slope without changing the early one, beautifully illustrated in Figure 6 of the paper (See Title Page of these Summaries, page 317) in overlapped traces recorded at different times of the ryanodine effect, with the last (169th) diastolic depolarization failing to generate an action potential. The authors also tested the combined action of ryanodine with either Bay K (resulting in dysrhythmic beating) or cesium (decreasing the early DD slope and hyperpolarizing late diastolic potential). The paper concluded: “Multiple mechanisms participate in subsidiary pacemaker automaticity. They include: (i) a cesium-sensitive component that contributes to a greater extent during the initial phase of diastolic depolarization; (ii) a component mediated via calcium released from the sarcoplasmic reticulum that contributes primarily during the latter half of diastolic depolarization; and (iii) possibly a direct contribution by the slow inward calcium current.”

While the exact mechanisms of Ca$^{2+}$ release-induced current remained unknown, an interesting speculation was that the discovered SR Ca$^{2+}$ release-mediated component might be similar, if not identical, to the transient inward current associated with delayed afterdepolarizations (DADs). More recent studies of the atrial pacemaker cells performed by the Lipsius group showed that disabling Ca cycling by ryanodine inhibits both diastolic Na+/Ca$^{2+}$ exchanger (NCX) current and its response to β-adrenergic stimulation. Ju and Allen discovered diastolic Ca$^{2+}$ sparks (local Ca$^{2+}$ releases, LCRs in recent terminology) in toad sinus venosus. LCRs and their NCX currents were also discovered in pacemaker cells of cat atria by Huser et al and in sinoatrial node cells by Bogdanov et al. While LCRs of the atrial cells are activated by Ca$^{2+}$ influx via T-type Ca$^{2+}$ channels, LCRs of sinoatrial node cells are not induced by the Ca$^{2+}$ current, but occur spontaneously. The diastolic LCR-activated NCX current accelerating diastolic depolarization represents a modern concept of cardiac pacemaker cell function.

1989

English novelist Daphne du Maurier, author of “Rebecca,” dies aged 82; 96 Liverpool football club supporters are crushed to death at the Hillsborough Stadium in England; and Labor union Solidarity is granted legal status in Poland
Beyond Bowditch: the convergence of cardiac chronotropy and inotropy

E. G. Lakatta

Cell Calcium. 2004;35:629-642

Undoubtedly a masterpiece, this essay written in 2004 by Lakatta reflects the author’s deep thinking and integration of knowledge culminating in a new concept of cardiac physiology. The ability of the heart to acutely beat faster and stronger was described as early as in 1872 by Bowditch, in “On the Peculiarities of Excitability Which Fibers of Cardiac Muscle Show.” This ability is central to the vertebrate survival instinct (fight-or-flight reflex). The reflex is mediated via autonomic nervous system and β-adrenergic receptors (β-AR) that regulate both excitation-contraction (EC) coupling and cardiac pacemaker function.

Analyzing the historical trends of studies of this fundamental phenomenon, Lakatta noted that one trend of studies, driven by a cult of scientists (Lakatta dubbed them “descendants of Bowditch”), had been to discover the mechanisms of cardiac myocyte performance in response to changes in rate of stimulation that was applied externally by experimenters. This major trend of cardiac research discovered the Ca2+ mechanisms of EC coupling. Mechanisms of how the heart beats faster, however, have not been addressed by the “Bowditch descendants,” but studied by another major cult of researchers, mainly electrophysiologists (“descendants of Hodgkin and Huxley”), to discover ion channels of cell surface membrane and how these channels might control the automaticity of cardiac pacemaker cells. Historically, cardiac research was specialized and basically disrupted by the reductionist approach. Based on recent data on the importance of intracellular Ca2+ for regulation of cardiac automaticity, demonstrated by his lab and others, Lakatta then sets a provocative question: “Is it possible that signals for both faster and stronger beating of the heart are transduced via common cell effectors? Sequestration and release of Ca2+ to and from internal stores is a pathway of intracellular signal transduction common to both ventricular myocytes and pacemaker cells.”

Lakatta’s conclusion is “Yes!” and available data to support this new concept are summarized. As recently shown by Huser et al in atrial pacemaker cells and by Bogdanov et al in sinoatrial node cells, local Ca2+ releases (LCRs) via Ryanodine receptors (RyRs) occurring during diastolic depolarization activate the Na+/Ca2+ exchanger (NCX) to produce an inward current that enhances their diastolic depolarization rate. In pacemaker cells, β-AR stimulation synchronizes spontaneous RyR activation during the diastolic depolarization, and augments both the LCR sizes and their resultant diastolic NCX inward current. This results in a faster beating rate of the pacemaker cells. In ventricular myocytes, β-AR stimulation synchronizes: (i) the unitary L-type Ca2+ channel activation during the action potential; (ii) activation of RyR Ca2+ releases induced by the L-type Ca2+ channels, and (iii) Ca2+ pumping loading into the SR, with the combined synchronizing effects leading to the generation of a global cytosolic Ca2+ transient of increased amplitude and accelerated kinetics. This results in a stronger contraction of the ventricular myocytes.

The paper concludes that β-AR stimulation induced synchronization of RyR activation (recruitment of additional RyRs to fire Ca2+) and of the ensuing Ca2+, and is thus a common mechanism that links both the gradations in cardiac inotropy and chronotropy. The concept of convergence of cardiac inotropy and chronotropy via the same Ca2+-dependent mechanisms must eventually unite the research of “descendants of Bowditch” and “descendants of Hodgkin and Huxley.” While arguments of electrophysiologists about the primary role of ion channels still persist (recent point-counterpoint paper of Lakatta and DiFrancesco), “Lakatta’s hypothesis” has been further supported by more recent experimental results and novel numerical modeling and formulated by Lakatta and his colleagues as “a general theory of the initiation and strength of the heart beat.”

2004

Venus passes between the Sun and the Earth, its first transit since 1882; Washington DC hosts the state funeral of former President Ronald Reagan; and a guitar owned by Eric Clapton raises $959,500 at a charity auction.
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