Calcium and Cardiac Rhythms
Physiological and Pathophysiological
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Calcium plays two pivotal roles in cardiac excitation-contraction (E-C) coupling. Ca\(^{2+}\) drives myofilament activation and carries or regulates ionic currents that are responsible for normal electrical rhythms as well as life-threatening arrhythmias. In this editorial, I will focus on Ca\(^{2+}\) and pacemaker activity and arrhythmogenesis.

Ca\(^{2+}\) entry via Ca\(^{2+}\) current (I\(_{Ca}\)) triggers sarcoplasmic reticulum (SR) Ca\(^{2+}\) release via ryanodine receptors (RyRs), and relaxation is driven by Ca\(^{2+}\) transport by the SR Ca\(^{2+}\)-ATPase and Na\(^{-}\)-Ca\(^{2+}\) exchange. Two I\(_{Ca}\) types occur in cardiac myocytes: L-type (I\(_{Ca,L}\)) activated at E\(_{m}\) > -40 mV and T-type (I\(_{Ca,T}\)) activated at E\(_{m}\) > -60 mV (near the pacemaker range). Inward I\(_{Ca,T}\) and I\(_{Ca,L}\) can contribute importantly to both normal and abnormal cardiac depolarization. I\(_{Ca,L}\) is crucial in E-C coupling in all cardiac myocytes. I\(_{Ca,L}\) is absent in most ventricular myocytes but is present in neonatal ventricular myocytes, some atrial myocytes, and in conducting and pacemaker cells. β-Adrenergic receptors (β-ARs) and cAMP-dependent protein kinase (PKA) increase I\(_{Ca,L}\) amplitude and shift activation to more negative E\(_{m}\) (closer to the pacemaker range). Parasympathetic stimulation of the heart (via muscarinic receptors) can offset the β-AR effect. Withdrawal of muscarinic activation can also cause a rebound overshoot in I\(_{Ca,L}\) and may contribute directly to postvagal tachycardia. I\(_{Ca,L}\) is rapidly inactivated by local [Ca\(^{2+}\)] at the inner channel mouth (mediated by calmodulin associated with the channel). As [Ca\(^{2+}\)] declines, I\(_{Ca,L}\) can recover partially from inactivation, even at depolarized E\(_{m}\). This can allow I\(_{Ca,L}\) reactivation before the action potential (AP) fully repolarizes, inducing early afterdepolarizations (EADs).

Resting myocytes exhibit spontaneous, localized SR Ca\(^{2+}\) release events (Ca\(^{2+}\) sparks), attributed to clusters of 6 to 20 RyRs localized at a single sarcoslemma-SR junction. During normal E-C coupling, Ca\(^{2+}\) entry via I\(_{Ca,L}\) triggers SR Ca\(^{2+}\) release (as sparks), but the temporal synchronization by the AP obscures individual Ca\(^{2+}\) sparks. Diastolic Ca\(^{2+}\) spark probability is increased by elevation of either local [Ca\(^{2+}\)] or intra-SR Ca\(^{2+}\) content. When cellular Ca\(^{2+}\) load is high, Ca\(^{2+}\) spark frequency and amplitude are high. At sufficiently high SR Ca\(^{2+}\) load, waves of Ca\(^{2+}\)-induced Ca\(^{2+}\) release propagate in myocytes. SR Ca\(^{2+}\) release can activate ionic currents that contribute to normal pacemaker activity, delayed afterdepolarizations (DADs), and triggered arrhythmias.

Na\(^{-}\)-Ca\(^{2+}\) exchange produces inward or outward current (I\(_{Na,Ca}\)) depending on E\(_{m}\) [Ca\(^{2+}\)], and [Na\(^{+}\)]. Depolarization favors Ca\(^{2+}\) influx (outward I\(_{Na,Ca}\)), but as [Ca\(^{2+}\)] rises and E\(_{m}\) repolarizes, Ca\(^{2+}\) efflux (inward I\(_{Na,Ca}\)) is more strongly favored. Moreover, high submembrane [Ca\(^{2+}\)] during I\(_{Ca}\) and SR Ca\(^{2+}\) release drives Ca\(^{2+}\) extrusion via I\(_{Na,Ca}\) at an earlier time during the AP than would be expected from the global Ca\(^{2+}\) transient. Inward I\(_{Na,Ca}\) activated by high [Ca\(^{2+}\)], can contribute to both normal pacemaker activity and arrhythmogenesis.

Ca\(^{2+}\)-Activated Currents: How Ca\(^{2+}\) Signals Change E\(_{m}\)

Three Ca\(^{2+}\)-activated currents have been reported in cardiac myocytes: I\(_{NS(Ca)}\), Ca\(^{2+}\)-activated Cl\(^{-}\} current (I\(_{Cl(Ca)}\)), and non-selective current (I\(_{NS(Ca)}\)). I\(_{NS(Ca)}\) is important in all cardiac myocytes, both as a Ca\(^{2+}\) transporter and as inward I\(_{Na,Ca}\) involved with pacemaker activity and arrhythmogenic transient inward current (I\(_{c}\)). I\(_{Cl(Ca)}\) occurs in many types of cardiac myocytes and has a low Ca\(^{2+}\) sensitivity, such that it is only activated by high local [Ca\(^{2+}\)].. The Cl\(^{-}\} reversal potential is generally near ~55 mV. Thus, I\(_{Cl(Ca)}\) would be depolarizing at E\(_{m}\) = -80 mV, have little effect around E\(_{m}\) = -55 mV, and be a repolarizing outward current at positive E\(_{m}\) during the AP. This allows I\(_{Cl(Ca)}\) to contribute to the early AP repolarizing notch (Ca\(^{2+}\)-activated transient outward current) and possibly to arrhythmogenic depolarizations. I\(_{NS(Ca)}\) would reverse near 0 mV, so like I\(_{Cl(Ca)}\), it could contribute to both repolarization and depolarization. However, there is less compelling evidence for any functional contribution of I\(_{NS(Ca)}\) in cardiac myocytes.

Ca\(^{2+}\)-activated K\(^{+}\} channels (I\(_{K(Ca)}\)) are present in many cell types but not in cardiac myocytes. Early work implicated I\(_{K(Ca)}\) as part of the transient outward current (I\(_{to}\)). However, it is now clear that I\(_{to}\) is caused by I\(_{Cl(Ca)}\) (Ca\(^{2+}\)-sensitive component) and several time- and E\(_{m}\)-dependent K\(^{+}\} channels (mainly coded by Kv4.2/4.3 and Kv1.4 genes). Thus, the main Ca\(^{2+}\)-activated currents in heart cells are I\(_{Na(Ca)}\) and I\(_{Cl(Ca)}\), which can contribute to both depolarization or repolarization.

Extracellular [Ca\(^{2+}\)] ([Ca\(^{2+}\)]\(_{o}\)) can also modify the gating of all E\(_{m}\}-dependent ion channels by reducing surface potential. High [Ca\(^{2+}\)]\(_{o}\), shifts channel activation to more positive E\(_{m}\) which typically reduces excitability. Conversely, low [Ca\(^{2+}\)]\(_{o}\), shifts activation to more negative E\(_{m}\) increasing excitability. Elevated [Ca\(^{2+}\)]\(_{o}\), can also, in principle, increase excitability, but this effect has been less well documented experimentally. These effects can shift the gating of Na\(^{+}\) and Ca\(^{2+}\) channels as much as 20 mV and thus effect excitability. Thus any inward current is more likely to activate I\(_{Na}\) or I\(_{Ca}\), when [Ca\(^{2+}\)]\(_{o}\) is low.
Ca$^{2+}$ and Normal Pacemaker Activity

Cells in the sinoatrial (SA) node and latent pacemakers in the atria, atrioventricular (AV) node, and Purkinje cells all exhibit spontaneous pacemaker activity. There is a normal hierarchy, where the fastest intrinsic pacemaker (SA node, 60 to 80/min) drives the whole heart. However, if SA-node firing frequency slows or conduction through the heart is blocked, other regions can take over (AV node ~40 to 60/min; His-Purkinje system ~20 to 30/min). This creates a functional fail-safe for activating the heart. There are also multiple cellular mechanisms involved in normal pacemaker activity (Figure 1) and these vary in different cells. This creates another type of mechanistic redundancy, such that complete failure of one channel type is unlikely to prevent pacemaker activity altogether. All of these pacemaker cells have relatively low levels of inward rectifier K$^+$ current ($I_{K1}$) compared with ventricular myocytes. $I_{K1}$ is largely responsible for stabilizing the resting E$_{m}$ near the K$^+$ equilibrium potential (E$_{K}\approx-90$ mV). Low $I_{K1}$ causes the more positive diastolic E$_{m}$ in SA- and AV-node cells and gives pacemaker cells high input impedance, such that small inward currents can cause relatively large depolarization.

Pacemaker depolarization can be caused by either increasing inward current or decreasing outward current (Figure 1). An example of the latter is a time-dependent decrease in delayed outward K$^+$ current ($I_{K}$). This can contribute to early pacemaker depolarization, especially in nodal cells where E$_{m}$ does not get very negative (so $I_{K}$ turns off slowly). The so-called pacemaker current ($I_{P}$) is a nonselective inward current (carried mostly by Na$^+$), activated during repolarization, and formed by hyperpolarization-activated cyclic nucleotide gated channels (HCN1, 2, and 4). The activation E$_{m}$ range for $I_{P}$ is progressively more negative going from SA-node to Purkinje cells to ventricular myocytes, and cAMP shifts the activation to more positive E$_{m}$. Controversy continues as to the quantitative role of $I_{P}$ in SA-node pacemaking, mainly because $I_{P}$ activation can be very slow at pacemaker E$_{m}$ in SA node. Nevertheless, this inward current undoubtedly contributes to pacemaker activity and especially so in Purkinje cells that have more negative diastolic E$_{m}$. The cAMP response also makes them a more likely contributor to sympathetic-induced chronotropy and enhanced automaticity. Although there is typically very little $I_{Na}$ available in atrial and nodal pacemaker cells (at the usual diastolic E$_{m}$), $I_{Na}$ might make a tiny contribution to pacemaker depolarization. There is also a sustained nonselective inward current ($I_{st}$) in some SA- and AV-nodal cells. $I_{st}$ activates at $-65$ mV (or more positive E$_{m}$) and inactivates only weakly, such that it may contribute during much of the pacemaker depolarization in these cells.

Both $I_{Ca,T}$ and $I_{Ca,L}$ can participate in pacemaker activity. The activation E$_{m}$ for $I_{Ca,T}$ is right in the range of the pacemaker potential, such that as depolarization proceeds, $I_{Ca,T}$ is progressively activated and inactivated. Indeed, blocking $I_{Ca,T}$ with μmol/L. Ni$^{2+}$ can slow pacemaker rates in SA-node and latent atrial pacemakers. The Ca$^{2+}$ that enters via $I_{Ca,T}$ can also trigger local SR Ca$^{2+}$ release, especially apparent in latent atrial pacemaker cells where broad subsarcolemmal SR junctions occur. This released Ca$^{2+}$ activates inward $I_{Na,Ca}$, which drives further depolarization. This may be particularly relevant late in diastolic depolarization. Inward $I_{Na,Ca}$ can also contribute to early depolarization because repolarization and high [Ca$^{2+}$], stimulate inward $I_{Na,Ca}$.

Ca$^{2+}$ sparks can also create an intrinsic rhythmicity, dependent on properties of the SR Ca$^{2+}$-ATPase and RyR. That is, after a local SR Ca$^{2+}$ release (spark), a finite time is required for local [Ca$^{2+}$], decline and reuptake into the SR (creating the driving force for another Ca$^{2+}$ spark). In addition, the RyR requires some recovery time after an initial release (Figure 1). Thus, Ca$^{2+}$ spark frequency recovers gradually after an SR Ca$^{2+}$ release. Indeed, with cellular Ca$^{2+}$ overload, myocytes can exhibit regular, stable Ca$^{2+}$ oscillations that are independent of E$_{m}$ (provided that Ca$^{2+}$ extrusion via Na$^+$/Ca$^{2+}$ exchange is blocked). β-AR activation stimulates SR Ca$^{2+}$ uptake (by PKA-dependent phosphorylation of phospholamban), and this can increase the resting Ca$^{2+}$ spark frequency, increasing diastolic depolarization rate.

In this issue of Circulation Research, Vinogradova et al show that this Ca$^{2+}$ spark-$I_{Na,Ca}$ system is very important for the basal rate of rabbit SA-nodal cells as well as the response to β-AR stimulation. They also indicated that by comparison, changes in $I_{Ca,L}$, $I_{Ca,T}$, and $I_{K}$ are less important to the isoproterenol-induced increase in SA-node cell firing. They conclude that the late diastolic Ca$^{2+}$ sparks are triggered by SR properties (rather than by $I_{Ca,T}$). The balance and timing of these various contributors to pacemaker activity is likely to vary in different cells and conditions, with different currents being more or less dominant in different cell types (eg, SA-node, latent atrial pacemakers, and Purkinje cells). This

Figure 1. Ionic currents involved in cardiac pacemaker activity. Several currents increase and/or decrease during the diastolic depolarization in cardiac myocytes (indicated by triangular shapes). See text for details and abbreviations.
reactivation of inward $\mathrm{AP}$. These factors combine to increase the likelihood of associate $\mathrm{Ca}^{2+}$/H$_{\text{9252}}$ at normal or high heart rates and especially with $\mathrm{I}_c$ and more slowly inactivating $\mathrm{I}_\mathrm{K}$. 29,30

spontaneous diastolic SR $\mathrm{Ca}^{2+}$ and delayed rectifier $\mathrm{I}_\mathrm{K}$. It should also be noted that a spontaneous diastolic SR $\mathrm{Ca}^{2+}$ release normally activates inward $\mathrm{I}_{\text{NaCa}}$ and $\mathrm{Ca}^{2+}$ extrusion. This may be a physiological part of pacemaker activity (and can serve to reduce $\mathrm{Ca}^{2+}$ overload), but it also creates arrhythmogenic $\mathrm{I}_c$ or delayed afterdepolarizations (DADs) in ventricular myocytes. Although we know several key contributors to cardiac pacemaker activity, there is likely to be tremendous heterogeneity, making absolute pronouncements of dominant mechanisms a continuing challenge.

$\mathrm{Ca}^{2+}$ and Triggered Cardiac Arrhythmias
Ventricular tachycardia (VT) is an immediate precursor of ventricular fibrillation and a major cause of sudden death in heart failure (HF). Three-dimensional mapping studies indicate that most VT in human HF initiates by nonreentrant mechanisms, especially in nonischemic HF.25 Triggered arrhythmias (DADs and EADs) are major initiators of VT. EADs are secondary depolarizations that occur before full AP repolarization (Figure 2). EADs are more common with long AP durations, during bradycardia and in patients with long-QT syndrome, where congenital mutations in specific ion channels have been directly implicated.26 In HF, there are reductions in $\mathrm{K}^+$ channel expression ($\mathrm{I}_{\text{KCa}}$, $\mathrm{I}_\mathrm{K1}$, and perhaps $\mathrm{I}_\mathrm{K}$) and more slowly inactivating $\mathrm{I}_{\text{NaCa}}$ and these cause AP prolongation.27,28 The smaller $\mathrm{Ca}^{2+}$ transients in HF may also cause less complete $\mathrm{I}_c$ inactivation during the early phases of the AP. These factors combine to increase the likelihood of reactivation of inward $\mathrm{I}_{\text{CaL}}$ late in the AP (a most likely cause of EADs).29,30

DADs initiate after repolarization and are caused by SR $\mathrm{Ca}^{2+}$ release and consequent $\mathrm{Ca}^{2+}$-activated $\mathrm{I}_c$. They are associated with cellular $\mathrm{Ca}^{2+}$ overload and are more common at normal or high heart rates and especially with $\beta$-AR activation. This makes sense because $\beta$-AR activation increases $\mathrm{I}_{\text{CaL}}$ and SR $\mathrm{Ca}^{2+}$ uptake. This tends to load the SR with $\mathrm{Ca}^{2+}$, overcoming the low SR $\mathrm{Ca}^{2+}$ load typical in HF (which contributes to the poor contractile function).3,31 In HF, Na$^+$-$\mathrm{Ca}^{2+}$ exchange is increased and $\mathrm{I}_\mathrm{K1}$ is decreased. This means that a given SR $\mathrm{Ca}^{2+}$ release in HF will produce more $\mathrm{I}_c$ (more inward $\mathrm{I}_{\text{NaCa}}$). Ventricular $\mathrm{I}_c$ and DADs are due almost entirely to $\mathrm{I}_{\text{NaCa}}$ (versus $\mathrm{I}_{\text{CaL}}$ or $\mathrm{I}_{\text{IbCa}}$).3 Further, any given $\mathrm{I}_c$ will produce a greater DAD because there is less $\mathrm{I}_{\text{K1}}$ to stabilize resting $E_m$. Thus, only half as much SR $\mathrm{Ca}^{2+}$ release is required in HF to cause a DAD that reaches the threshold to trigger an arrhythmogenic AP.3 Indeed, this arrhythmogenic mechanism in ventricle is similar to the pacemaker mechanism in SA node described by Vinogradova et al.2 Thus, $\mathrm{I}_c$ and $\mathrm{I}_{\text{NaCa}}$ are centrally important in the genesis of life-threatening arrhythmias as well as in normal pacemaker activity in the heart.

In conclusion, there are multiple ways in which $\mathrm{Ca}^{2+}$ alters cellular cardiac rhythms (normal and abnormal). Traditionally, there has been some segregation between investigation of cardiac rhythms/arrhythmias, myocyte $\mathrm{Ca}^{2+}$ regulation, and cardiac mechanics. These perspectives must be merged to develop a modern, comprehensive understanding of how the heart works.

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