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Intracellular Ca\(^{2+}\) is the central regulator of cardiac contractility. Moreover, it is becoming increasingly apparent that alterations in myocyte Ca\(^{2+}\) regulation may be critically important in both the mechanical dysfunction and arrhythmogenesis associated with congestive heart failure.\(^1,2\) Thus, it is imperative to have a clear and relatively quantitative understanding of how cellular Ca\(^{2+}\) levels are regulated during the normal contraction-relaxation cycle. The scope and relevant references in this field are far too large for this format, so my focus here is narrower and more personal than elsewhere.\(^3-5\)

Figure 1A shows the key pathways involved in myocyte Ca\(^{2+}\) transport. During the cardiac action potential (AP) L-type Ca\(^{2+}\) channels are activated and Ca\(^{2+}\) enters the cell via Ca\(^{2+}\) current (\(I_{Ca}\)) and also a much smaller amount enters via Na\(^{+}-Ca^{2+}\) exchange (NCX). Ca\(^{2+}\) influx triggers Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) and, to some extent, can also contribute to activation of the myofilaments directly. The Ca\(^{2+}\) entry plus the amount released from the SR via Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) raises cytosolic free [Ca\(^{2+}\)] (\([\text{Ca}^{2+}]_c\)) and also a much smaller amount enters via Na\(^{+}-Ca^{2+}\) exchange (NCX). Ca\(^{2+}\) influx triggers Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) and, to some extent, can also contribute to activation of the myofilaments directly. The Ca\(^{2+}\) entry plus the amount released from the SR via Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) raises cytosolic free [Ca\(^{2+}\)] (\([\text{Ca}^{2+}]_c\)), causing Ca\(^{2+}\) binding to multiple cytosolic Ca\(^{2+}\) buffers. One of the most functionally important cytosolic Ca\(^{2+}\) buffers is the thin-filament protein troponin C (TnC). When Ca\(^{2+}\) binds to TnC, it switches on the myofilaments in a cooperative manner activating contraction. For relaxation and diastolic filling to occur, [Ca\(^{2+}\)] must decline such that Ca\(^{2+}\) dissociates from TnC, thereby turning off the contractile machinery. Four Ca\(^{2+}\) transporters remove Ca\(^{2+}\) from the cytosol: (1) SR Ca\(^{2+}\)-ATPase, (2) sarcolemmal NCX, (3) sarcolemmal Ca\(^{2+}\)-ATPase, and (4) mitochondrial Ca\(^{2+}\) uniporter. The SR Ca\(^{2+}\)-ATPase and NCX are most important quantitatively.

Data on Ca\(^{2+}\) binding, functional effects, and transport from multiple laboratories and with different experimental approaches allow consideration of Ca\(^{2+}\) cycling in relatively quantitative terms. Although these numbers will continue to be refined, they are useful to consider. For consistency, cellular Ca\(^{2+}\) will be discussed below in units of \(\mu\)mol/L cytosol (where cytosol is \(\approx 65\%\) of cell volume and excludes mitochondrial volume that is \(\approx 30\%\) of cell volume).\(^3\)

Ca\(^{2+}\) Requirements for Activation of Myofilaments

How much Ca\(^{2+}\) is required to activate the myofilaments to produce contraction? Usually myofilament response to Ca\(^{2+}\) is shown as a function of free [Ca\(^{2+}\)], (Figure 1B, inset), and the myofilaments respond cooperatively to [Ca\(^{2+}\)]. Indeed, in intact ventricular muscle, the half-activating [Ca\(^{2+}\)] is \(\approx 600\) nmol/L with a Hill coefficient \(\approx 4.6.6-7\) This is very important information, and we know that myofilament Ca\(^{2+}\) sensitivity can be decreased by protein kinase A (PKA) phosphorylation of TnI, low pH, and reduced sarcomere length. Indeed, increased myofilament Ca\(^{2+}\) sensitivity at longer sarcomere lengths is crucial in Starling’s law of the heart, whereby...
increased diastolic filling results in stronger ventricular contraction.

The \([Ca^{2+}]\) dependence of force, however, does not indicate how much total \([Ca^{2+}]\) is required to activate the myofilaments. This issue is complicated by the fact that there are many other \(Ca^{2+}\)-binding moieties in the cell that are in dynamic competition with TnC. Indeed, \([Ca^{2+}]\) is heavily buffered such that it takes \(100\, \text{mmol} \text{L}^{-1}\) cytosol to raise \([Ca^{2+}]\) from a diastolic level of \(100\, \text{nmol} \text{L}^{-1}\) to a peak systolic level of \(1\, \text{mmol} \text{L}^{-1}\). Figure 1B incorporates the titration of the other cellular \(Ca^{2+}\) buffers (including \(70\, \text{nmol} \text{L}^{-1}\) regulatory TnC and \(47\, \text{nmol} \text{L}^{-1}\) SR \(Ca^{2+}\)-ATPase sites per L cytosol). This shows the amount of total \(Ca^{2+}\) influx plus release that is required for a given level of contractile force, starting from diastolic \([Ca^{2+}]\). Little force is developed below \(30\, \text{nmol} \text{L}^{-1}\), but then the curve becomes much steeper. During a typical twitch (at \(23^\circ\text{C} \text{ to } 30^\circ\text{C}\)), contractile force reaches \(40\%\) of maximum, and this would require \(60\, \text{nmol} \text{L}^{-1}\) cytosol, and \([Ca^{2+}]\), would be \(540\, \text{nmol} \text{L}^{-1}\). So where does this \(\Delta[Ca^{2+}]\) go during relaxation?

**Figure 1.** \(Ca^{2+}\) transport and requirements for activation of myofilament force. A, Schematic diagram of cellular \(Ca^{2+}\) fluxes. B, \(Ca^{2+}\) requirements for contractile activation, based on diastolic \([Ca^{2+}]\)\(=150\, \text{nmol} \text{L}^{-1}\) and total cytosolic \(Ca^{2+}\) buffering\(=244/(1+673/[Ca^{2+}])\). This includes TnC (\(Ca^{2+}\) and \(Ca^{2+}\)-\(Mg^{2+}\) sites), myosin, SR \(Ca^{2+}\)-ATPase, calmodulin, ATP, creatine phosphate, and sarcolemmal sites. Force is also shown as a function of \([Ca^{2+}]\) in inset \(\text{Force}=100/[1+(600/[Ca^{2+}])^4]\). For more details, see Bers.3

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**Ca^{2+}** Removal From the Cytosol During Cardiac Relaxation

For relaxation and ventricular filling to occur, the \(Ca^{2+}\) that activated the myofilaments must be removed from the cytosol by the 4 \(Ca^{2+}\) transport systems mentioned above. We analyzed the contributions of each system in quantitative detail by selective inhibition of each transporter during myocyte relaxation and \([Ca^{2+}]\) decline.12-15 SR \(Ca^{2+}\) uptake was prevented by either thapsigargin or 10 mmol/L caffeine, NCX was prevented by complete removal of extracellular Na\(^+\) and Ca\(^{2+}\), sarcolemmal (SL) \(Ca^{2+}\)-ATPase and mitochondrial \(Ca^{2+}\) uniporter (Mito). Percentages indicate the fraction of the total cytosolic \(Ca^{2+}\) removal attributable to each system when they dynamically interact in the cell. Data in panels A and B are from Bassani et al.12 For panel C, rabbit heart failure (HF), the \(V_\text{max}\) for NCX was increased 116% and SR \(Ca^{2+}\)-ATPase was reduced by 24% as indicated by Pogwizd et al.2

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**Figure 2.** Integrated \(Ca^{2+}\) fluxes during twitch relaxation in rabbit and rat ventricular myocytes. Free \([Ca^{2+}]\) during twitch relaxation was used as a driving function to calculate \(Ca^{2+}\) flux via each system, using the \([Ca^{2+}]\) dependence of transport rates measured for each system studied in isolation. SR is SR \(Ca^{2+}\)-ATPase, and the slow systems are a combination of sarcolemmal (SL) \(Ca^{2+}\)-ATPase and mitochondrial \(Ca^{2+}\) uniporter (Mito). Percentages indicate the fraction of the total cytosolic \(Ca^{2+}\) removal attributable to each system when they dynamically interact in the cell. Data in panels A and B are from Bassani et al.12 For panel C, rabbit heart failure (HF), the \(V_\text{max}\) for NCX was increased 116% and SR \(Ca^{2+}\)-ATPase was reduced by 24% as indicated by Pogwizd et al.2

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transport rate (in \( \mu \text{mol/L cytosol/sec} \)) is plotted to define \( \text{Ca}^{2+} \) influx as a function of \([\text{Ca}^{2+}]_i\), for each system. Then the normal \([\text{Ca}^{2+}]_i\), transient can be used as a driving function, and \( \text{Ca}^{2+} \) transport by each system can be calculated. Figure 2A shows that in rabbit ventricular myocytes, the SR \( \text{Ca}^{2+} \)-ATPase removes 70% of the activator \( \text{Ca}^{2+} \) from the cytosol, whereas the NCX removes 28%, with only \( \approx 1\% \) each for the sarcosomemal \( \text{Ca}^{2+} \)-ATPase and mitochondrial \( \text{Ca}^{2+} \) uniporter (referred to collectively as the slow systems). In rat ventricle, the SR \( \text{Ca}^{2+} \)-ATPase activity is higher (presumably due to more pump molecules per unit cell volume)\(^{16}\) and \( \text{Ca}^{2+} \) removal via NCX is less, resulting in a balance of 92:7:1% for SR \( \text{Ca}^{2+} \)-ATPase, NCX, and slow systems (Figure 2B). In mouse ventricle, the situation is quantitatively similar to rat, whereas the balance of \( \text{Ca}^{2+} \) fluxes in guinea pig, ferret, and human ventricle are more similar to rabbit.\(^{13–15,18,19}\) The total amount of \( \text{Ca}^{2+} \) transported during \([\text{Ca}^{2+}]_i \) decline in both rabbit and rat ventricular myocytes is similar to the 60 \( \mu \text{mol/L} \) cytosol discussed above for \( \text{Ca}^{2+} \) requirements of contractile activation.

In failing versus nonfailing human heart, there is also typically a reduction in SR \( \text{Ca}^{2+} \)-ATPase expression\(^{20,21}\) and an increase in NCX expression.\(^{22–24}\) This would shift the balance of \( \text{Ca}^{2+} \) fluxes during relaxation in favor of \( \text{Ca}^{2+} \) extrusion via NCX and reduce SR \( \text{Ca}^{2+} \) uptake. Thus, in the failing human (and rabbit) heart, the SR \( \text{Ca}^{2+} \)-ATPase and NCX may contribute nearly equally to \([\text{Ca}^{2+}]_i \), decline (Figure 2C) as opposed to a 2- to 3-fold dominance of the SR in the normal heart. This shift in competition from the SR \( \text{Ca}^{2+} \)-ATPase toward NCX will also tend to limit SR \( \text{Ca}^{2+} \) loading in heart failure. Such a limitation of SR \( \text{Ca}^{2+} \) loading could also contribute to the mechanical dysfunction in heart failure.\(^{24}\) In addition, the SR \( \text{Ca}^{2+} \) ATPase transports two \( \text{Ca}^{2+} \) ions per ATP consumed, whereas extrusion by NCX only pumps one \( \text{Ca}^{2+} \) per ATP used (indirectly, to pump out the 3 \( \text{Na}^+ \) ions via \( \text{Na}^+/\text{K}^+ \)-ATPase which had entered in exchange for one \( \text{Ca}^{2+} \) via NCX). Thus, transsarcolemmal \( \text{Ca}^{2+} \) cycling is energetically more expensive than SR transport.

**Figure 3.** \( \text{Ca}^{2+} \) influx during AP in rabbit ventricular myocyte. A, APs measured under physiological conditions were used as a voltage-clamp command with all other currents blocked (including \( I_{\text{Ca}} \)). Contraction and \( I_{\text{Ca}} \) were measured during 10 pulses approaching steady state (starting after SR \( \text{Ca}^{2+} \) had been depleted by caffeine exposure, 25°C C). B, Derivative of the difference current (versus pulse 1 where there was no SR \( \text{Ca}^{2+} \) release), indicative of the rate of SR \( \text{Ca}^{2+} \) release sensed by the \( \text{Ca}^{2+} \) channel (25°C). C, Integrated \( I_{\text{Ca}} \) during APs as the SR reloads at 25°C and 35°C. Inset shows steady-state \( I_{\text{Ca}} \) during AP at both temperatures (data from Puglisi et al).\(^{34}\)**

**\( \text{Ca}^{2+} \) Influx During the Cardiac AP**

If 28% of the activator \( \text{Ca}^{2+} \) in rabbit is extruded from the rabbit myocyte by NCX at a steady-state twitch, there must be a similar amount of \( \text{Ca}^{2+} \) entry (eg, via \( I_{\text{Ca}} \)) at each beat. Otherwise, there would be progressive loss or gain of cellular \( \text{Ca}^{2+} \) (ie, this would not be a steady state). Indeed, during an AP in rabbit, there is more \( \text{Ca}^{2+} \) entry via \( I_{\text{Ca}} \) and less SR \( \text{Ca}^{2+} \) release than in rat.\(^{3,25}\) \( \text{Ca}^{2+} \) influx via NCX during a rabbit AP is \( \approx 10 \mu \text{mol/L} \) cytosol in cells where SR \( \text{Ca}^{2+} \) load (based on integration of NCX current; see Figure 4A) was 87 \( \mu \text{mol/L} \) cytosol.\(^{26}\) For a fractional SR \( \text{Ca}^{2+} \) release during the twitch of 43%,\(^{27}\) this indicates an SR \( \text{Ca}^{2+} \) release of 37 \( \mu \text{mol/L} \) cytosol or 77% of activator \( \text{Ca}^{2+} \) from SR versus 23% for \( I_{\text{Ca}} \). Similar estimations in rat yielded 92% SR \( \text{Ca}^{2+} \) release and 8% \( \text{Ca}^{2+} \) entry via \( I_{\text{Ca}} \) (with total activator \( \text{Ca}^{2+} \approx 80 \mu \text{mol/L} \) cytosol).\(^{25,28}\) These values agree remarkably well with those for \( \text{Ca}^{2+} \) removal above (based on \([\text{Ca}^{2+}]_i \), decline), especially considering differences in methodology and inherent assumptions. Thus, although these quantitative estimates continue to be refined, we are getting an increasingly clear picture of exactly how many \( \text{Ca}^{2+} \) ions are going where, and when in ventricular myocytes.

\( I_{\text{Ca}} \) is subject to \( \text{Ca}^{2+} \)-dependent inactivation, and this is readily appreciated by the faster current inactivation when \( \text{Ca}^{2+} \) is the charge carrier versus \( \text{Ba}^{2+} \).\(^{5}\) \( \text{Ca}^{2+} \) entering the cell through the channel produces this effect very locally, because it cannot be abolished even by high intracellular \( \text{Ca}^{2+} \) buffering with EGTA or BAPTA. Recent studies have also shown that calmodulin, which may be bound to the carboxy terminal, mediates \( \text{Ca}^{2+} \)-dependent inactivation.\(^{29–31}\)

In addition to \( \text{Ca}^{2+} \) entering the cell, SR \( \text{Ca}^{2+} \) release can also contribute importantly to this \( \text{Ca}^{2+} \)-dependent inactivation.\(^{32,33}\) This is because SR \( \text{Ca}^{2+} \) is released into the same restricted junctional space where most of the L-type \( \text{Ca}^{2+} \) channels probably reside (see SR-sarcolemmal junction in Figure 1A). Figure 3A shows how the \( I_{\text{Ca}} \) time course during an AP changes when there is no SR \( \text{Ca}^{2+} \) release (small pulse 1 contraction after SR \( \text{Ca}^{2+} \) depletion) and as the SR \( \text{Ca}^{2+} \) release gradually recovers to steady state (pulse 10).\(^{34}\)
analysis of this difference current (Figure 3B) suggested that the rate of local SR Ca\(^{2+}\) release (as sensed by the Ca\(^{2+}\) channel) was maximal in \(~5\) ms at 25°C and 2 to 3 ms at 35°C. This is fast compared with fluorescence changes from indicators that are distributed throughout the cytosol. These times are similar to the time to peak \(I_{Ca}\), which emphasizes that there is very little delay between \(I_{Ca}\) and SR Ca\(^{2+}\) release. As the SR refills with Ca\(^{2+}\) and contractions approach steady state (from pulse 1 to 10), this SR Ca\(^{2+}\) release--dependent inactivation of \(I_{Ca}\) causes the integrated Ca\(^{2+}\) influx via \(I_{Ca}\) to decrease by \(~50\%\) (Figure 3C). Thus, SR Ca\(^{2+}\) release creates a negative feedback on Ca\(^{2+}\) influx, such that when there is ample SR Ca\(^{2+}\) release, further Ca\(^{2+}\) influx is turned off. Of course, Ca\(^{2+}\) entry via \(I_{Ca}\) also participates in this feedback, and much of the inactivation of \(I_{Ca}\) at pulse 1 is probably due to inactivation that is Ca\(^{2+}\) influx--dependent (versus voltage--dependent). Figure 3C also shows that the integrated \(I_{Ca}\) behaves similarly at both 25°C and 35°C. Although at 35°C peak \(I_{Ca}\) is much larger, \(I_{Ca}\) inactivation is also faster, resulting in nearly the same \(I_{Ca}\) integral as at 25°C.

The foregoing discussion has not considered Ca\(^{2+}\) influx via NCX (ie, outward \(I_{Na/Ca}\)). The direction of \(I_{Na/Ca}\) depends on the concentrations of Na\(^{+}\) and Ca\(^{2+}\) on both sides of the membrane and also on membrane potential (\(E_m\)). Indeed, like ion channels, \(I_{Na/Ca}\) has a reversal potential (\(E_{Na/Ca} = -3E_{Na} - 2E_{Ca}\), where \(E_{Na}\) and \(E_{Ca}\) are equilibrium or Nernst potentials for Na\(^{+}\) and Ca\(^{2+}\)). At \(E_{Na/Ca}\), the energy in the [Na\(^{+}\)] and [Ca\(^{2+}\)] gradients is exactly balanced such that no net Ca\(^{2+}\) transport occurs. In a resting cardiac myocyte, \(E_{Na/Ca}\) is typically \(-40\) mV. When \(E_{Na} > E_{Na/Ca}\), Ca\(^{2+}\) influx via \(I_{Na/Ca}\) is favored thermodynamically. Thus, at the upstroke of the AP, Ca\(^{2+}\) entry is thermodynamically favored and outward \(I_{Na/Ca}\) is expected. If one does not consider spatial [Ca\(^{2+}\)] gradients and calculates outward \(I_{Na/Ca}\) with the useful equation described by Luo and Rudy,\(^{35}\) one could infer Ca\(^{2+}\) influx of 0.3 to 1 \(\mu\)mol/L cytosol. However, as \(I_{Ca}\) and SR Ca\(^{2+}\) release activate rapidly and raise local [Ca\(^{2+}\)], very rapidly (especially near the membrane), this changes \(E_{Na}\) and \(E_{Na/Ca}\) and greatly limits Ca\(^{2+}\) entry via NCX. Inclusion of these local [Ca\(^{2+}\)] concentrations might reduce the amount of Ca\(^{2+}\) entry expected during a normal AP to \(\leq 0.2\) \(\mu\)mol/L cytosol, concentrated in the first 1 to 3 ms of the AP. This is negligible in comparison to the \(~10\) \(\mu\)mol/L cytosol Ca\(^{2+}\) entry via \(I_{Ca}\).

The amount of Ca\(^{2+}\) influx via \(I_{Na/Ca}\) can be increased greatly when [Na\(^{+}\)] is elevated (eg, in response to digitals glycosides) and also if SR Ca\(^{2+}\) release and/or \(I_{Ca}\) is inhibited. Moreover, if the AP is very long and [Ca\(^{2+}\)], declines at plateau potentials, then \(I_{Na/Ca}\) can produce additional late Ca\(^{2+}\) influx.\(^{36}\) There is also work that suggests that Ca\(^{2+}\) entry via NCX can trigger SR Ca\(^{2+}\) release.\(^{37-40}\) Although this may not be important under normal physiological conditions because of the dominant role of \(I_{Ca}\),\(^{41,42}\) it may become more important when [Na\(^{+}\)] is elevated, \(I_{Ca}\) is depressed, or NCX expression is elevated.

**SR Ca\(^{2+}\) Load and Release**

SR Ca\(^{2+}\) load can be raised by increasing Ca\(^{2+}\) influx or decreasing Ca\(^{2+}\) efflux (eg, elevated stimulation frequency, AP duration, \(I_{Ca}\) [Ca\(^{2+}\)], [Na\(^{+}\)], or reduced [Na\(^{+}\)]) . Stimulation of the SR Ca\(^{2+}\)-ATPase (by phospholamban phosphorylation or gene knockout) also increases SR Ca\(^{2+}\) load as well as speeding relaxation and [Ca\(^{2+}\)] decline.\(^{43}\) In heart failure, the combination of reduced SR Ca\(^{2+}\)-ATPase and elevated NCX may contribute to the lower SR Ca\(^{2+}\) load observed,\(^{2,24}\) because the SR Ca\(^{2+}\)-ATPase would compete less effectively with NCX for Ca\(^{2+}\) during relaxation and diastole.

High SR Ca\(^{2+}\) load increases the amount of Ca\(^{2+}\) available for release, but it can also dramatically increase the fraction of SR Ca\(^{2+}\) that is released for a given \(I_{Ca}\) trigger.\(^{27,45}\) This latter effect may be attributable to a stimulatory effect of high intra-SR [Ca\(^{2+}\)] on ryanodine receptor open probability.\(^{46,47}\) This effect of luminal SR [Ca\(^{2+}\)] may also contribute to the apparently spontaneous SR Ca\(^{2+}\) release observed with cellular Ca\(^{2+}\) overload. This is the basis of aftercontractions, transient inward current, and delayed afterdepolarizations that can trigger arrhythmias. At moderately low SR Ca\(^{2+}\) load, CICR appears to fail.\(^{27,45}\) This property may help the SR reload if it becomes relatively depleted, and it could even contribute dynamically to the turnoff of SR Ca\(^{2+}\) release during excitation-contraction coupling (ECC) (see review published earlier in this series\(^{16}\)).

Measuring SR Ca\(^{2+}\) load online is less direct than [Ca\(^{2+}\)], \(I_{Ca}\), or force. One useful approach is to rapidly apply caffeine (10 to 20 mmol/L), which releases all SR Ca\(^{2+}\) and prevents net uptake because of open SR Ca\(^{2+}\) release channels. Then, quantitative measures of SR Ca\(^{2+}\) load can be obtained from the amplitude of contraction or \(\Delta [Ca^{2+}]\), or by integrating \(I_{Na/Ca}\) (given that most of the SR Ca\(^{2+}\) is removed from the cell this way, see Figure 4A). Maximal SR Ca\(^{2+}\) content under relatively physiological conditions is \(~100\) \(\mu\)mol/L cytosol or about twice the amount of Ca\(^{2+}\) required to activate a twitch.\(^{26,49,50}\) Rapid cooling contractures (RCCs) are also useful for assessing SR Ca\(^{2+}\), especially in multicellular preparations where slow caffeine diffusion to all the cells limits the utility of the caffeine approach.\(^{18,19}\) Cooling to \(~0°C\) inhibits Ca\(^{2+}\) pumping and also causes rapid SR Ca\(^{2+}\) release (presumably due to very long ryanodine receptor openings\(^{25}\)). Then, one can measure either \(\Delta [Ca^{2+}]\), or contractile force (which develops slowly at 0°C). This technique is less quantitative concerning absolute amounts of Ca\(^{2+}\) but is useful for measuring changes in SR Ca\(^{2+}\) content under different conditions (Figure 4B).

**Restitution and Force-Frequency Relationship**

During CICR, SR Ca\(^{2+}\) release turns off because the channel either inactivates\(^{52,53}\) or adapts to the high local [Ca\(^{2+}\)].\(^{44}\) Thus, recovery from this state requires time and low [Ca\(^{2+}\)], to return to its normal resting Ca\(^{2+}\) sensitivity. There is a fast phase of restitution after an AP and twitch (\(\tau = 50\) to 300 ms), which is probably due to recovery of \(I_{Ca}\), availability of Ca\(^{2+}\) in the SR, and partial recovery of ryanodine receptors. Some early ECC models included slow diffusion of SR Ca\(^{2+}\) from uptake sites to release sites\(^{35}\) in explaining rest potentiation, but this diffusion should be very fast (\(~1\) to 2 ms), and we now know that longer times are required for Ca\(^{2+}\) release channel recovery.\(^{3,52-54,56}\) There is also a much slower phase of ECC restitution (\(\tau = 5\) to 15 seconds), which is largely respon-
Increased fractional SR Ca\(^{2+}\) release, and these species normally exhibit more prominent failing (F) and nonfailing (NF) hearts are from Pieske et al.\(^{19}\) those conditions (force normalized to that at 0.2 Hz). Data from and mouse SR Ca\(^{2+}\) rest is prolonged, SR Ca\(^{2+}\) preparation that normally exhibits marked rest decay.\(^57\) Rat inertent, long-lasting rest potentiation in rabbit myocytes, a modified NCX expression in the failing heart.\(^{19}\) Notably, this post–rest potentiation can occur without any increase in SR Ca\(^{2+}\) load, \(I_{\text{Ca}}\), or AP duration. Indeed, SR Ca\(^{2+}\) content can be declining while twitch SR Ca\(^{2+}\) release is increasing for a given \(I_{\text{Ca}}\) trigger (ie, increased fractional release).\(^{19,56}\) As rest is prolonged, SR Ca\(^{2+}\) load decreases in rabbit, guinea pig, ferret, and failing human ventricle. This is because as Ca\(^{2+}\) leaks from the SR, some fraction is extruded by NCX rather than being pumped back into the SR. This resting SR Ca\(^{2+}\) loss is the basis of rest decay of twitch amplitude in these species and can be prevented by blocking NCX by 0Na–0Ca solution. In fact, blocking NCX produces prominent, long-lasting rest potentiation in rabbit myocytes, a preparation that normally exhibits marked rest decay.\(^{57}\) Rat and mouse SR Ca\(^{2+}\) content does not decline much with rest, and these species normally exhibit more prominent and longer-lasting rest potentiation (reflecting mainly the increased fractional SR Ca\(^{2+}\) release as ECC recovers completely). Thus, for a given \(I_{\text{Ca}}\), the main factors that determine SR Ca\(^{2+}\) release are the amount of SR Ca\(^{2+}\) available and the fraction of SR Ca\(^{2+}\) released (which depends on recent history).

Increasing frequency alters SR Ca\(^{2+}\) release in two major ways: (1) It increases SR Ca\(^{2+}\) load (due to more frequent \(I_{\text{Ca}}\) influx and less time for extrusion by NCX), and (2) there is less time for the ryanodine receptor to recover from inactivated or adapted states. In rat and mouse ventricular muscle, the SR is often found to be relatively full even at very low stimulation frequency, possibly a consequence of relatively high [Na\(^{+}\)], which limits Ca\(^{2+}\) extrusion via NCX.\(^{58}\) Thus, increasing frequency in rat or mouse usually causes little or no further increase in SR Ca\(^{2+}\), such that the dominant frequency-dependent effect is the encroachment into full recovery time of ECC. Thus, rat and mouse myocytes often show negative force-frequency relationships. If, for some reason, the rat cells start with lower SR Ca\(^{2+}\) at low frequency, then a positive force-frequency relationship can also be seen (with increasing [Na\(^{+}\]), and SR Ca\(^{2+}\)).\(^{59}\) In most other species (rabbit, guinea pig, ferret, and nonfailing human), the force-frequency relationship is normally positive and coincides with dramatic increases in SR Ca\(^{2+}\) content (Figure 4B). In these cases, the increase in SR Ca\(^{2+}\) is more than enough to compensate for a reduction in fractional release at high frequency. In the failing human heart, the SR Ca\(^{2+}\) content increases only slightly with increasing frequency, mostly between 0.2 and 1 Hz. Although this is associated with some increase in twitch force, SR Ca\(^{2+}\) does not increase further at higher frequency, and so the relationship there is dominated by the intrinsic depressant effect of higher frequency on fractional SR Ca\(^{2+}\) release. This results in a flat or negative force-frequency relationship (Figure 4B). This would, of course, limit the functional reserve of the failing human heart and could be a direct consequence of reduced levels of SR Ca\(^{2+}\)-ATPase and increased levels of NCX expression in the failing heart.\(^{19}\)

In conclusion, Ca\(^{2+}\) in cardiac myocytes is in a dynamic yet delicate balance, and the interaction of numerous cellular processes orchestrates many aspects of cardiac function at the cellular level. Many of these systems are also subject to many regulatory influences (not discussed here). The result is a rich variation in functional behavior that allows the heart to function effectively, but this also continues to pose many challenges to understanding this complex system under diverse conditions. Important remaining questions include the molecular mechanism of ECC, how the release channel is regulated physiologically, how alterations in Ca\(^{2+}\) handling in disease states lead to mechanical dysfunction and arrhythmias, and what are the best molecular targets for therapeutic strategies.

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