Cardiac Excitation-Contraction Coupling

Fotios G. Pitoulis and Cesare M. Terracciano

6.1 Introduction to Excitation-Contraction Coupling – 62

6.2 Ca^{2+} Influx – 62
6.2.1 L-Type Ca^{2+} Channels (LTCCs) – 62
6.2.2 Na^{+}-Ca^{2+} Exchanger (NCX) – 63

6.3 Ca2+ Efflux – 65
6.3.1 SERCA Protein – 65
6.3.2 NCX – 66
6.3.3 Other Extrusion Mechanisms – 67

6.4 Excitation-Contraction Coupling – 68
6.4.1 Calcium Sparks – 68
6.4.2 Calcium-Induced Calcium Release – 68

6.5 What We Don't Know – 70
6.5.1 Ca^{2+} Spark Theories Interlude – 70
6.5.2 CICR Termination – Stopping the Domino Effect – 71

6.6 Where We're Heading – 72
6.6.1 ECC as a Measure of Cardiac Maturity – 72
6.6.2 ECC in Pathology – 73

References – 74

© Springer Nature Switzerland AG 2019
C. Terracciano, S. Guymer (eds.), *Heart of the Matter*, Learning Materials in Biosciences,
https://doi.org/10.1007/978-3-030-24219-0_6

What You Will Learn in This Chapter

This chapter will provide you with an understanding of the regulation of Ca^{2+} in the myocardium, its physiological implication as well as its role in orchestrating myocardial contraction. The chapter explores the processes of excitation-contraction coupling (ECC) and calcium-induced calcium release (CICR) whilst appreciating the relevance of ECC in pathology and in engineering heart tissue.

Learning Objectives

- Understand the molecular mechanism that underlie calcium-induced calcium release
- Appreciate and assess the different theories for the calcium-induced calcium release termination process
- Be able to discuss the issues with calcium handling in induced pluripotent stem cells

6.1 Introduction to Excitation-Contraction Coupling

Of the array of ions involved in the workings of the heart, calcium (Ca^{2+}) is perhaps the most important [1]. During the cardiac action potential, Ca^{2+} entry through the sarcolemmal Ca^{2+} channels stimulates Ca^{2+} release from the sarcoplasmic reticulum (SR), causing a rise in cytosolic Ca^{2+} and the subsequent activation of troponin on myofilaments (see ► Chap. 10), resulting in the development of force to eject blood out of the ventricles [2]. The process that links myocyte electrical excitation to contraction is known as excitation-contraction coupling (ECC). Appreciating ECC is crucial as it forms the basis of physiology, is dysregulated in almost all pathology and acts as a marker of the robustness of novel experimental cardiac models such as stem cell-derived cardiomyocytes [1, 3–5].

In each heartbeat, the cytoplasmic Ca^{2+} concentration of a healthy cardiomyocyte (CM) oscillates from ≅100 nM to 1 μM [6]. Precise Ca^{2+} regulation is a matter of life and death, and improper cytoplasmic Ca^{2+} rise and/or removal can lead to defective systole and diastole, respectively (known as systolic and diastolic dysfunction).

6.2 Ca^{2+} Influx

During the ventricular action potential, influx of Ca^{2+} from the extracellular to subsarcolemmal space generates a Ca^{2+} current, known as I_{Ca}, which triggers Ca^{2+} release from the SR, mediated by SR-release channels known as ryanodine receptors (RyRs). I_{Ca} occurs in two main ways [5]:

- Voltage-sensitive sarcolemmal Ca^{2+} channels (LTCCs)
- Na^{+}-Ca^{2+} exchanger (NCX)

6.2.1 L-Type Ca^{2+} Channels (LTCCs)

LTCCs are activated by the initial membrane potential (V_m) depolarisation cause by the opening of voltage-gated Na^{+} channels [1]. Following LTCC opening, deactivation occurs by both time-dependent, V_m-dependent, and cytosolic calcium ($[Ca^{2+}]_i$)-dependent mechanisms [5]. The V_m-dependent inactivation of the LTCCs can be demonstrated by administering depolarisation pulses and measuring LTCC inactivation kinetics [8].

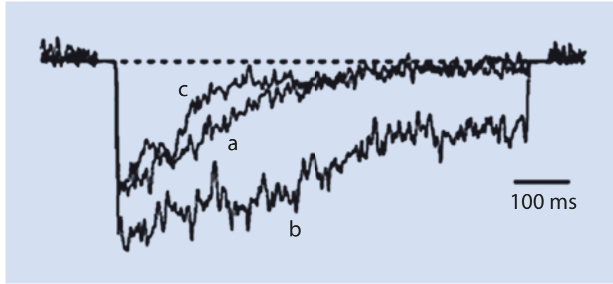


Fig. 6.1 Ensemble currents demonstrating Ca^{2+}-dependent LTCC inactivation in planar lipid bilayer experiments; a, b, and c show currents with 10 µM-, 20 nM-, and 15 µM-[Ca^{2+}] respectively. Increasing concentrations of [Ca^{2+}] accelerate the rate of LTCC inactivation. (Image from [7])

Similarly, increasing $[Ca^{2+}]_i$ accelerates the inactivation rate of LTCCs, suggesting the precense of a negative-feedback system that prevents excess Ca^{2+} influx [7]. This is observed when Ca^{2+} is replaced with Ba^{2+} and the LTCC inactivation rate decelerates as Ca^{2+}-dependent inactivation is minimised [5].

Enzymatic and non-enzymatic mechanisms have been proposed to explain Ca^{2+}-dependent inactivation. In the former, dephosphorylation of the LTCC by Ca^{2+}-activated phosphatases deactivates the channel [7]. In the latter, a Ca^{2+}-calmodulin complex on the -COOH terminal of the α1 subunit of the LTCC binds Ca^{2+} when local $[Ca^{2+}]_i$ increases, altering the channel's conformation and thus inactivating it [5, 7] (Fig. 6.1).

6.2.2 Na^+-Ca^{2+} Exchanger (NCX)

The second contributor of Ca^{2+} influx during the action potential is the Na^+-Ca^{2+} exchanger (NCX): a counter-transport system that operates by exchanging 3 Na^+ for 1 Ca^{2+}. This net movement of positive charge in the direction of Na^+ makes NCX electrogenic (i.e. it generates current). Typically, NCX moves Na^+ ions in whilst Ca^{2+} is effluxed out of the cell – known as the '*forward mode*' and producing an inward current. NCX can also function in '*reverse mode*', loading the cell with Ca^{2+} whilst Na^+ ions are effluxed out of the cell (outward current). This can be summarised mathematically in a few equations:

$$E_{\text{rev}} = E_{\text{NCX}} = 3E_{\text{Na}} - 2E_{\text{Ca}} \quad (6.1)$$

$$3(E_{\text{Na}} - V_{\text{m}}) > 2(E_{\text{Ca}} - V_{\text{m}}) \quad (6.2)$$

$$V_{\text{m}} < E_{\text{NCX}} \quad (6.3)$$

Let's work through those:

1. E_{rev} or E_{NCX} is the reversal potential of NCX – that is, the V_m at which NCX will switch from '*forward*' to '*reverse*' mode. As the equation shows, this depends on the individual equilibrium potentials of Na^+ and Ca^{2+}. This is exactly the same as the reversal potential of an ion channel, meaning that the current produced by NCX (I_{NCX}) when the membrane potential is equal to the reversal potential ($V_m = E_{\text{NCX}}$) is *zero* – there is no net movement of charge through the sarcolemma.

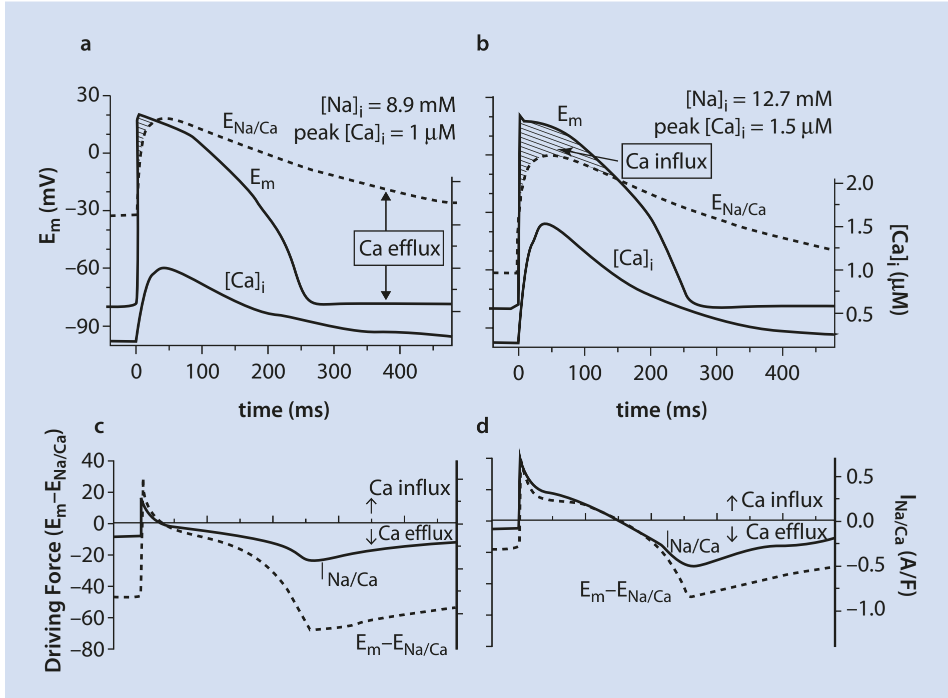


Fig. 6.2 Mode of operation of NCX: (**a**, **b**) V_m shown as E_m on graph, intracellular Ca^{2+} ($[Ca^{2+}]_i$) and E_{NCX}, with **a** low intracellular Na^+ ($[Na^+]_i$) and **b** high $[Na^+]_i$. Notice that with higher $[Na^+]_i$, $V_m > E_{NCX}$ for a greater period of time, meaning NCX functions in reverse mode for longer, promoting higher $[Ca^{2+}]_i$. **c** Driving force corresponds to the thermodynamic drive, which determines the mode of operation of NCX according to Eq. 6.2. When $V_m - E_{NCX} > 0$ there is Ca^{2+} influx due to the transporter operating in reverse mode. **d** Current generated by NCX (I_{NCX}). Notice that $I_{NCX} > 0$ (i.e. outward) when $V_m > E_{NCX}$ and $I_{NCX} < 0$ (i.e. inward) when $V_m < E_{NCX}$. Also, notice that when $E_{NCX} = V_m$, $I_{NCX} = 0$. ($I_{NCX} = I_{NA/Ca}$)(Image from [5])

2. This equation shows the thermodynamic basis for the transport that governs NCX, suggesting that when the energy for the inward movement of three Na^+ ions exceeds the energy for the inward movement of one Ca^{2+} ion, Na^+ influx and Ca^{2+} efflux are favoured (i.e. '*forward mode*'). Conversely, if $3(E_{Na} - V_m) < 2(E_{Ca} - V_m)$, then the reverse is thermodynamically favoured, and Ca^{2+} influx occurs [5].
3. This is a rearranged form of Eq. 6.2, demonstrating that when V_m is more negative than E_{NCX}, the exchanger functions in forward mode, and vice versa (Fig. 6.2). That is:
   - $V_m < E_{NCX}$ – NCX operates in forward mode
   - $V_m > E_{NCX}$ – NCX operates in reverse mode

Ultimately, whether NCX promotes Ca^{2+} influx or efflux depends on its mode of operation, determined by (a) V_m, (b) E_{Na}, and (c) E_{Ca}. Thus, although intuitively it is sensible (and typically correct) to proclaim that when the subsarcolemmal Ca^{2+} is high NCX will favour Ca^{2+} extrusion, the mode of operation is not merely a function of Ca^{2+} (and by extension its equilibrium potential), but also E_{Na} and V_m. Evidently, the ability of NCX to operate bidirectionally makes it a pivotal player in Ca^{2+} homeostasis.

6.3 Ca^{2+} Efflux

The dissociation of Ca^{2+} from troponin on myofilaments allows relaxation to take place. For this to happen, Ca^{2+} must be removed from the cytoplasm. This occurs via four mechanisms:
1. Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA)
2. NCX (forward mode)
3. Sarcolemmal Ca^{2+}-ATPase
4. Mitochondrial Ca^{2+} transporters (into mitochondria)

6.3.1 SERCA Protein

Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is a protein pump concentrated on the longitudinal component of the SR, transporting Ca^{2+} from the cytoplasm to the SR lumen [9]. It has three different isoforms (SERCA1, 2 and 3), with SERCA2a expressed abundantly in the heart [10]. The transport reaction involves multiple steps, beginning with the binding of 2 Ca^{2+} ions and 1 ATP molecule on the pump's cytoplasmic side, in addition to phosphorylation. This triggers conformational alterations that facilitate the release of Ca^{2+} into the SR lumen, and H^{+} into the cytoplasm [11]. Perhaps counterintuitively then, relaxation, and not merely contraction, is energy dependent [12].

In general, the ATP concentration required to saturate SERCA is 1000× fold lower than the cytoplasmic ATP of a healthy CM at any given time, meaning that except in the energy-starved heart (e.g. failing, dysrhythmic), lack of ATP is not the rate-limiting factor for Ca^{2+} removal [12]. However, ATP can also allosterically modulate SERCA activity via a lower affinity binding site on the pump, such that in ischaemia it is the lack of this allosteric effect, rather than of ATP available for hydrolysis, that may disrupt relaxation kinetics [5, 12].

The main SERCA2a activity regulator is a homopentameric protein known as phospholamban (PLN) [5, 13]. When PLN is dephosphorylated, it tonically inhibits SERCA by increasing its $K_m(Ca^{2+})$, meaning more Ca^{2+} is required to attain the same Ca^{2+} transport rate [14]. When phosphorylated (e.g. in response to adrenergic stimulation), this tonic inhibition is lifted, enhancing SERCA affinity for Ca^{2+} by decreasing its $K_m(Ca^{2+})$, thus accelerating Ca^{2+} SR sequestration and relaxation.

PLN phosphorylation is at least in part accountable for the positive lusitropic effects observed in the presence of adrenergic stimulation. Phosphorylation of PLN has been demonstrated in three sites including (a) serine-16, (b) threonine-17 and (c) serine-10 by cAMP-dependent protein kinase A, Ca^{2+}/Calmodulin protein kinase II and Ca^{2+}-activated protein kinase C, respectively [14]. Dephosphorylation of PLN by SR-associated phosphatases restores PLN's tonic inhibition [14].

Sarcolipin (SLN) (a PLN homologue) is another SERCA regulator, albeit less well understood. It has been suggested that when SLN is co-expressed with PLN, SERCA2a Ca^{2+} affinity decreases more than with PLN alone (◘ Fig. 6.3) [15, 16]. This may be due to the increased concentration of active (inhibitory) PLN monomers in the presence of SLN [17]. In particular, PLN is present either in homopentameric or monomeric form, the latter of which exhibits increased inhibitory activity [15]. When SLN is co-expressed, it holds PLN in a monomeric form (preventing it from polymerizing into pentamers),

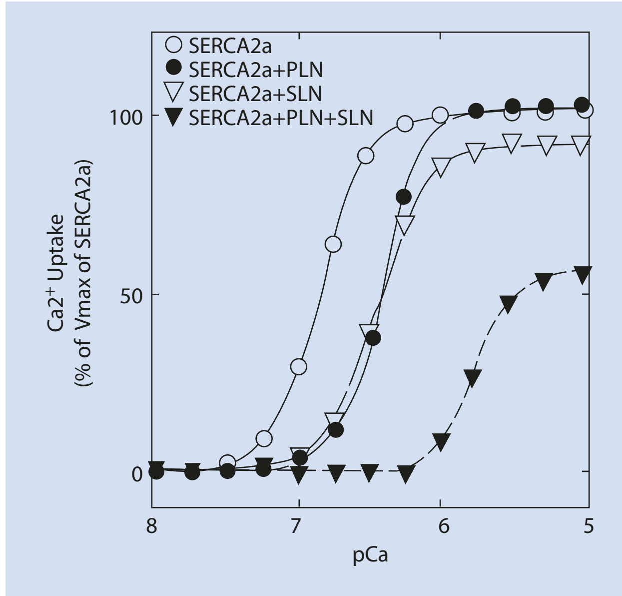


Fig. 6.3 Rate of Ca^{2+} uptake in HEK-293 cells expressing SERCA2a alone, SERCA2a and PLN, SERCA2a and SLN, SERCA2a and PLN and SLN. Note that the Ca^{2+} uptake rate is considerably decreased in the latter. (Image from [16])

enhancing its inhibitory activity and further decreasing SERCA Ca^{2+} uptake [15]. However, SLN has also been proposed to inhibit SERCA by a mechanism independent of PLN, as seen with SLN overexpression in PLN($^{-/-}$) (null mutants), which display impaired contractility, altered Ca^{2+} handling and relaxation [17, 18].

6.3.2 NCX

We previously considered the different modes of operation of NCX, demonstrating that when $V_m < E_{NCX}$, NCX extrudes Ca^{2+}. In general, SERCA and NCX are the two main transporters responsible for relaxation, and at any one time are in the state of constant competition for Ca^{2+}. This has important physiological implications and can be demonstrated by considering Ca^{2+} efflux in different species. For example, after a period of rest (i.e. no electrical stimulation), rabbit cardiac preparations show a decline in the amplitude of the first post-rest contraction, termed *rest decay* [19, 20] (Fig. 6.4).

In contrast, rat CMs have an increased contraction amplitude after a period of rest – known as post-rest potentiation [19]. Such phenomena are explained by the dynamic relationship of Ca^{2+} extruders. In both rabbits and rats during rest, Ca^{2+} leaks from the SR due to the random openings of RyRs (see *Ca^{2+} sparks*, below). Ca^{2+} is then subjected to two opposing forces – SERCA and NCX. In rabbits, NCX moves Ca^{2+} out of the cell, progressively decreasing the SR Ca^{2+} content, which results in less Ca^{2+} available for myofilament activation and a diminished contraction post-rest [5, 19, 20]. In contrast, rats have high intracellular Na^{+} (resulting in $V_m > E_{NCX}$), slow NCX, and fast SERCA transport rates, the summation of which leads to increased SR Ca^{2+} content during rest and a potentiated contraction post-rest [19].

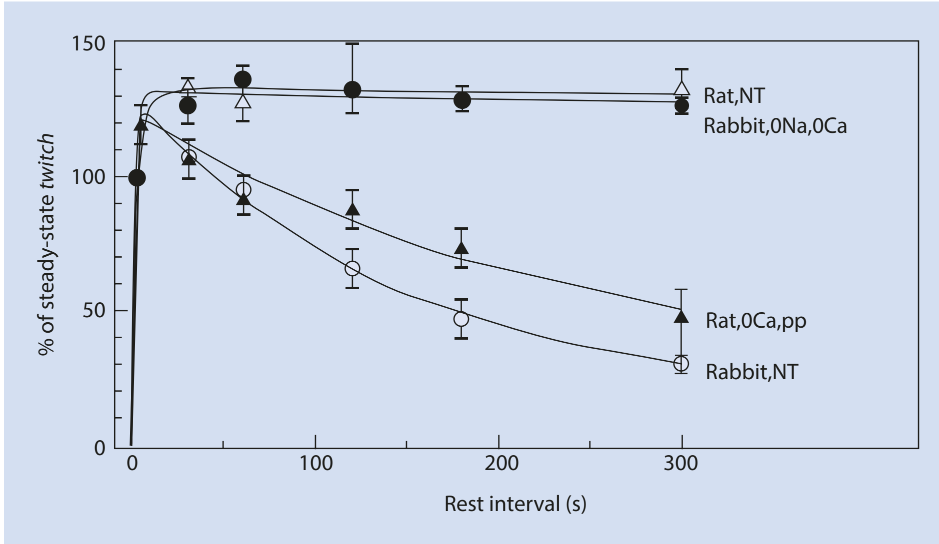


Fig. 6.4 Rest decay and rest-potentiation in rabbits and rats, respectively. After a period of electrical rest for 100 s, the first contraction after rest is decreased to 75% of contraction during steady state in rabbits. In contrast, rats exhibit post-rest potentiation, increasing their contraction amplitude to approximately 140% after a period of electrical rest. Notice that thermodynamic inhibition of NCX in rabbits (0Na, 0Ca) converts the post-rest decay into post-rest potentiation. Similarly, applying a 0Ca^{2+} extracellular solution in rats favours Ca^{2+} extrusion by NCX and converts the typical post-rest potentiation into post-rest decay (NT = Normal Tyrode's solution). (Image from [19])

6.3.3 Other Extrusion Mechanisms

When caffeine is applied on cardiac preparations Ca^{2+} stored in the SR is released. If NCX and SERCA are blocked, the rate of cytoplasmic Ca^{2+} removal is significantly slowed but not completely abolished [21].

This is because in addition to NCX and SERCA, Ca^{2+} removal also occurs by sarcolemmal Ca^{2+}-ATPase and the mitochondrial Ca^{2+} uniporter (MCU). Sarcolemmal Ca^{2+}-ATPase utilises ATP to efflux Ca^{2+} out of the cell whilst MCUs facilitate the flux of Ca^{2+} in mitochondria down a large electrochemical gradient [22, 23]. With the exception of a few species (e.g. ferret), the contribution of these in removing Ca^{2+} on a beat-to-beat basis is marginal when compared to NCX or SERCA [24]. As such, they are known as *slow extruders*.

However, their role in maintaining CM health and function is anything but marginal. For instance, increased mitochondrial Ca^{2+} loading activates energy production (e.g. via ATP synthase) allowing CMs to cope with increased energy demands, yet prolonged periods of elevated intracellular Ca^{2+} can trigger mitochondrial dysfunction and acute CM death [25]. Ultimately, the rate of Ca^{2+} removal from the CM cytosol can be quantified to highlight the rate of removal by each transporter, such that the total rate of Ca^{2+} removal is equal to:

$$\frac{d[\mathrm{Ca}]t}{dt} = J_{\mathrm{SERCA}} + J_{\mathrm{NCX}} + J_{\mathrm{slow}} \tag{6.4}$$

where J is the rate of removal of extrusion components, each governed by a nonlinear function dependent on Ca^{2+} concentration [5].

6.4 Excitation-Contraction Coupling

I_{Ca} generated during the AP enhances Ca^{2+} release from the SR via an RyR-mediated process termed *calcium-induced calcium release* (*CICR*) that forms the basis of ECC [26]. Ryanodine receptors (RyRs) are Ca^{2+} release channels embedded on the SR membrane, which open in response to cytosolic Ca^{2+} [1]. There are three different RyR isoforms, with RyR2 being mainly expressed in the heart [27]. Particularly, RyRs are found in discrete groups on the junctional SR (i.e. the part of the SR in close proximity to the sarcolemma), establishing functional Ca^{2+}-release units known as *couplons* [1]. Ca^{2+} influx causes multiple couplons to open, resulting in the release of Ca^{2+} from the SR and the development of the Ca^{2+}-transient.

Each couplon consists of approximately 100 RyRs closely apposed to approximately 10–25 sarcolemmal LTCCs, forming the cardiac dyad and separated by a nanometres-wide cleft known as the dyadic space (or cleft) [2].

6

The close proximity between RyRs and LTCCs in the dyad is made possible by deep invaginations of the sarcolemma known as t-tubules (TT) and ensures efficient coupling between I_{Ca} and SR-Ca^{2+} release [1, 2, 6]. Specifically, one LTCC opening within a cardiac dyad is sufficient to trigger SR-Ca^{2+} release from a whole couplon, meaning that the ≅10–25 LTCCs for ≅100 RyRs ensures a safety margin for triggering SR-Ca^{2+} release [26–28]. In addition to the role of cytosolic Ca^{2+} in triggering Ca^{2+} release via RyRs, SR luminal Ca^{2+} also plays a pivotal role in SR-Ca^{2+} release [29]. For instance, increased SR Ca^{2+} content can stimulate Ca^{2+} release, whilst RyR2 activity is diminished as luminal Ca^{2+} decreases [29].

6.4.1 Calcium Sparks

In 1993, Cheng et al. used fluorescent Ca^{2+} indicators and laser scanning confocal microscopy to describe the concept of Ca^{2+} sparks for the first time [26]. Ca^{2+} sparks are microscopic elevations of cytoplasmic Ca^{2+} reflecting the synchronous opening of a cluster of RyRs [1]. They occur by either of two mechanisms described below:

1. Stochastic openings of RyRs [26–30]. The open probability of a single or a small number of RyRs can randomly become non-zero, triggering nano-elevations of the resting $[Ca^{2+}]_i$ to about 170 nM [26]. This is important, as in pathological states associated with Ca^{2+} overload (supranormal SR Ca^{2+}-content), spontaneous SR Ca^{2+} release events can cause spark-induced spark-release, leading to high $[Ca^{2+}]_i$ ("macrosparks", ≅ 500 nM) and the successive development of dysrhythmogenic waves [26, 28].
2. Evoked by I_{Ca} that raises local subsarcolemmal Ca^{2+} and activates RyRs [26, 30]. During the cardiac action potential, I_{Ca} evokes multiple Ca^{2+} sparks by stochastically activating clusters of RyRs. The spatiotemporal summation of ≅10^4 individual Ca^{2+} sparks results in the production of the seeming spatially uniform Ca^{2+} transient [28].

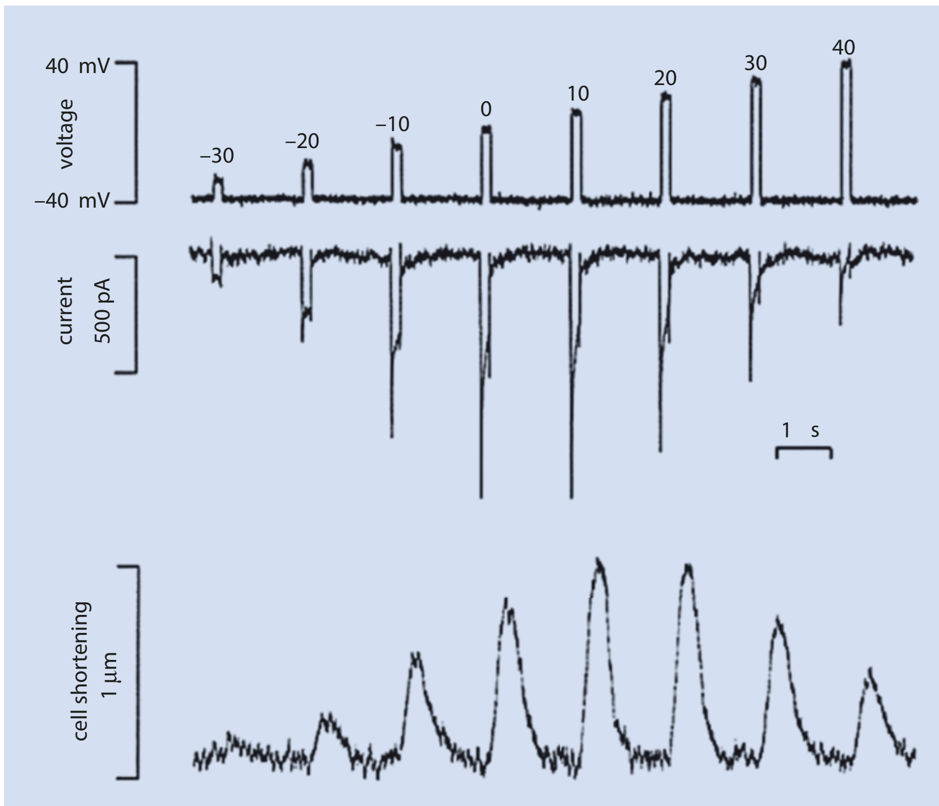
6.4.2 Calcium-Induced Calcium Release

The idea that the Ca^{2+} transient consists of many individual 'atomic' subevents (i.e. Ca^{2+} sparks) revolutionised the ECC paradigm. Previously, many models of cardiac CICR

assumed a common pool of cytosolic Ca^{2+}, consisting of homogenously distributed Ca^{2+} from I_{Ca} and SR Ca^{2+} release, with the former controlling the latter [31].

According to that model, when I_{Ca} stimulates SR Ca^{2+} release and the pool begins to fill with Ca^{2+}, a positive feedback loop is established in which Ca^{2+} released from the SR triggers more SR-Ca^{2+} release [31]. This makes SR-Ca^{2+} release an all-or-none response such that once the SR-Ca^{2+} release process commences, CICR is expected to evolve autonomously, irrespective of sarcolemmal Ca^{2+} influx [32].

However, experimental evidence does not support the notion that SR-Ca^{2+} release becomes autonomous. Instead, it is accepted that the magnitude of SR Ca^{2+}-release is a function of I_{Ca} [31, 33]. As I_{Ca} is primarily carried by the LTCCs, SR Ca^{2+} release is dependent on membrane potential [34]. If I_{Ca} is abruptly terminated by depolarisation above the LTCC reversal potential, SR Ca^{2+} release is also terminated [31, 35]. Therefore, SR Ca^{2+} release is graded – meaning it is a function of Ca^{2+} influx through LTCCs (I_{Ca}) (i.e. dependent on duration and magnitude of Ca^{2+} entry) [31, 36]. This is depicted in ◘ Fig. 6.5, which shows the typical characteristic bell-shaped voltage dependency of (a) I_{Ca} and (b) cell shortening (reflecting Ca^{2+} transient magnitude). Such a graded response is not in accordance with common pool models, which would be expected to cause an 'all-or-none' SR-Ca^{2+} release.



◘ **Fig. 6.5** Bell shaped LTCC current (I_{Ca}) in response to increasing membrane potential (voltage-clamp). Notice bell-shaped relationship of cell-shortening as well, reflecting bell-shaped Ca^{2+} transient amplitude (peaking at approximately 10 V)

To explain this gradation, Stern et al. proposed the local control theory of ECC, whereby Ca^{2+} sensed by RyRs is not the same as the average cytoplasmic $[Ca^{2+}]_i$ [33]. In particular, the opening of LTCCs causes a very high and rapid local rise of $[Ca^{2+}]$ within a cardiac dyad to >10 μM [33]. This activates RyRs within a couplon, causing SR-Ca^{2+} release to further elevate local $[Ca^{2+}]_i$ [33].

It is also proposed that the sensitivity of RyRs to Ca^{2+} is much less than the ambient cytosolic Ca^{2+}, preventing an 'all-or-none' regenerative calcium release [33]. Therefore, although CICR may be regenerative within an individual couplon (i.e. Ca^{2+} released by one RyR in a couplon triggering Ca^{2+} release by other RyRs in the same couplon – a positive feedback loop), Ca^{2+} released from one couplon does not spread in sufficiently high amounts to trigger Ca^{2+} release from neighbouring couplons [36].

These Ca^{2+} release events triggered by individual stochastic openings of LTCCs are in essence Ca^{2+} sparks, the spatial and temporal summation of which leads to the whole-cell Ca^{2+} transient [30]. Gradation of Ca^{2+} transient then occurs by the stochastic recruitment of more or less Ca^{2+} sparks according to the membrane potential (and by extension I_{Ca}) [34]. Ultimately, the distinction between common pool and local control models of ECC is highlighted by the fact that in the latter, elementary Ca^{2+}-sparks are recruited not by the mean $[Ca^{2+}]_i$ in the cell, but rather by the amount of Ca^{2+} flowing through the sarcolemmal LTCC, elevating local $[Ca^{2+}]_i$ in the cardiac cleft nanodomain [37] (Fig. 6.6).

6.5 What We Don't Know

6.5.1 Ca^{2+} Spark Theories Interlude

But what determines whether a Ca^{2+} spark will actually be evoked? Santana et al. proposed that the probability of triggering a Ca^{2+} spark is dependent upon the square of the local Ca^{2+} concentration in the nanodomain, and that opening of a single LTCC is sufficient for this to happen [30, 38] – that is:

$$P(\text{spark}) = \left(\text{local}\left[\text{Ca}^{2+}\right]\right)^2$$

Appreciating the role of local $[Ca^{2+}]$ in triggering a Ca^{2+} spark is critical, as alterations in the microarchitecture of the ECC apparatus (e.g. in the geometric arrangement of LTCCs and RyRs) seen with pathology, can affect the P(spark), leading to Ca^{2+} handling abnormalities with implications for cardiac contractility [30].

By far the most accepted mechanism of CICR is the RyR-mediated release of SR Ca^{2+}, triggered by the influx of Ca^{2+} through the LTCCs [1]. Yet, NCX has been postulated to be involved in CICR [39]. In isolated CMs, LeBlanc et al. blocked LTCC Ca^{2+} influx using nisoldepine, demonstrating that voltage-clamp depolarisations caused an initial rapid inward current, followed by a rise in $[Ca^{2+}]_i$, both of which were abolished with application of tetrodotoxin (TTX, a Na^+ channel inhibitor), suggesting that the observed Ca^{2+} transient was Na^+ channel-dependent [39]. Following further experiments, they concluded that the initial depolarisation upstroke due to the inward Na^+ current, coupled with the increasingly positive V_m, promotes transient '*reverse mode*' in NCX operation, leading to Ca^{2+} influx and providing the trigger for CICR [9, 39].

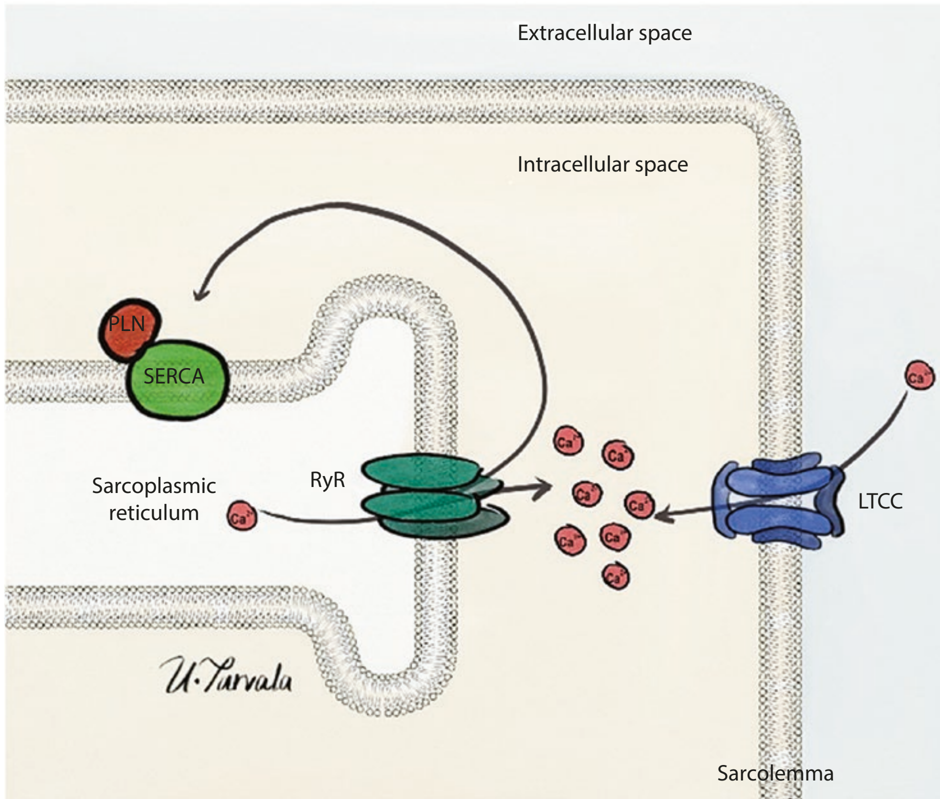


Fig. 6.6 Close apposition of sarcolemmal LTCCs and RyRs on the junctional SR promote efficient coupling. During an AP, I_{Ca} increases $[Ca^{2+}]_i$ in the cardiac dyadic cleft, evoking Ca^{2+} sparks via RyR activation. Local $[Ca^{2+}]_i$ in the cardiac dyadic cleft is much higher than $[Ca^{2+}]$ in the bulk cytosolic space, allowing for RyR activation in couplons despite their low Ca^{2+} sensitivity

Another example comes from $NCX^{-/-}$ isolated ventricular myocytes. These cells display normal ECC, however in the presence of heavy Ca^{2+} buffering (minimising the effect of Ca^{2+} influx from LTCCs), reduced coupling efficiency is observed vs. wild-type CMs. This suggests there is an increased proportion of couplons failing to activate during the AP in the $NCX^{-/-}$ myocytes compared to wild type [40]. Accordingly, Goldhaber et al. proposed that NCX has a role in maintaining coupling during depolarisation by priming the dyadic space with a subthreshold amount of Ca^{2+}, meaning only a small amount of further Ca^{2+} from LTCCs is required to trigger CICR [40]. Others have remained sceptical of the role of NCX in ECC [41, 42], as (a) Na^+ channels may be excluded from the dyadic cleft, and (b) NCX as a transporter (and not an ion channel) is notably slower than LTCCs, meaning that when both co-exist, CICR is dominated by the latter [1, 43].

6.5.2 CICR Termination – Stopping the Domino Effect

We have seen that couplons are separated from each other and that according to the local control theory of ECC, RyR Ca^{2+} sensitivity is low enough so that Ca^{2+} released from one

couplon does not trigger Ca^{2+} release from neighbouring couplons. Yet, as Ca^{2+} is both the cause and effect of the release, SR-Ca^{2+} release should still be inherently regenerative within a couplon [5]. However, with approximately 50% (i.e. not the whole amount) of SR Ca^{2+} released in each contraction, what terminates the release of Ca^{2+} from the SR?

Proposed mechanisms include stochastic attrition, ryanodine receptor inactivation, adaption and local depletion of Ca^{2+} in the SR [1, 36]. Despite the number of approaches to explain CICR termination, a single unifying mechanism does not exist, with a weighted combination of the different theories likely responsible.

Stochastic Attrition Proposed by Stern et al. and suggests that random simultaneous closure of RyRs in a couplon could abruptly break the positive feedback cycle, halting the regenerative nature of CICR [36, 44]. As RyR are stochastically oscillating between closed and open states, there is always a chance that all channels close at the same time, degrading the local Ca^{2+} gradient [36]. The probability of this happening is dependent upon (a) the probability of the RyR being open (P_o), (b) the average time they remain open (τ_o) and (c) the number of RyR in a couplon (n) [36]. Generally, it can be shown as P_o, τ_o and n increase, the probability of simultaneous, stochastic closure of all RyRs drops exponentially [1, 36]. Thus, although stochastic attrition is a mechanism that is always present, it is rather improbable that it singlehandedly terminates the CICR of a highly active calcium synapse [36].

6

Ryanodine Receptor Inactivation A Ca^{2+}-induced inactivation mechanism was originally proposed by Fabiato, who used skinned myocytes to suggest that binding of Ca^{2+} to a high-affinity site on RyR inactivated the channel, stopping the Ca^{2+} release process [45]. Therefore, and perhaps conveniently, much like Ca^{2+}-dependent inactivation of LTCCs, Ca^{2+}-dependent inactivation of RyR may terminate CICR. In reality however, with most experiments being performed in planar bilayers and never validated in intact cells, this mechanism's contribution to SR-Ca^{2+} release termination remains unclear [36, 45].

Adaption RyRs relax to a lower P_o after activation. Strictly speaking, adaption is not RyR inactivation per se as the channels can be reactivated with exposure to higher $[Ca^{2+}]_i$ [1, 45].

Depletion of Local SR Luminal Ca^{2+} Opening of RyRs could lead to depletion of SR lumincal Ca^{2+} near the channel, resulting in either the flux of Ca^{2+} out of the SR to become zero (i.e. there is no more Ca^{2+} to be released), halting the positive feedback loop and ending CICR; or, causing the probability of RyR opening (in part determined by luminal Ca^{2+}) to be decreased such that no more Ca^{2+} is released [36]. As cytosolic Ca^{2+} is constantly being pumped into the SR, typically at a different location than the one releasing Ca^{2+}, the validity of this mechanism is dictated by (a) the rate of Ca^{2+} reuptake and (b) the rate of diffusion of Ca^{2+} from the 'uptake SR compartment' to the 'SR release compartment' [36].

6.6 Where We're Heading

6.6.1 ECC as a Measure of Cardiac Maturity

Stemcell-derived cardiomyocytes hold significant therapeutic promise for the treatment of diseased myocardium. These are stem cells that have been differentiated in to cardiomyocytes. A problem that has beset this field is the immaturity of such cells, in particular the absence of adult-like functional and morphological cardiomyocyte characteristics [46].

Components of the ECC seem particularly affected, with stem cell-derived cardiomyocytes having few or even no t-tubules, which can at least in part explain the abnormal Ca^{2+} dynamics and considerably weaker contractile force amplitudes than those developed by the adult myocardium (e.g. human ventricular strips twitch tension is ≅44 mN/mm^2 vs. 0.08 mN/mm^2 in human pluripotent stem cell-derived cardiomyocytes – a 550-fold decrease) [46, 47].

In cell therapy, stem cell-derived cardiomyocytes are transplanted onto the injured myocardium in an effort to increase the contractile ability of the heart. Lack of robust ECC apparatus questions whether beneficial effects (if any) of such approaches reflect the addition of force-generating cardiomyocytes or merely the release of nurturing paracrine mediators from these cells [48].

Furthermore, transplantation of immature cells that beat asynchronously, have improper Ca^{2+} homeostasis, and/or are comparatively weaker to human cardiomyocytes may increase the dysrhythmogenic risk and by extension the safety of such approaches [4].

6.6.2 ECC in Pathology

Abnormal ECC underlies many pathological processes. In the failing heart, characterized by an inability to maintain a cardiac output sufficient to meet the metabolizing needs of the body [49, 50], disrupted geometrical arrangements of the ECC components may compromise the fidelity of ECC [51, 52]. For example, among the most well-known phenotypical changes seen in failing cardiomyocytes is the loss of t-tubules [53, 46]. Such morphological changes can disrupt the tight coupling between the LTCC and RyRs, and diminish the ability of a given I_{Ca} to trigger a Ca^{2+} spark, (and by extension a Ca^{2+} transient), hampering contractile performance [4, 52, 53]. Ingenious computational models have shown that for any spatial arrangement of LTCC and RyRs, there is an optimal amount of Ca^{2+} influx required to maximally activate RyRs [31]. If that becomes suboptimal (e.g. excessive Ca^{2+} influx which only minimally activates RyRs) in pathology then renormalizing this relationship between Ca^{2+} influx and RyR response with drugs or interventions may be of therapeutic benefit by enhancing contraction while simultaneously minimizing supranormal Ca^{2+} influx. This is important as excess Ca^{2+} influx raises the probabilities of Ca^{2+}-induced pathological states (e.g. Ca^{2+} overload-induced arrhythmias & activation of Ca^{2+} mediated pathological hypertrophy signalling pathways).

Take-Home Message

- During ventricular action potentials, Ca^{2+} influx from the extracellular to subsarcolemmal space generates a Ca^{2+} current, I_{Ca}, which initiates Ca^{2+} release from the sarcoplasmic reticulum via a process known as calcium-induced calcium release.
- I_{Ca} is generated by two main mechanisms: voltage-sensitive sarcolemmal Ca^{2+} channels (LTCCs) and Na^+/Ca^{2+} exchangers (NCX).
- Calcium-induced calcium release is mediated by ryanodine receptors interacting in complex microdomains with LTCCs.
- The therapeutic usefulness of transplanting stem cell-derived cardiomyocyte onto injured myocardium is hindered in part by the deficient Ca^{2+} handling exhibited by these cells.

References

1. Bers DM (2002) Cardiac excitation-contraction coupling. Nature 415:198–205
2. Eisner DA, Caldwell JL, Kistamás K, Trafford AW (2017) Calcium and excitation-contraction coupling in the heart. Circ Res 121:181–195
3. Ibrahim M, Al Masri A, Navaratnarajah M, Siedlecka U, Soppa GK, Moshkov A et al (2010) Prolonged mechanical unloading affects cardiomyocyte excitation-contraction coupling, transverse-tubule structure, and the cell surface. FASEB J 24(9):3321–3329
4. Kane C, Couch L, Terracciano CMN (2015) Excitation–contraction coupling of human induced pluripotent stem cell-derived cardiomyocytes. Front Cell Dev Biol 3:59
5. Bers DM. Excitation-contraction coupling and cardiac contractile force. Springer Science; 1991
6. Marks AR (2003) Calcium and the heart: a question of life and death. J Clin Investig 111:597–600
7. Haack JA, Rosenberg RL (1994) Calcium-dependent inactivation of L-type calcium channels in planar lipid bilayers membrane preparation planar lipid bilayers. Biophys J 66(April):1051–1060
8. Zhang JF, Ellinor PT, Aldrich RW, Tsien RW (1994) Molecular determinants of voltage-dependent inactivation in calcium channels. Nature 372:97–100
9. Barry WH, Bridge JH (1993) Intracellular calcium homeostasis in cardiac myocytes. Circulation 87(6):1806–1815
10. Periasamy M, Kalyanasundaram A (2007) SERCA pump isoforms: their role in calcium transport and disease. Muscle Nerve 35:430
11. MacLennan DH, Green NM (2000) Pumping ions. Nature 405:633–634
12. Katz a M, Lorell BH (2000) Regulation of cardiac contraction and relaxation. Circulation 102(20 Suppl 4):IV69–IV74
13. Gustavsson M, Verardi R, Mullen DG, Mote KR, Traaseth NJ, Gopinath T et al (2013) Allosteric regulation of SERCA by phosphorylation-mediated conformational shift of phospholamban. Proc Natl Acad Sci 110(43):17338–17343
14. Frank KF, Bolck B, Erdmann E, Schwinger RHG (2003) Sarcoplasmic reticulum Ca2+ -ATPase modulates cardiac contraction and relaxation. Cardiovasc Res 57(April):20–27
15. MacLennan DH, Asahi M (2003) Tupling a R. The regulation of SERCA-type pumps by phospholamban and sarcolipin. Ann N Y Acad Sci 986(1):472–480
16. Asahi M, Kurzydlowski K, Tada M, MacLennan DH (2002) Sarcolipin inhibits polymerization of phospholamban to induce superinhibition of sarco(endo)plasmic reticulum Ca2+-ATPases (SERCAs). J Biol Chem 277(30):26725–26728
17. Periasamy M, Bhupathy P, Babu GJ (2008) Regulation of sarcoplasmic reticulum Ca2+ ATPase pump expression and its relevance to cardiac muscle physiology and pathology. Cardiovasc Res 77(2):265–273
18. Gramolini AO, Trivieri MG, Oudit GY, Kislinger T, Li W, Patel MM et al (2006) Cardiac-specific overexpression of sarcolipin in phospholamban null mice impairs myocyte function that is restored by phosphorylation. Proc Natl Acad Sci U S A 103(7):2446–2451
19. Bassani RA, Bers DM (1994) Na-ca exchange is required for rest-decay but not for rest-potentiation of twitches in rabbit and rat ventricular myocytes. J Mol Cell Cardiol 26:1335–1347
20. Bers DM (1991) Ca regulation in cardiac muscle. Med Sci Sport Exerc 23(10):1157–1162
21. Bassani RA, Bassani JW, Bers DM (1992) Mitochondrial and sarcolemmal Ca2+ transport reduce [Ca2+]i during caffeine contractures in rabbit cardiac myocytes. J Physiol 453(1):591–608
22. Choi HS, Eisner DA (1999) The role of sarcolemmal Ca^{2+}-ATPase in the regulation of resting calcium concentration in rat ventricular myocytes. J Physiol 515(1):109–118
23. Kirichok Y, Krapivinsky G, Clapham DE (2004) The mitochondrial calcium uniporter is a highly selective ion channel. Nature 427(6972):360–364
24. Bassani RA, Bassani JWM, Bers DM (1995) Relaxation in ferret ventricular myocytes: role of the sarcolemmal Ca ATPase. Pflugers Arch Eur J Physiol 430(4):573–578
25. Kwong JQ, Lu X, Correll RN, Schwanekamp JA, Vagnozzi RJ, Sargent MA et al (2015) The mitochondrial calcium uniporter selectively matches metabolic output to acute contractile stress in the heart. Cell Rep 12(1):15–22
26. Cheng H, Lederer W, Cannell M (1993) Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. Science 262(5134):740–744
27. Lehnart SE, Wehrens XHT, Kushnir A, Marks AR (2004) Cardiac ryanodine receptor function and regulation in heart disease. Ann N Y Acad Sci 1015:144

28. Cheng H, Lederer WJ (2008) Calcium Sparks. Physiol Rev 88(4):1491–1545
29. Györke S, Terentyev D Modulation of ryanodine receptor by luminal calcium and accessory proteins in health and cardiac disease. Cardiovasc Res 2008, 77(2):245–255
30. Cannell MB, Soeller C (1998) Sparks of interest in cardiac excitation-contraction coupling. Trends Pharmacol Sci 19:16–20
31. Stern MD (1992) Theory of excitation-contraction coupling in cardiac muscle. Biophys J 63(2):497–517
32. Cannell MB, Kong CHT (2012) Local control in cardiac E-C coupling. J Mol Cell Cardiol 52:298
33. Stern MD, Song LS, Cheng H, Sham JS, Yang HT, Boheler KR et al (1999) Local control models of cardiac excitation-contraction coupling. A possible role for allosteric interactions between ryanodine receptors. J Gen Physiol 113(3):469–489
34. Hinch R, Greenstein JL, Tanskanen a J, Xu L, Winslow RL (2004) A simplified local control model of calcium-induced calcium release in cardiac ventricular myocytes. Biophys J 87(6):3723–3736
35. Barcenas-Ruiz L, Wier WG (1987) Voltage dependence of intracellular [ca 2+]i transients in guinea pig ventricular myocytes. Circ Res 61:148–154
36. Stern MD, Cheng H (2004) Putting out the fire: what terminates calcium-induced calcium release in cardiac muscle? Cell Calcium 35(6):591–601
37. Wier WG, Balke CW (1999) Ca2+ release mechanisms, Ca2+ sparks, and local control of excitation-contraction coupling in normal heart muscle. Circ Res 85(9):770–776
38. Santana LF, Cheng H, Gómez AM, Cannell MB, Lederer WJ, Scott JD et al (1996) Relation between the sarcolemmal Ca2+ current and Ca2+ sparks and local control theories for cardiac excitation-contraction coupling. Circ Res 78(1):166–171
39. Hume LN (1990) Sodium current-induced release of calcium from cardiac sarcoplasmic reticulum. Science 248(4953):372–376
40. Goldhaber JI, Philipson KD (2013) Cardiac sodium-calcium exchange and efficient excitation-contraction coupling: implications for heart disease. Adv Exp Med Biol 961:355–364
41. López-López JR, Shacklock PS, Balke CW, Wier WG (1995) Local calcium transients triggered by single L-type calcium channel currents in cardiac cells. Science 268(5213):1042–1045
42. Bers DM, Lederer WJ, Berlin JR (1990) Intracellular Ca transients in rat cardiac myocytes: role of Na-Ca exchange in excitation-contraction coupling. Am J Physiol Physiol 258(5):C944–C954
43. Sham JSK, Cleemann L, Morad M (1992) Gating of the cardiac Ca2+ release channel: the role of Na+ current and Na+ -Ca2+ exchange. Science 255:850–853
44. Sobie EA, Duly KW, Cruz JDS, Lederer WJ, Jafri MS (2002) Termination of cardiac Ca2+ sparks: an investigative mathematical model of calcium-induced calcium release. Biophys J 83(1):59–78
45. Sham JS, Song LS, Chen Y, Deng LH, Stern MD, Lakatta EG et al (1998) Termination of Ca2+ release by a local inactivation of ryanodine receptors in cardiac myocytes. Proc Natl Acad Sci U S A 95(25):15096–15101
46. Yang X, Pabon L, Murry CE (2014) Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. Circ Res 114:511–523
47. Mannhardt I, Breckwoldt K, Letuffe-Brenière D, Schaaf S, Schulz H, Neuber C et al (2016) Human engineered heart tissue: analysis of contractile force. Stem Cell Reports 7:29
48. Malliaras K, Marbán E (2011) Cardiac cell therapy: where weve been, where we are, and where we should be headed. Br Med Bull 98(1):161–185
49. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS et al (2016) 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 37:2129–2200m
50. Zima AV, Bovo E, Mazurek SR, Rochira JA, Li W, Terentyev D (2014) Ca handling during excitation-contraction coupling in heart failure. Pflugers Archiv Eur J Physiol 466:1129–1137
51. Gomez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB et al (1997) Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. Science 276(5313): 800–806
52. Ibrahim M, Terracciano CM (2013) Reversibility of T-tubule remodelling in heart failure: mechanical load as a dynamic regulator of the T-tubules. Cardiovasc Res 98:225–232
53. Lyon AR, MacLeod KT, Zhang Y, Garcia E, Kanda GK, Lab MJ et al (2009) Loss of T-tubules and other changes to surface topography in ventricular myocytes from failing human and rat heart. Proc Natl Acad Sci U S A 106(16):6854–6859