Ca\textsuperscript{2+} Release Mechanisms, Ca\textsuperscript{2+} Sparks, and Local Control of Excitation-Contraction Coupling in Normal Heart Muscle

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It is well established that most of the Ca\textsuperscript{2+} that activates contraction in mammalian heart is released from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR) and that the RyR are themselves activated by Ca\textsuperscript{2+} in the mechanism known as “Ca\textsuperscript{2+} induced Ca\textsuperscript{2+} release” (CICR).\textsuperscript{1} Confocal imaging has made possible the visualization of localized Ca\textsuperscript{2+} release through RyR, in the form of Ca\textsuperscript{2+} sparks.\textsuperscript{2} It appears that Ca\textsuperscript{2+} sparks are triggered by a local [Ca\textsuperscript{2+}]\textsubscript{i}, which is different from the spatial average [Ca\textsuperscript{2+}]\textsubscript{i}, and which is established first in the region of the RyR by the opening of a single L-type Ca\textsuperscript{2+} channel.\textsuperscript{3,4} These phenomena are the basis of the theory of excitation-contraction (E-C) coupling known as “local control,” which was predicted so presciently by Michael D. Stern in 1992.\textsuperscript{5} Nevertheless, the molecular mechanisms of Ca\textsuperscript{2+} sparks and the nature of the triggering by Ca\textsuperscript{2+} entry are still obscure. To complicate matters further, other possible sources of Ca\textsuperscript{2+} that activate, or “trigger,” this release have been proposed recently, and it has even been suggested that a voltage-sensitive release mechanism, which does not require Ca\textsuperscript{2+}, may exist in cardiac muscle, similar to that in skeletal muscle.\textsuperscript{6} It is our intention here to review the evidence for local control of E-C coupling in normal heart muscle and to evaluate critically the evidence for additional sources of trigger Ca\textsuperscript{2+} or mechanisms of SR Ca\textsuperscript{2+} release. We emphasize, however, that concepts about cardiac Ca\textsuperscript{2+} sparks, and their possible role in cardiac E-C coupling, do not necessarily extend to Ca\textsuperscript{2+} sparks that occur in smooth muscle and skeletal muscle. Local Ca\textsuperscript{2+} release in smooth muscle cells has also been called Ca\textsuperscript{2+} sparks,\textsuperscript{7} but is thought to modulate relaxation,\textsuperscript{8} rather than contraction. Local release events similar to Ca\textsuperscript{2+} sparks are not observed in adult mammalian skeletal muscle at all during E-C coupling.\textsuperscript{9}

“Local Control” Theory of Cardiac E-C Coupling

The essence of the “local control” theory of E-C coupling in cardiac muscle is that SR Ca\textsuperscript{2+} release is controlled by the L-type Ca\textsuperscript{2+} current because independent, elementary events of SR Ca\textsuperscript{2+} release are “recruited” by Ca\textsuperscript{2+} flowing through single L-type Ca\textsuperscript{2+} channels, and not by the average [Ca\textsuperscript{2+}]\textsubscript{i}, within the cell. One of the structural bases of local control is the separation of SR Ca\textsuperscript{2+} release channels (RyR) at the ends of sarcomeres, near L-type Ca\textsuperscript{2+} channels in transverse tubules. There, RyR can be activated strictly by the Ca\textsuperscript{2+} in their own immediate (local) molecular environment. In local control theory, the [Ca\textsuperscript{2+}]\textsubscript{i} in that environment is established first by Ca\textsuperscript{2+} entry via L-type Ca\textsuperscript{2+} channels, and the release in separate clusters of RyR is independent of release in other such clusters, by virtue of physical separation. Such triggered local release appears to be manifest as the so-called Ca\textsuperscript{2+}“sparks,”\textsuperscript{2} first observed as spontaneous events of SR Ca\textsuperscript{2+} release. Although the exact nature and origin of Ca\textsuperscript{2+} sparks remain uncertain, the prevailing view is that the whole-cell Ca\textsuperscript{2+} transient may be explained as the spatial and temporal summation of Ca\textsuperscript{2+} sparks, each triggered locally by the flow of current through a single L-type Ca\textsuperscript{2+} channel.

Received July 2, 1999; accepted August 25, 1999.

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(Circ Res. 1999;85:770-776.)

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Indirect evidence of local control of cardiac SR Ca\(^{2+}\) release was obtained from analysis of the relationship between whole-cell Ca\(^{2+}\) current and whole-cell SR Ca\(^{2+}\) release flux.\(^{10,11}\) Work of the previous years had produced a detailed description and theory of the whole-cell Ca\(^{2+}\) transient, in terms of the various cellular processes determining cytoplasmic free [Ca\(^{2+}\)].\(^{12-16}\) Control of SR Ca\(^{2+}\) release by L-type Ca\(^{2+}\) current was established by similarities in the voltage dependence of peak SR Ca\(^{2+}\) release flux and peak Ca\(^{2+}\) current,\(^{10}\) and by the fact that SR Ca\(^{2+}\) release can be turned off by stopping the Ca\(^{2+}\) current rapidly,\(^{10}\) either by repolarization (where current deactivates) or by depolarization to very positive voltages (where driving force for Ca\(^{2+}\) entry is small). SR Ca\(^{2+}\) release can also be triggered by “tails” of Ca\(^{2+}\) current through L-type Ca\(^{2+}\) channels on repolarization from positive pulse potentials.\(^{12}\) (Repolarization would be expected only to deactivate a voltage-dependent release mechanism.) The key observation in suggesting local control, however, was that although both peak SR Ca\(^{2+}\) release and peak L-type Ca\(^{2+}\) current displayed a bell-shaped dependence on membrane voltage during voltage-clamp pulses, the relationships were, in fact, different. Small Ca\(^{2+}\) currents at negative potentials (where single-channel currents are relatively large) were much more efficacious in triggering SR Ca\(^{2+}\) release than Ca\(^{2+}\) currents at positive potentials (where single channel currents are more frequent but smaller). On the basis of these data, we\(^{11}\) postulated that SR Ca\(^{2+}\) release flux was controlled locally, by single L-type Ca\(^{2+}\) channel currents. Local release events could not be resolved, however, with the methods available at that time.

**Visualization of Local SR Ca\(^{2+}\) Release:**

**Ca\(^{2+}\) Sparks**

The first observation of local SR Ca\(^{2+}\) release (aside from Ca\(^{2+}\) waves) was that of Cheng et al,\(^2\) who used confocal microscopy to see small, spontaneous, nonpropagating fluorescence transients in flou-3-loaded rat cardiac cells. They named the fluorescent events “Ca\(^{2+}\) sparks.” They concluded from the effects of ryanodine that the Ca\(^{2+}\) sparks arose from the SR and represented release of Ca\(^{2+}\) from one or a small group of RyRs. It was postulated in that work that such events might also occur with electrical excitation.

**Ca\(^{2+}\) Sparks Evoked by Electrical Depolarization:**

**Relation to L-type Ca\(^{2+}\) Current**

After the initial report of spontaneous Ca\(^{2+}\) sparks, several studies appeared in which Ca\(^{2+}\) sparks were evoked by electrical stimulation, thus providing the first direct evidence that Ca\(^{2+}\) sparks might underlie SR Ca\(^{2+}\) release during normal E-C coupling. The first study combining whole-cell voltage clamp and confocal microscopy\(^17\) revealed local, ryanodine-sensitive, inhomogeneities in Ca\(^{2+}\) during small depolarizing pulses that evoked only small Ca\(^{2+}\) currents. These evoked local transients appeared to be identical to spontaneous Ca\(^{2+}\) sparks, recorded in the same cells. Spatially averaged [Ca\(^{2+}\)], obtained by integrating line-scan images, was identical to that obtained in the earlier studies in which whole-cell Ca\(^{2+}\) had been measured. These results made abundantly clear the fact that observations of spatially averaged [Ca\(^{2+}\)] would be misleading for the understanding of E-C coupling, given that the spatially averaged [Ca\(^{2+}\)] was clearly different from that in the region of the RyR. The sparks occurred near t-tubules.\(^{18}\) Similar results were obtained during action potentials,\(^{19}\) and cadmium, a blocker of L-type Ca\(^{2+}\) channels, increased the inhomogeneity of the Ca\(^{2+}\) transient, suggesting that individual SR release events were distinguishable.

In a detailed study,\(^3\) it was shown that Ca\(^{2+}\) sparks were evoked with a time and voltage dependence similar to that expected for first latency histograms of L-type Ca\(^{2+}\) channels.\(^{20}\) When the probability of L-type Ca\(^{2+}\) channel activation is made extremely low through the use of Ca\(^{2+}\) channel blockers, only a few, widely separated L-type Ca\(^{2+}\) channels will open. Changes in spatial average [Ca\(^{2+}\)], were negligibly small under this condition, rendering it unlikely that the Ca\(^{2+}\) sparks could be triggered by anything other than the opening of a nearby L-type Ca\(^{2+}\) channel. Although single-channel currents could not be recorded or localized, it seems reasonable that the only factors that could explain the voltage and time dependence of the spatially isolated sparks were the voltage and time dependence of single L-type Ca\(^{2+}\) channels. A different study at the same time reached the same conclusion by showing that the number of Ca\(^{2+}\) sparks increased with voltage during ramped depolarization (−70 to −40 mV), similarly to the increase of the Ca\(^{2+}\) current with voltage during the ramp.\(^4\) Recently, two new studies\(^21,22\) have confirmed the original Ca\(^{2+}\) spark latency histograms\(^3\) and presented the experimental relationship between Ca\(^{2+}\) currents and Ca\(^{2+}\) spark probability in much more detail.

Several attempts have been made to describe precisely the mathematical relationship between the probability of evoking Ca\(^{2+}\) sparks and the Ca\(^{2+}\) current. The first of these\(^6\) provided the basic equation for the probability (P) that a Ca\(^{2+}\) spark will be triggered by the opening of a single L-type Ca\(^{2+}\) channel. A subsequent attempt used a similar analysis\(^23\) but failed to take into account the time dependence of the probability of L-type Ca\(^{2+}\) channel opening. This problem has now been recognized and clarified.\(^23\) The most detailed analysis of experimental data so far has been that of Collier et al,\(^21\) who found that the time constants describing Ca\(^{2+}\) current and Ca\(^{2+}\) spark occurrence were not significantly different at membrane potentials between −30 and +30 mV. From comparison of the experimental results to a simple model, these authors were able to confirm that the opening of a single L-type Ca\(^{2+}\) channel initiates a Ca\(^{2+}\) spark. Thus, the body of experimental and theoretical work, to date, supports the idea that Ca\(^{2+}\) sparks are triggered by the opening of a single L-type Ca\(^{2+}\) channel.

The question of how many Ca\(^{2+}\) ions must bind to an RyR to initiate a Ca\(^{2+}\) spark remains controversial. Mathematical modeling of the dyadic space suggested that the local [Ca\(^{2+}\)], would be proportional to the single L-type Ca\(^{2+}\) channel current, \(i\).\(^25\) Some data have been presented\(^23\) that spark probability is dependent on the square (ie, power, 2) of the single channel current (and, therefore, possibly, on the square of local [Ca\(^{2+}\)]) as might arise if two Ca\(^{2+}\) bind to an RyR to trigger a spark. A recent study\(^26\) using whole-cell Ca\(^{2+}\) transients and Ca\(^{2+}\) currents, however, demonstrated a linear
relationship between peak Ca\(^{2+}\) currents and the maximum rate of rise of the Ca\(^{2+}\) transient, and the results were interpreted to mean that only one Ca\(^{2+}\) need bind to activate an RyR and Ca\(^{2+}\) release. The use of whole-cell fluorescence transients for this calculation seems inherently less reliable a method, as opposed to observing Ca\(^{2+}\) sparks directly.

Finally, activation of Ca\(^{2+}\) sparks by patch depolarization of cardiac cells has been achieved,\(^2\) although the probabilities of activation were lower than would be required for normal E-C coupling. It seems likely that formation of the membrane patch recording disrupted the normal coupling between L-type Ca\(^{2+}\) channel and RyR. Thus, direct evidence, in the form of simultaneous recordings, that a single opening of a single L-type Ca\(^{2+}\) channel can trigger a Ca\(^{2+}\) spark, is still lacking.

### Ca\(^{2+}\) Sparks Sum in Space and Time to Produce the Whole-Cell Ca\(^{2+}\) Transient

The work described above supported the concept that Ca\(^{2+}\) sparks are activated by L-type Ca\(^{2+}\) channel currents but did not establish directly that Ca\(^{2+}\) sparks summed independently to produce the whole-cell Ca\(^{2+}\) transient. Cannell et al\(^6\) had provided theoretical evidence of the feasibility of this idea. The fact that the total number of Ca\(^{2+}\) sparks depended on voltage in the same manner as the whole-cell Ca\(^{2+}\) transient\(^1\) also implied that this was true. Nevertheless, direct evidence on this point has been lacking, until recently, when local Ca\(^{2+}\) release has been imaged with fast confocal microscopes with line scanning\(^28\) and with 2D (full frame) imaging.\(^22\) The line-scan images\(^28\) showed local release, at z lines, during the initial phase of a normal Ca\(^{2+}\) transient. Furthermore, if a Ca\(^{2+}\) spark had occurred spontaneously just previously at a particular z line, then release failed at that z line, indicating directly the role of Ca\(^{2+}\) sparks in E-C coupling (and that the processes producing Ca\(^{2+}\) sparks may experience refractoriness). The fast 2D images\(^22\) also showed localized release, probably at dyadic junctions. It has been suggested that the nonlinear relationship between single-channel current and SR Ca\(^{2+}\) release (possibly dependent on \(I\)) provides a mechanism whereby relatively small local changes in [Ca\(^{2+}\)], can increase spark probability by a factor of 10\(^6\) during normal E-C coupling.\(^24\)

Whole-cell Ca\(^{2+}\) release flux has been measured directly recently\(^29\) using a novel fluorescence method, in which cytoplasmic [Ca\(^{2+}\)], is reduced through the use of high concentrations of EGTA, and localized SR Ca\(^{2+}\) release is observed with the low-affinity fast Ca\(^{2+}\) indicator, Oregon Green 488 BAPTA-5N. This method permits visualization of release at specific sites during voltage-clamp pulses. The overall waveform of the release and its absolute value were reported similar to that obtained earlier,\(^10,11,16\) through mathematical analysis of the whole-cell Ca\(^{2+}\) transient, in the absence of EGTA. The number of Ca\(^{2+}\) sparks involved in the total release flux remains to be determined.

### Molecular Origin and Mechanisms of Cardiac Ca\(^{2+}\) Sparks

The molecule responsible for Ca\(^{2+}\) sparks in cardiac muscle is the RyR\(_2\) isoform of the ryanodine receptor. Cheng et al\(^2\) clearly favored the hypothesis that Ca\(^{2+}\) sparks arose from the opening of a single RyR, which would certainly be an “elementary event,” because it would arise from one molecule. Of course, the possibility that Ca\(^{2+}\) sparks arose from a small number of RyR “acting in concert” could not be excluded by their data. The distinction between these possibilities is extremely important, however, for our understanding of the mechanism of SR Ca\(^{2+}\) release during E-C coupling. If, in fact, a Ca\(^{2+}\) spark arises from just one RyR, then we are left with the very puzzling question of why the others in the group are not activated by the Ca\(^{2+}\) released from one. Similarly, if they “act in concert,” then the very interesting question of cooperativity among a large group of macromolecules arises. Much of the work appearing to establish Ca\(^{2+}\) sparks as elementary events of E-C coupling relied on accurately counting the numbers of Ca\(^{2+}\) sparks in confocal line-scan images. Sparks were typically identified subjectively, or on the basis of some arbitrary criterion, such as minimum spatial half-width or an amplitude threshold. This enabled counting Ca\(^{2+}\) sparks, and when such criteria were used, it appeared that spontaneous and evoked Ca\(^{2+}\) sparks were identical. Furthermore, the mean amplitude of (counted) evoked Ca\(^{2+}\) sparks was independent of voltage,\(^3\) a finding confirmed again recently.\(^21\) However, confocal line-scan images invariably show small changes in fluorescence that are difficult to categorize. Are these Ca\(^{2+}\) sparks occurring off the laser scan line, or are they different types of events of SR Ca\(^{2+}\) release? The possible existence of events of SR Ca\(^{2+}\) release different from Ca\(^{2+}\) sparks or yet smaller than Ca\(^{2+}\) sparks throws into question the notion of Ca\(^{2+}\) sparks as truly “elementary” events of E-C coupling. Furthermore, it was recognized early on that the limitations of confocal imaging will make it difficult to distinguish out-of-focus Ca\(^{2+}\) sparks from possible small Ca\(^{2+}\) sparks.\(^30,31\) At present, the question of the number of RyR and their gating pattern underlying cardiac Ca\(^{2+}\) sparks remains unresolved.\(^32\) Nevertheless, the theory of Ca\(^{2+}\) spark amplitude distributions is now better understood, both in cardiac muscle\(^33\) and in skeletal muscle.\(^34\) In addition, “automatic Ca\(^{2+}\) spark detection” programs\(^29,33\) can be used to eliminate bias of the observer. The best available data from analysis of Ca\(^{2+}\) spark amplitude distributions suggest that they represent a distribution of “source strengths.”\(^33\) Here, “source strength” refers to the combination of RyR open time and current amplitude. At present, however, it cannot be distinguished reliably whether or not such Ca\(^{2+}\) spark amplitude distributions are fit better by a gaussian distribution or an exponential distribution of “source strengths.”

The first substantive indication that cardiac Ca\(^{2+}\) sparks may not arise from single RyR came when multiple sites of origin were resolved in ventricular cells\(^35\) and in atrial cells.\(^36\) In ventricular cells, transverse scanning revealed multiple sites of origin, perhaps corresponding to separate clusters of RyR.

Ca\(^{2+}\) sparks with multiple sites of origin are distinct from the postulated Ca\(^{2+}\) quarks, which may represent release from single RyR. When SR Ca\(^{2+}\) release was evoked by photolysis of caged Ca\(^{2+}\) in the whole cell, Ca\(^{2+}\) sparks were not observed, leading to the suggestion that release occurred as
unresolvable events. The existence of Ca\(^{2+}\) quarks was postulated, units of SR release smaller than Ca\(^{2+}\) sparks. This release gave rise to spatially uniform changes in Ca\(^{2+}\), a puzzling observation because of the lack of any known uniformly distributed SR Ca\(^{2+}\) release channels. When Ca\(^{2+}\) was released in a small volume by two-photon photolysis, small events of SR Ca\(^{2+}\) release were observed directly, for the first time. These were abolished by SR depletion (and therefore not due directly to photolytically released Ca\(^{2+}\)) and were smaller in amplitude than typical Ca\(^{2+}\) sparks.

The original computations of the flux of Ca\(^{2+}\) underlying Ca\(^{2+}\) sparks was consistent with the idea that a Ca\(^{2+}\) spark could arise from a single RyR, if it was assumed that the flux through a single RyR was \(\approx 4\) pA. However, the most recent data from lipid bilayer experiments under quasiphysiological conditions suggest that the unitary Ca\(^{2+}\) current should be <0.6 pA. This implies that multiple RyR are involved in the generation of a Ca\(^{2+}\) spark. Although comparisons between cardiac Ca\(^{2+}\) sparks and frog skeletal muscle Ca\(^{2+}\) sparks may not be valid, a detailed model of E-C coupling in this tissue suggests that Ca\(^{2+}\) sparks arise from multiple RyR. Recently, “coupled” gating of isolated RyR has been demonstrated, and the potential of “coordinated” gating of cardiac RyR to explain cardiac E-C coupling has been noted.

Termination of the Ca\(^{2+}\) spark and/or refractoriness in spark generation is expected to be extremely important in E-C coupling. A mechanism must exist by which RyR are inactivated and not available to release Ca\(^{2+}\) again, in order for a Ca\(^{2+}\) transient to be produced. This mechanism appears not to be either SR depletion or “stochastic inactivation.” Ca\(^{2+}\) release appears to be terminated by an “active extinguishing mechanism” such as Ca\(^{2+}\)-dependent inactivation or adaptation. The possible roles in terminating release of the accessory proteins, sorcin and FKBP12, have been discussed recently.

**Other Putative Sources of Ca\(^{2+}\) to Trigger SR Ca\(^{2+}\) Release**

Although there is a consensus that the L-type Ca\(^{2+}\) current is the major trigger for SR Ca\(^{2+}\) release, other triggers have been suggested. In guinea pig ventricular myocytes, T-type Ca\(^{2+}\) current can trigger SR Ca\(^{2+}\) release, although much less efficiently than L-type. The ability of the Na\(^{+}/Ca\(^{2+}\) exchanger to operate in the “reverse” mode and cause SR Ca\(^{2+}\) release has been reported in a number of mammalian ventricular cell types, most recently, in the study of Litwin et al. The location of the Na\(^{+}/Ca\(^{2+}\) exchanger in the membranes of cardiac cells remains controversial, with some studies interpreted to show that exchanger molecules are located preferentially in t-tubules, whereas other studies are interpreted to show a more uniform distribution of exchanger molecules. Under experimental conditions during which L-type Ca\(^{2+}\) channels were open but not conducting, Ca\(^{2+}\) entry via “reverse” mode of the Na\(^{+}/Ca\(^{2+}\) exchanger did not induce Ca\(^{2+}\) sparks but rather caused a slow uniform increase in \([Ca^{2+}]\) throughout the cell. The activity of local Na\(^{+}/Ca\(^{2+}\) exchange could set a local \([Ca^{2+}]\), that would shift the sensitivity of the RyR to Ca\(^{2+}\) entering via L-type Ca\(^{2+}\) channels. Recently, a new and functionally distinct Na\(^{+}\) current component (\(I_{Ca(TTX)}\)) has been identified in rat ventricular cells. This new component displays different kinetics, different voltage ranges for both activation and inactivation, and different permeability properties from the classical cardiac Na\(^{+}\) channel. Specifically, \(I_{Ca(TTX)}\) activates over a more negative voltage range than classical cardiac Na\(^{+}\) channels and is highly permeable to Ca\(^{2+}\). Under nonphysiological experimental conditions (ie, Na\(^{+}\)-free external and internal solutions), Ca\(^{2+}\) permeation of \(I_{Ca(TTX)}\) is capable of triggering SR Ca\(^{2+}\) release. These \(I_{Ca(TTX)}\)-evoked Ca\(^{2+}\) transients are delayed markedly in onset and have slower upstrokes compared with Ca\(^{2+}\) transients elicited by L-type Ca\(^{2+}\) currents of similar current density. It is not yet known whether \(I_{Ca(TTX)}\) channels are permeable to Ca\(^{2+}\) in the presence of physiological concentrations of Na\(^{+}\). To the extent that \(I_{Ca(TTX)}\) is relevant to E-C coupling, it will probably have a modulatory role rather than provide a major component of the Ca\(^{2+}\) trigger for SR Ca\(^{2+}\) release and the “gain” of cardiac E-C coupling.
HEK293 cells expressing either the α and β₁ subunit or the α, β_, and β₂ subunits of the Na⁺ channel.

**Putative Protein Kinase A–Dependent Voltage-Sensitive Ca²⁺ Release Mechanism (VSRM)**

Recently, it has been postulated that a component of SR Ca²⁺ release in mammalian cardiac muscle is activated by changes in membrane voltage or to be “sensitive” to membrane voltage.⁶⁻¹⁻⁶¹ ⁶² These experiments have been performed under different conditions than those earlier, in which the detailed relationship between I₈ and SR Ca²⁺ release was studied through the use of holding potentials that activated I₈, and permitted good control of membrane voltage during voltage-clamp pulses. Although temperature and internal monovalent cations (K⁺ versus Cs⁺) were once thought to be important in observing VSRM, it now appears that the most important conditions for observing VSRM (in dialyzed cells) are the inclusion of cAMP and the use of relatively negative prepulse potentials.⁶³ In these experiments, whole-cell L-type Ca²⁺ currents and Ca²⁺ transients (or contraction) are measured at 37°C in single adult rat, rabbit, or guinea pig ventricular cells. Typically Na⁺ channels are inhibited with lidocaine (300 μM), and Ca²⁺ entry via Na⁺/Ca²⁺ exchange is prevented with Na⁺-free pipette solutions. In the presence of 8-Bromo-cAMP, a two-step protocol (from −65 to −40 to 0 mV) elicits two contractions, of approximately equal magnitude.⁶⁵ In this “two-step” protocol, the first contraction (elicited by depolarization to −40 mV) is absent if cAMP is absent but persists in the presence of Ca²⁺ channel blockers (Cd²⁺). The second is absent if Ca²⁺ channels are blocked but persists in the absence of CaMP and the use of relatively positive holding potentials (−40 mV). Because the putative VSRM was shown to be completely inactivated at −40 mV,⁶³ the two-step protocol is thought to produce an initial contraction (at −40 mV) that is dependent solely on the VSRM and does not require Ca²⁺ entry via Ca²⁺ channels, whereas the second (to 0 mV) is thought to produce a contraction that is dependent solely on Ca²⁺ entry. In earlier work, it was shown that ryanodine (30 mmol/L) abolished the VSRM contraction but not those triggered by I₈, and that the VSRM contractions were abolished in the total absence of external Ca²⁺ but persisted in the presence of external [Ca²⁺] of 50 μmol/L. It is not known yet whether such release might occur via Ca²⁺ sparks.

The experiments described above are intriguing, but we have several reservations about them. (1) Voltage-activated Ca²⁺ release should occur in the absence of external Ca²⁺. This has not been demonstrated adequately in any of the experiments cited above. On the contrary, Nabauer et al⁶⁴ have already shown (rather convincingly) that contractions elicited by clamp pulses from −60 mV (and more negative) to 0 mV fail completely after switching rapidly to Ca²⁺ free solutions. Most importantly, neither Na⁺ nor Ba²⁺, which do flow through L-type Ca²⁺ channels and which do support voltage-activated Ca²⁺ release in skeletal muscle, was capable of eliciting contraction. These elegant and conclusive experiments⁶⁴ should be repeated in the presence of high concentrations of intracellular cAMP. (2) Ca²⁺ channels are not totally unavailable at −40 mV and therefore, steps to −40 mV will activate L-type Ca²⁺ channels, particularly in the highly potentiated state produced by high concentrations of cAMP. The small currents flowing at −40 mV should be identified unequivocally. (3) The VSRM should be studied mainly at positive potentials, where it is well established that Ca²⁺ entry is not sufficient to trigger contraction, but a VSRM should be fully activated. In fact, recent work⁶⁵ using similar conditions to those used by Ferrier et al⁶⁶ failed to show Ca²⁺ release at very positive membrane potentials. (4) The amount of contraction in the experiments cited above is relatively small. For example, contractions were only about 3 μm at 0 mV from −65 mV with 50 μmol/L cAMP (Figure 10 of Reference 63). Such small contractions, under such highly potentiated conditions, are puzzling. (5) The L-type Ca²⁺ currents in the presence of 50 μmol/L cAMP are extremely large (eg, 7 nA, Figure 1 of Reference 63). Under these conditions, the SR must be highly loaded, particularly because the SERCA will be highly stimulated. Under these conditions, it may be “trigger happy.” Regenerative releases could be stimulated by the opening of just a few Ca²⁺ channels. (6) It will be difficult to study the putative VSRM selectively, because any release activated by voltage may inevitably be amplified by CICR. In frog skeletal muscle, the voltage-activated release is thought to provide the initial change in Ca²⁺ that activates the RyR, via CICR, that are not facing voltage sensors.⁴⁰ It is not possible to separate totally one type of release from the other. (7) Ca²⁺ transients should be measured, and the rate of SR Ca²⁺ release flux should be calculated.¹⁰,²⁹ Maximum shortening is not a reliable indicator of the peak rate of SR Ca²⁺ release in mechanically unloaded myocytes. (8) Organic L-type Ca²⁺ channel blockers should be compared with the inorganic blockers (Cd²⁺, Ni²⁺, and Co²⁺), because the mode of block of these substances is quite different. (9) The experiments demonstrating VSRM in dialyzed cells use cAMP in the intracellular perfusion solution, and L-type Ca²⁺ currents are very large. We wonder, therefore, whether cells in such a condition are capable of responding further to β₁-agonist stimulation. Within the living organism, the heart is capable of increasing output substantially over basal levels as a result of β₁-agonist stimulation. If cells demonstrating VSRM are not responsive to β₁-agonist stimulation, the implication is that the VSRM is not important in normal E-C coupling in the basal state.

From the above, we conclude that, if VSRM exists in cardiac muscle, it must differ substantially from voltage-activated release in skeletal muscle. Finally, the difficulty of controlling or regulating such a putative mechanism has been pointed out recently.⁶⁶

**Summary**

The body of experimental work on E-C coupling in normal cardiac muscle supports a number of conclusions. (1) Ca²⁺ entry via L-type Ca²⁺ channels is the predominant source of the Ca²⁺ that triggers SR Ca²⁺ release under conditions
that support normal E-C coupling. (2) Whole-cell L-type Ca\(^{2+}\) currents and whole-cell Ca\(^{2+}\) transients can be understood in terms of the recruitment and summation of their respective independent local events (namely, single L-type Ca\(^{2+}\) currents and Ca\(^{2+}\) sparks). (3) Ca\(^{2+}\) sparks almost certainly arise from Ca\(^{2+}\) released from a cluster of RyR. (4) The local [Ca\(^{2+}\)] in the region of the RyR is undoubtedly determined by several molecular species, including the L-type Ca\(^{2+}\) channels, the RyR themselves, the Na/Ca exchanger, Na\(^{+}\) channels, Ca\(^{2+}\) pumps, and others. It is important to note that spontaneous Ca\(^{2+}\) sparks do occur in mammalian cardiac muscle under physiological conditions.\(^6^7\) Thus, the concepts derived from single-cell studies appear to be relevant to the intact tissue.

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**Key Words:** Ca\textsuperscript{2+} spark □ excitation-contraction coupling □ L-type Ca\textsuperscript{2+} channel □ sarcoplasmic reticulum □ ryanodine receptor
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doi: 10.1161/01.RES.85.9.770

\textit{Circulation Research} is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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