Integrative Analysis of Calcium Cycling in Cardiac Muscle


Abstract—The control of intracellular calcium is central to regulation of contractile force in cardiac muscle. This review illustrates how analysis of the control of calcium requires an integrated approach in which several systems are considered. Thus, the calcium content of the sarcoplasmic reticulum (SR) is a major determinant of the amount of Ca\(^{2+}\) released from the SR and the amplitude of the Ca\(^{2+}\) transient. The amplitude of the transient, in turn, controls Ca\(^{2+}\) fluxes across the sarcolemma and thence SR content. This control of SR content influences the response to maneuvers that modify, for example, the properties of the SR Ca\(^{2+}\) release channel or ryanodine receptor. Specifically, modulation of the open probability of the ryanodine receptor produces only transient effects on the Ca\(^{2+}\) transient as a result of changes of SR content. These interactions between various Ca\(^{2+}\) fluxes are modified by the Ca\(^{2+}\) buffering properties of the cell. Finally, we predict that, under some conditions, the above interactions can result in instability (such as alternans) rather than ordered control of contractility. (Circ Res. 2000;87:1087-1094.)

Key Words: excitation-contraction coupling | sarcoplasmic reticulum | ryanodine receptor

Contraction of cardiac muscle is initiated by an increase of \([\text{Ca}^{2+}]_i\), the magnitude of which is released from the sarcoplasmic reticulum (SR) through a specialized release channel, the ryanodine receptor (RyR), via the process of Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR). The entry of a small amount of \((\text{"trigger"})\text{ Ca}^{2+}\) through the sarcolemmal L-type Ca\(^{2+}\) current \((I_{\text{Ca}})\) produces a localized increase of \([\text{Ca}^{2+}]_i\) in the small space between the surface and SR membranes. This then increases the open probability of the RyR, resulting in the release of Ca\(^{2+}\) from the SR into the cytoplasm. Obviously, for the heart to function as a pump, it must relax as well as contract. Relaxation is initiated by a reduction of \([\text{Ca}^{2+}]_i\), produced either by pumping back into the SR by the SR Ca\(^{2+}\)-ATPase (SERCA) or out of the cell, largely by the sarcolemmal Na\(^+\)-Ca\(^{2+}\) exchange.

Therefore, each time the heart contracts, Ca\(^{2+}\) enters the cytoplasm both from the extracellular fluid and from the SR. For the heart to be in a steady state it is essential that, during each cardiac cycle, exactly that amount of Ca\(^{2+}\) that had entered from outside the cell is pumped back out and that which is released by the SR is returned. In the rest of this article, we will show that this requirement for Ca\(^{2+}\) flux balance has significant implications for the regulation of contraction. However, before dealing with this regulation, we will address a few comments to the properties of intracellular Ca\(^{2+}\) buffers, because these determine the magnitude of the changes of free Ca\(^{2+}\) produced by a given Ca\(^{2+}\) flux.

Ca\(^{2+}\) Buffering

The magnitude of the systolic rise of \([\text{Ca}^{2+}]_i\), depends not only on the magnitude of the fluxes but, in addition, on the Ca\(^{2+}\) buffering power of the cell. Buffering properties of cardiac cells have been measured in a variety of ways. Relatively direct measurements can be made in permeabilized cells, but because some cytoplasmic constituents will have been lost, there is a need for data from intact cells. The general approach is to estimate the change of total Ca\(^{2+}\) (\(\Delta\text{Ca}_T\)) by integrating sarcolemmal Ca\(^{2+}\) fluxes (under conditions where the SR does not contribute) and compare this with the change of \([\text{Ca}^{2+}]_i\), \((\Delta[\text{Ca}^{2+}]_i)\) obtained with a fluorescent indicator. One method measures the increase of \([\text{Ca}^{2+}]_i\), produced by a given \(I_{\text{Ca}}\). The use of pulses that activate different amounts of \(I_{\text{Ca}}\), therefore allows a buffer curve to be produced. An alternative and more rapid method uses caffeine to release Ca\(^{2+}\) from the SR, resulting in an increase of \([\text{Ca}^{2+}]_i\), which decays as Ca\(^{2+}\) is pumped out of the cell on Na\(^+\)-Ca\(^{2+}\) exchange. The integral of the Na\(^+\)-Ca\(^{2+}\) exchange current (after correction for the electroneutral Ca\(^{2+}\)-ATPase) gives a measure of the total amount of Ca\(^{2+}\) pumped out of the cell, and the change of free Ca\(^{2+}\) is obtained from a fluorescent indicator. One caffeine application then gives an entire...
curve (see Figure 3B). This method also has the advantage that it provides a quantitative measure of SR content. To a first approximation, the buffering in the cell can be described with a single $K_s$, although for more precise work, note should be taken of the fact that the overall buffering is made up of contributions from various components with different $K_s$.

These include troponin and calmodulin as well as membrane binding sites. Values for the overall $I_{\text{Ca}}$ depends on at least 2 factors, as follows: (1) the properties of the SR and thence the Ca$^{2+}$ release flux, when a channel opens. In the remainder of this article, we will discuss the contributions of these 2 factors.

Sites at Which CICR May Be Regulated

The amount of Ca$^{2+}$ released from the SR for a given entry on $I_{\text{Ca}}$ depends on at least 2 factors, as follows: (1) the properties of the RyR, in particular the relationship between [Ca$^{2+}$], and the open probability of the RyR, and (2) the Ca$^{2+}$ content of the SR and thence the Ca$^{2+}$ release flux when a channel opens. In the remainder of this article, we will discuss the contributions of these 2 factors.

What Regulates SR Ca$^{2+}$ Content?

Much attention has been focused on the effects of directly modulating the activity of SERCA and, in particular, its interaction with the inhibitory accessory protein phospholamban. Phosphorylation of phospholamban relieves the inhibition of SERCA, thereby stimulating its activity. This occurs, for example, during sympathetic stimulation of $\beta$-receptors resulting in an enhanced SR Ca$^{2+}$ content and increased rate of decay of the Ca$^{2+}$ transient. As would be expected, animals deficient in phospholamban (phospholamban knockout mice) have elevated SR Ca$^{2+}$ contents.

Ca$^{2+}$ transients with faster rates of decay than controls, and a smaller response to $\beta$ stimulation.

Autoregulation of SR Ca$^{2+}$ Content

Another important factor that controls the SR Ca$^{2+}$ content is the level of cytoplasmic Ca$^{2+}$. The higher the [Ca$^{2+}$], the greater the rate of Ca$^{2+}$ pumping into the SR. In addition, Ca$^{2+}$ release from the SR influences sarcolemmal Ca$^{2+}$ fluxes. This can be seen experimentally if the SR Ca$^{2+}$ content is altered. In the experiment illustrated in Figure 1, the SR had initially been emptied by exposure to 10 mmol/L caffeine. When stimulation was begun, the Ca$^{2+}$ transient was initially very small but then recovered over the next minute as the SR refilled with Ca$^{2+}$ (Figure 1A). Figure 1B shows expanded records of these Ca$^{2+}$ transients. Trace b was recorded in the steady state. Accompanying the large Ca$^{2+}$ content is Ca$^{2+}$ entry via the L-type Ca$^{2+}$ current and efflux on the Na$^+$/Ca$^{2+}$ exchange on repolarization (Figure 1C). It is clear that the entry via the L-type Ca$^{2+}$ current balances the efflux on Na$^+$/Ca$^{2+}$ exchange. In other words, in the steady state, Ca$^{2+}$ entry equals efflux. A very different result is seen for the first stimulus (a). The Ca$^{2+}$ entry is larger than in the steady state and the efflux smaller. Therefore, instead of being in Ca$^{2+}$-flux balance, the cell gains Ca$^{2+}$. The Ca$^{2+}$ movements on each pulse are shown in the lower panels of Figure 1A. The larger Ca$^{2+}$ currents when the SR is empty and the decrease in size on refilling result from decreased Ca$^{2+}$-induced inactivation of the L-type Ca$^{2+}$ current. The increase of efflux on Na$^+$/Ca$^{2+}$ exchange with increased systolic Ca$^{2+}$ simply represents the fact that the rate of Ca$^{2+}$ pumping by Na$^+$/Ca$^{2+}$ exchange increases with an increase in the amplitude of the systolic Ca$^{2+}$ transient. The third panel of Figure 1A shows that, on starting stimulation, there is a net gain of Ca$^{2+}$ on each pulse and that this gain disappears in the steady state. Finally, the bottom panel sums the net influx on each pulse. This shows that, by the end of the period shown, the cell had gained $\approx 80$ mmol/L as a result of stimulation.
will result in greater release, thereby decreasing Ca\textsuperscript{2+} entry into and increasing efflux out of the cell. This will tend to decrease SR content toward the initial level. The effectiveness or “gain” of this system will depend on the relationship between SR Ca\textsuperscript{2+} content and sarclolemmal fluxes. The greater the slope of this relationship, the more tightly controlled will be the SR content. However, as discussed later in this article, there may be reasons for not wanting too steep a relationship.

The above focus on the control of sarclolemmal fluxes by SR content does not mean that control of the SERCA by, for example, phosphorylation of phospholamban is unimportant, but rather that such control mechanisms will simply adjust the set point produced by this autoregulation. It is worthwhile noting that the phosphorylamban mechanism itself cannot control SR content, as, with the exception of the results of one study,\textsuperscript{20} phosphorylation depends on parameters other than SR content such as the concentration of cAMP and consequent activation of protein kinase A. In contrast, autoregulation senses the SR content via effects on the amplitude of the systolic Ca\textsuperscript{2+} transient. In this sense, this mechanism carries out a function similar to that of “capacitative” control of Ca\textsuperscript{2+} entry in nonexcitable cells. In capacitative entry, when the endoplasmic reticulum Ca\textsuperscript{2+} content decreases, a signal is produced to increase Ca\textsuperscript{2+} influx across the surface membrane of the cell. This increases Ca\textsuperscript{2+} entry via a channel known as IC\textsubscript{RAC} (Ca\textsuperscript{2+} release–activated Ca\textsuperscript{2+} channel). The Ca\textsuperscript{2+} flux through this channel is somehow increased by depletion of the Ca\textsuperscript{2+} content of the endoplasmic reticulum (for review see Barritt\textsuperscript{27}). It may be that the large magnitude of the transmembrane Ca\textsuperscript{2+} fluxes in the heart makes modulation of IC\textsubscript{S} and the Na\textsuperscript{+}–Ca\textsuperscript{2+} exchange a more suitable strategy than IC\textsubscript{RAC}.

Figure 1A shows that increasing the SR Ca\textsuperscript{2+} content (bottom panel) results in a large increase of the amplitude of the systolic Ca\textsuperscript{2+} transient (top). This is emphasized in Figure 3A, which shows that the amount of Ca\textsuperscript{2+} released from the SR increases steeply with increased content such that, at higher contents, a greater fraction of the SR content is released.\textsuperscript{7,21,28–30} This steep dependence has 2 consequences, as follows: (1) it makes it imperative that SR content be controlled and (2) it means that an increase of SR Ca\textsuperscript{2+} content is an effective positive inotropic maneuver.

The above considerations mean that, in the steady state, the systolic Ca\textsuperscript{2+} transient must be of a magnitude to produce a Ca\textsuperscript{2+} efflux that exactly balances the Ca\textsuperscript{2+} influx into the cell. This can explain the effects of many inotropic maneuvers without any knowledge of the internal mechanisms of the cell. Thus, an increase of the L-type Ca\textsuperscript{2+} current will increase Ca\textsuperscript{2+} influx. In the steady state, this will require an increased Ca\textsuperscript{2+} transient to support an increased efflux. Catecholamines accelerate the rate of decay of the systolic Ca\textsuperscript{2+} transient.\textsuperscript{16} This acceleration will leave less time for the surface membrane to remove calcium, and therefore a larger Ca\textsuperscript{2+} transient is required to support the same Ca\textsuperscript{2+} efflux.

### Diastolic Ca\textsuperscript{2+} Fluxes

In the examples presented above, the cell is in calcium balance at the end of systole. It is, however, possible that there may be a net flux of Ca\textsuperscript{2+} during the systolic period that is compensated by an equal and opposite flux during diastole. An example is provided by the effects of cardiac glycosides. These have long been known to increase the magnitude of the systolic Ca\textsuperscript{2+} transient.\textsuperscript{31–33} This results from an increase of intracellular Na\textsuperscript{+} concentration and consequent effects on Na\textsuperscript{+}–Ca\textsuperscript{2+} exchange leading to an increase of SR Ca\textsuperscript{2+} content.\textsuperscript{34,35} One might expect that this would be due to a decreased ability of Na\textsuperscript{+}–Ca\textsuperscript{2+} exchange to remove Ca\textsuperscript{2+} requiring a larger Ca\textsuperscript{2+} transient to produce the same efflux. It appears, however, that the cell loses Ca\textsuperscript{2+} during systole in strophanthidin (as the larger Ca\textsuperscript{2+} transient increases Ca\textsuperscript{2+} efflux and decreases influx) and that this is compensated for by increased diastolic Ca\textsuperscript{2+} entry presumably on Na\textsuperscript{+}–Ca\textsuperscript{2+} exchange.\textsuperscript{36} This emphasizes the potential importance of the diastolic as well as the systolic period. Longer-term\textsuperscript{37} modulation must also be considered. For example, altering the rate of stimulation can produce gradual changes of contraction that have been linked to changes of intracellular Na\textsuperscript{+} concentration. Such longer-term effects will alter the steady states referred to above by altering SR content.

### Limitations on SR Ca\textsuperscript{2+} Content as the Only Regulator of Systolic Ca\textsuperscript{2+}

One unanswered question is the extent to which SR Ca\textsuperscript{2+} content can be increased to increase the force of contraction. There is presumably a maximum level of SR Ca\textsuperscript{2+} content. It has been suggested that the maximum SR free Ca\textsuperscript{2+} concentration is limited only by the energy available from ATP.\textsuperscript{12} In contrast to this result, agents that inhibit opening of the RyR increase the SR content,\textsuperscript{38} suggesting that leak of Ca\textsuperscript{2+} out of the SR may limit the maximum content. Furthermore, the maximum content also appears to be limited by spontaneous Ca\textsuperscript{2+} release (waves) occurring at high Ca\textsuperscript{2+} loads.\textsuperscript{39,40} Whatever the origin of the maximum SR content, its existence means that mechanisms in addition to an increase of SR content are required to increase the magnitude of the systolic Ca\textsuperscript{2+} transient.

### Modulation of the RyR

It is well established that the open probability of the RyR can be affected by substances other than cytoplasmic Ca\textsuperscript{2+} concentration.\textsuperscript{41} Among the substances that increase the open probability are caffeine,\textsuperscript{52} 2,3-butanedione monoxime (BDM),\textsuperscript{53} and cADP-ribose.\textsuperscript{44,45} The open probability is also increased by phosphorylation.\textsuperscript{46–48} Finally, the RyR is also associated with the FK506 binding protein (FKBP12.6). These FK binding proteins stabilize the RyR and, in their absence, long-lasting subconductance states appear, resulting in increased Ca\textsuperscript{2+} flux.\textsuperscript{49} Dissociation of FKBP12.6 from the RyR is increased by immunophilsins such as FK506 or phosphorylation.\textsuperscript{48} The open probability can be decreased by local anesthetics such as tetracaine.\textsuperscript{51–53} Of potential relevance to ischemia, the open probability is decreased by acidification\textsuperscript{54} or a decrease of cytoplasmic ATP concentration.\textsuperscript{54} For convenience, we will consider this area in 2 parts, modulation of the RyR during systole and diastole.
Figure 2. Changes of sarcolemmal fluxes and SR Ca\(^{2+}\) release during potentiation of CICR by caffeine (500 \(\mu\)mol/L). A, Time traces show the following (from top to bottom): a, \([Ca^{2+}]_i\); b, changes in total Ca; c, net sarcolemmal flux calculated as Ca\(^{2+}\) entry on \(I_{sc}\) minus efflux on Na\(^+-Ca^{2+}\) exchange; d, fractional Ca\(^{2+}\) efflux, fraction of the total Ca\(^{2+}\) transient that is pumped out of the cell; e, SR Ca\(^{2+}\) content; and f, fractional release, fraction of the SR Ca\(^{2+}\) content that is released. B, Specimen records from a control transient (i) and the first in caffeine (ii). For each transient, records show the following (from top to bottom): \([Ca^{2+}]_i\), membrane current, and calculated movement of Ca\(^{2+}\) across the surface membrane. Note that for transient i, influx \(\approx\) efflux, whereas for transient ii, the efflux is greater than the influx. Data are from a rat myocyte; adapted from Reference 30.

Systolic Regulation of the RyR

Many previous studies have suggested that maneuvers or circumstances that affect either the level of expression or the open probability of the RyR will alter systolic Ca\(^{2+}\). Thus, the depression of contraction in heart failure has been linked to either a decrease in the number of RyRs\(^{35-61}\) or a change in their properties such that fewer are opened by a given trigger increase of \([Ca^{2+}]_i\).\(^{62,63}\) This conclusion does not, however, fit well with results examining the effects of maneuvers that alter the RyR pharmacologically. Figure 2A shows that increasing the open probability with a low concentration of caffeine produces a purely transient increase in the amplitude of the systolic Ca\(^{2+}\) transient.\(^{30}\) Similarly, BDM produces only a transient increase.\(^{54-66}\) Other work shows that a decrease of open probability of the RyR produced by either tetracaine\(^67\) or decreased pH\(^{68}\) produces a decrease of systolic \([Ca^{2+}]_i\), that is also transient.

The explanation of the transient effects of modulation of the RyR lies with the interactions between SR content and sarcolemmal fluxes reviewed above. This is shown in Figure 2. The application of caffeine produces a transient increase of systolic \([Ca^{2+}]_i\), that decays to resting levels. The steady-state Ca\(^{2+}\) transient in caffeine is identical to that of the control.\(^{30}\) The specimen traces of Figure 2B show the accompanying membrane currents and calculated Ca\(^{2+}\) fluxes for both the control and the first transient in caffeine. In control conditions (Figure 2Bi), Ca\(^{2+}\) influx and efflux are equal and the cell is therefore in Ca\(^{2+}\) flux balance. However, when caffeine is applied (Figure 2Bii), the increase of the systolic Ca\(^{2+}\) transient results in an increase of Ca\(^{2+}\) efflux on Na\(^+-Ca^{2+}\) exchange such that the Ca\(^{2+}\) efflux is greater than the influx. This results in a predicted loss of Ca\(^{2+}\) from the cell. Panel c of Figure 2A shows the net sarcolemmal Ca\(^{2+}\) flux (ie, Ca\(^{2+}\) entry minus exit). In control conditions, Ca\(^{2+}\) entry and exit are balanced. However, the increase in the amplitude of the Ca\(^{2+}\) transient in caffeine results in an increase of Ca\(^{2+}\) efflux and thence a net loss of Ca\(^{2+}\) from the cell and therefore the SR (panel e).

The transient nature of the response to low concentrations of caffeine therefore arises as follows. In the steady state, Ca\(^{2+}\) influx equals Ca\(^{2+}\) efflux. Initially in caffeine, the larger Ca\(^{2+}\) transient results in greater efflux (to a level greater than the influx), resulting in a net loss of Ca\(^{2+}\) from the cell and SR. This, in turn, will decrease the amplitude of the Ca\(^{2+}\) transient, thereby decreasing the amount of Ca\(^{2+}\) pumped out of the cell. Eventually, a new steady state will be reached at which the Ca\(^{2+}\) efflux equals the influx. It is important to realize that, so long as neither the Ca\(^{2+}\) influx nor the properties of Na\(^+-Ca^{2+}\) exchange are altered, this steady state can only be reached when the Ca\(^{2+}\) transient is the same size as the control one.

Direct measurements of SR Ca\(^{2+}\) content show that the decrease of the potentiation of systolic Ca\(^{2+}\) produced by caffeine or BDM are, indeed, accompanied by a decrease of SR content.\(^{30,64,66}\)

The transient decrease of systolic Ca\(^{2+}\) produced by agents that decrease the open probability of the RyR has a similar explanation. In brief, the decrease of the Ca\(^{2+}\) transient will decrease the amount of Ca\(^{2+}\) that leaves the cell (to a value less than that of the influx). This will then result in an increase of SR content\(^{67,68}\) and a consequent increase of systolic Ca\(^{2+}\) until both systolic Ca\(^{2+}\) and Ca\(^{2+}\) efflux return to control levels, accompanied by an increase of SR Ca\(^{2+}\) content.
If, for example, stimulation of the RyR has no steady-state effect on the amplitude of systolic Ca\(^{2+}\) transient, one should consider why it is regulated. There are 2 possible explanations, which can be understood by considering the inotropic effects of increasing the L-type Ca\(^{2+}\) current.\(^{25,30}\) (1) Maneuvers that increase the amplitude of \(I_{\text{Ca}}\) will increase the loading of the cell, and therefore the SR, with Ca\(^{2+}\). This will produce a slowly developing increase of systolic Ca\(^{2+}\) and cannot produce a physiologically useful rapid increase of contractility. However, the potentiation of the RyR open probability by the increased “trigger” Ca\(^{2+}\) entry will result in a transient increase of systolic Ca\(^{2+}\). The combination of a slow (but maintained) increase and a transient effect will result in a rapidly developing and maintained increase of systolic Ca\(^{2+}\). (2) As pointed out above, excessive increase of SR content results in spontaneous Ca\(^{2+}\) release and also an increase of the gradient against which SERCA pumps. Potentiation of RyR open probability will decrease SR Ca\(^{2+}\) content and avoid these problems.

**Diastolic Modulation of the RyR**

In the above analysis, we assumed that the only effect of modulation of the RyR occurs during systole and, therefore, that any efflux of Ca\(^{2+}\) from the SR through the RyR during diastole can be ignored. As reviewed above, low concentrations of caffeine produce a purely transient potentiation of SR Ca\(^{2+}\) release. In contrast, high concentrations produce a dose-dependent decrease of systolic [Ca\(^{2+}\)].\(^{69-71}\) This is because, at these concentrations, caffeine produces a large increase of SR Ca\(^{2+}\) permeability, even at rest, and thereby depletes the SR. Even if all of the SR content is released, the amplitude of the Ca\(^{2+}\) transient will still be less in control.

A recent study has suggested that such an increase of diastolic Ca\(^{2+}\) release can account for the decrease of Ca\(^{2+}\) transient in heart failure.\(^{48}\) It was found that the RyR from failing hearts was hyperphosphorylated. This resulted in increased sensitivity to activating Ca\(^{2+}\) and the occurrence of subconductance levels. This will increase the Ca\(^{2+}\) leak of the SR and thereby decrease its Ca\(^{2+}\) content. This is an attractive explanation for decreased contraction that does not suffer from the problems reviewed above for the idea that either the number of RyR or their systolic activation are modified. Nevertheless, flux balance must still be maintained. This might happen in 2 ways. (1) Increased diastolic leak may slow the rate of relaxation of the systolic Ca\(^{2+}\) transient. This is the case for high concentrations of caffeine and arises because the increase of leak makes it harder for the SR to remove Ca\(^{2+}\) from the cytoplasm. This leads to a decrease in the rate of decay of the residual Ca\(^{2+}\) transient. The decay of the Ca\(^{2+}\) transient will also be slowed simply as a consequence of the increased Ca\(^{2+}\) buffering at lower [Ca\(^{2+}\)].\(^{13}\) Although the Ca\(^{2+}\) transient is smaller than is the case under control conditions, the fact that it lasts longer means that the Na\(^{-}\)-Ca\(^{2+}\) exchange has longer to pump Ca\(^{2+}\) out of the cell and therefore the efflux can still equal the influx. (2) An alternative explanation is that there may be an increase of diastolic Ca\(^{2+}\). This will support an increased Ca\(^{2+}\) efflux from the cell during diastole that will compensate for decreased efflux in systole.

**Integration of SR Release, Buffering, and Sarcolemmal Ca\(^{2+}\) Transport**

The contributions of all of the processes mentioned above can be seen by considering, again, the effects of increasing the open probability of the RyR with caffeine. The data of Figure 2Aa show the transient increase of systolic Ca\(^{2+}\) produced by caffeine. The change of cytoplasmic total Ca\(^{2+}\) is shown in Figure 2Ab. It is clear that the fractional increase of the amplitude of the free Ca\(^{2+}\) transient is larger than that of the total. This (see above) is due to the [Ca\(^{2+}\)] dependence of Ca\(^{2+}\) buffering. Figure 2Ac shows the net sarcolemmal Ca\(^{2+}\) flux on each pulse. As explained above, this is initially zero as Ca\(^{2+}\) influx and efflux are in balance. However, during the onset of the effects of caffeine, there is a net Ca\(^{2+}\) efflux. Figure 2Ad shows Ca\(^{2+}\) efflux as a fraction of the total Ca\(^{2+}\) transient. This is increased but not by as much as the amplitude of the free Ca\(^{2+}\) transient (see below). Figure 2Ae shows the calculated SR Ca\(^{2+}\) content. This was obtained as follows: sarcolemmal Ca\(^{2+}\) flux minus change of total cytoplasmic Ca\(^{2+}\). As is emphasized in Figure 2Af, the fraction of SR Ca\(^{2+}\) content that is released increases initially in caffeine but then decreases (to a level still higher than that of control) during continued exposure to caffeine. The initial increase is a direct effect of caffeine on the RyR, whereas the subsequent decrease is a consequence of the decrease of SR content. In the steady state, the fraction released is still, however, greater than that of the control, as the same total amount of Ca\(^{2+}\) is released from a reduced SR content. This figure therefore shows the complicated consequences of a simple variation of one cellular parameter, the Ca\(^{2+}\) sensitivity of the RyR.

The various relationships underlying this behavior are shown in Figure 3. Figure 3A summarizes the dependence of the amplitude of the systolic Ca\(^{2+}\) transient on SR Ca\(^{2+}\) content. The change of free Ca\(^{2+}\) is a much steeper function of SR content than is the total Ca\(^{2+}\). The reason for this is shown in Figure 3B; as [Ca\(^{2+}\)] increases, it becomes a very steep function of total Ca\(^{2+}\). The Ca\(^{2+}\) efflux from the cell is plotted as a function of the amplitude of the free Ca\(^{2+}\) transient in Figure 3C. This relationship is tending toward saturation, reflecting the finite \(K_s\) of the Na\(^{-}\)-Ca\(^{2+}\) exchange. The graph of Figure 3D shows the dependence of Ca\(^{2+}\) efflux on SR content. This therefore involves the following 3 processes: the dependence of the total Ca\(^{2+}\) transient on SR content, the buffering properties of the cell, and the dependence of Ca\(^{2+}\) efflux on [Ca\(^{2+}\)]. This relationship is less steep than that of [Ca\(^{2+}\)], on SR content because of the flattening effect produced by saturation of Na\(^{-}\)-Ca\(^{2+}\) exchange. The effect of this is visible in Figure 2Ab; the fractional increase of the Ca\(^{2+}\) efflux is much less than that of the free Ca\(^{2+}\) transient.

In some cases, more than one of the Ca\(^{2+}\) regulation processes can be directly affected by a single factor. For example, the activity of both the SR Ca\(^{2+}\) ATPase and the RyR are regulated by ATP. During ischemia, therefore, when [ATP] falls, uptake of Ca\(^{2+}\) into the SR would be compromised, but inhibition of the RyR might compensate. Some
The above discussion has described a system whereby an increase of SR Ca\(^{2+}\) content leads to an increase of Ca\(^{2+}\) influx and decrease of Ca\(^{2+}\) influx that, in turn, compensate for the increased Ca\(^{2+}\) content. This autoregulation is a classic negative feedback system. It does, however, involve a delay, as the change of SR Ca\(^{2+}\) content on one beat only influences Ca\(^{2+}\) fluxes on the next beat. It is well known that delays can cause instability in negative feedback systems. The potential effect of this can be seen qualitatively as follows. Imagine that there is a very steep relationship between SR content and Ca\(^{2+}\) influx. If the cell begins with a large SR content, then the Ca\(^{2+}\) transient will result in a large loss of Ca\(^{2+}\) from the cell. This will decrease the SR content. The next beat will therefore arise from a depleted SR, resulting in a smaller Ca\(^{2+}\) transient and influx and therefore a net gain of Ca\(^{2+}\) by the cell and thence, on the next beat, a large Ca\(^{2+}\) transient. If this continues, alternating small and large Ca\(^{2+}\) transients will be produced. A simple model of this is shown in Figure 4.


24. Hohenecker M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.

25. Randić M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.

26. Randić M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.

27. Randić M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.

28. Randić M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.

29. Randić M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.

30. Randić M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.

31. Randić M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.

32. Randić M, Suko J. Phosphorylation of the purified cardiac ryanodine receptor Ca2+ release channel by the PKA, PKC and PKG. J Gen Physiol. 1995;101:345–368.


54. Xu L, Mann G, Meissner G. Regulation of cardiac Ca\(^{2+}\) release channel by Ba\(^{2+}\), H\(^+\), Mg\(^{2+}\), and adenine nucleotides under normal and simulated ischemic conditions. *Circ Res*. 1996;79:1100–1109.


Integrative Analysis of Calcium Cycling in Cardiac Muscle


Circ Res. 2000;87:1087-1094
doi: 10.1161/01.RES.87.12.1087

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/87/12/1087

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/