Intracellular Calcium Homeostasis in Cardiac Myocytes

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Calcium homeostasis in cardiac myocytes is of functional importance for at least three reasons. First, cardiac myocytes must achieve a resting cytosolic calcium ion concentration ([Ca\textsuperscript{2+}]) of <200 nmol/L if the contractile elements are to relax. With extracellular [Ca\textsuperscript{2+}] at 1 mmol/L, this low [Ca\textsuperscript{2+}] must be maintained in the presence of a 5,000-fold gradient for Ca\textsuperscript{2+} across the sarcolemma. Second, coupling of excitation to contraction (E-C coupling) in heart involves a complex interaction of membrane electric events mediated by specific ion channels in the sarcolemma. This results in calcium influx, the release of calcium from intracellular stores in the sarcoplasmic reticulum (SR) via Ca\textsuperscript{2+}-specific channels in the SR membrane, and subsequent extrusion of the calcium. This in turn is coordinated with reuptake of calcium into the SR stores. A fundamental principle of this process is that to maintain steady-state calcium homeostasis, the amount of calcium entering the cell with each contraction must be extruded before the subsequent contraction. Third, the force of contraction in cardiac myocytes is modulated by variations in the magnitude of the calcium transient. Drugs that modify calcium homeostasis may significantly alter the force of contraction of an individual myocyte and thus of the intact heart. In this discussion, we review recent work that has increased our understanding of the structure and function of the ion channels and transport proteins that are most critically involved in [Ca\textsuperscript{2+}] homeostasis. We consider how they interact during the processes of E-C coupling, contraction, and relaxation and briefly discuss how a number of positively inotropic drugs produce an increase in force. The reader is also referred to several other recent reviews that deal with these issues.1-3

Control of Resting [Ca\textsuperscript{2+}]

Various ion channels and transport proteins involved in calcium homeostasis in the cardiac myocyte are shown schematically in Figure 1. In the resting ventricular myocyte (Figure 1A), the [Ca\textsuperscript{2+}] is determined by a Ca\textsuperscript{2+} leak that is compensated for by an ATP-dependent sarcolemmal Ca\textsuperscript{2+} pump (sarcolemmal Ca\textsuperscript{2+}-ATPase) and the sarcolemmal Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger. This exchanger has recently been cloned.4 The complementary DNA encodes a protein of 970 amino acids with a molecular mass of 108 kd. The native exchanger protein has a maximal apparent molecular mass of about 160 kd, the discrepancy possibly a result of glycosylation of the native protein. Hydropathy analysis indicates that the protein can be divided into three regions: a hydrophobic NH\textsubscript{2} terminal portion containing six potential membrane-spanning segments; a long hydrophilic region that is modeled as a large cytoplasmic loop; and a hydrophilic COOH terminal portion comprising six potential membrane-spanning segments. At this time, little is known about relations between structure and function of this molecule. However, deletion mutants in which the putative cytoplasmic loop has been removed have been expressed in Xenopus oocytes. Whereas the native molecules have a requirement for activating Ca\textsuperscript{2+}, these mutant exchangers lack this requirement (K. Philipson, personal communication). Some information is also available on the distribution of this exchanger. Recent experiments investigating the binding of antibodies to the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger with immunofluorescence and immunoelectron microscopy indicate that although the exchanger is detectable over the entire myocyte surface, antibody binding sites appear to be concentrated in the T tubule region of the myocyte.6 The functional significance of this arrangement is not yet clear.

The exchanger is a counterion transporter on which three Na\textsuperscript{+} are exchanged for each Ca\textsuperscript{2+}.7 This results in positive charge movement that opposes the direction of calcium transport. The net electrochemical force producing the exchange is given by the following equation:

\[ A = 3\Delta\mu_{\text{Na}} - \Delta\mu_{\text{Ca}} \]  

where A is the total electrochemical driving force (free energy), \( \Delta\mu_{\text{Na}} \) is the free energy available in the electrochemical gradient for Na\textsuperscript{+}, and \( \Delta\mu_{\text{Ca}} \) is the free energy in the electrochemical gradient for Ca\textsuperscript{2+}. Thus, if three times the energy in the inward Na\textsuperscript{+} gradient exceeds that in the Ca\textsuperscript{2+} gradient, Ca\textsuperscript{2+} will be extruded. If, on the other hand, the Na\textsuperscript{+} gradient is collapsed relative to the Ca\textsuperscript{2+} gradient, Ca\textsuperscript{2+} entry and Na\textsuperscript{+} extrusion takes place. Equation 1 can be stated in a more familiar form as

\[ A = 3E_{\text{Na}} - 2E_{\text{Ca}} - E_m \]  

where \( E_m \) is membrane potential and \( E_{\text{Na}} \) and \( E_{\text{Ca}} \) are the equilibrium potentials for Na\textsuperscript{+} and Ca\textsuperscript{2+}. If the exchange is at equilibrium, \( A = 0 \), and we obtain

\[ E_m = 3E_{\text{Na}} - 2E_{\text{Ca}} \]  

The reversal potential is the membrane potential at which exchange changes direction, and in the case of the...
Na\(^+\)–Ca\(^{2+}\) exchange, it is identical to the equilibrium potential, \(E_{eq}\) (around \(-40\) mV) in resting ventricular cells. The Na\(^+\)–Ca\(^{2+}\) exchanger is known to be voltage sensitive.\(^8\) If the membrane potential is more negative than the reversal potential, as is normally the case in the resting myocyte, the exchanger functions in a Na\(^+\)-in/Ca\(^{2+}\)-out ("forward") mode and thus produces calcium extrusion. The importance of the Na\(^+\)–Ca\(^{2+}\) exchanger in regulating the resting level of calcium within cardiac myocytes was demonstrated most directly by the experiments of Sheu and Fozzard,\(^8\) who measured intracellular sodium and calcium activities in cardiac Purkinje cells with ion-sensitive microelectrodes. These experiments revealed a direct relation between \([\text{Na}^+]\) and \([\text{Ca}^{2+}]\). Thus, increases in \([\text{Na}^+]\) cause dissipation of the electrochemical energy in the \([\text{Na}^+]\) gradient available to extrude \([\text{Ca}^{2+}]\), resulting in an increase in \([\text{Ca}^{2+}]\).

The level of \([\text{Na}^+]\), in myocytes is controlled largely by Na\(^+\)–K\(^{+}\)-ATPase, and thus the Na\(^+\) pump is also an important indirect regulator of Na\(^+\)–Ca\(^{2+}\) exchange. The activity of the Na\(^+\)–Ca\(^{2+}\) exchanger can also be modulated by a variety of other mechanisms. For example, the exchanger is inhibited by acidosis\(^10\) and by severe ATP depletion.\(^11\) It appears likely that ATP increases the affinity of the exchanger for \([\text{Ca}^{2+}]\);\(^12\) but it has not yet been demonstrated that protein kinase-mediated phosphorylation of the exchanger has any regulatory influence. Calmodulin is a 148-amino acid regulatory protein involved in many Ca\(^{2+}\)-dependent signaling pathways.\(^13\) In some transport proteins, including the sarcolemmal calcium ATPase,\(^14\) there is an autoinhibitory calmodulin-binding domain. Calmodulin can increase the activity of the protein by binding to this amino acid sequence, presumably interfering with the interaction of this domain with other components of the molecule and thus relieving the autoinhibition. Recently, Li et al.\(^15\) have synthesized a Na\(^+\)–Ca\(^{2+}\) exchanger inhibitory peptide. This molecule is identical to the calmodulin-binding sequence of the exchanger and effectively inhibits exchange.\(^15\) It is not established, however, that this calmodulin-binding peptide sequence within the exchanger exerts any autoinhibitory effect under normal physiological conditions.

Another Ca\(^{2+}\) transport system that contributes to maintenance of the low cytosolic concentration in ventricular myocytes is the sarcoplasmic calcium ATPase. The structure and function of plasma membrane calcium ATPases have recently been reviewed by Carafoli.\(^16\) The plasmalemmal calcium ATPases are found in most cells and use the free energy released by the hydrolysis of one ATP to transport one Ca\(^{2+}\) out of the cell against its concentration gradient. General properties of the plasma membrane calcium ATPases include a molecular mass of 134,000 kd; a \(K_m\) for \([\text{Ca}^{2+}]\) of <0.5 \(\mu\)mol/L in the optimally activated state; and as mentioned above, stimulation by Ca\(^{2+}\)-calmodulin and possibly kinase-induced phosphorylation. In cultured cardiac myocytes, the rate at which the sarcoplasmic calcium ATPase can extrude the Ca\(^{2+}\) from the myocyte appears to be about 1/10 that of the Na\(^+\)–Ca\(^{2+}\) exchanger over the range of physiological cytosolic calcium concentrations.\(^17\) Cannell\(^18\) has estimated that the Na\(^+\)–Ca\(^{2+}\) exchanger accounts for as much as 75% of resting Ca\(^{2+}\) efflux. Thus, although the sarcoplasmic calcium ATPase probably does contribute to mainte-

nance of \([\text{Ca}^{2+}]\), in resting myocytes, its importance in this regard relative to the Na\(^+\)–Ca\(^{2+}\) exchanger seems to be minor. The intracellular Ca\(^{2+}\) storage sites, such as mitochondria,\(^19,20\) as well as calcium-binding proteins\(^21\) can buffer short-term (seconds to minutes) changes in calcium. Steady-state loading of these storage sites is determined by the \([\text{Ca}^{2+}]\), which in turn is regulated largely by transsarcolemmal calcium entry and extrusion by Na\(^+\)–Ca\(^{2+}\) exchange and perhaps to a lesser extent by the sarcolemmal calcium ATPase, as discussed above.

**Excitation–Contraction Coupling**

In cardiac myocytes, the transition from the resting relaxed state with low \([\text{Ca}^{2+}]\) to a contraction occurs because a small quantity of \([\text{Ca}^{2+}]\) crosses the sarcolemma and induces a much larger release of \([\text{Ca}^{2+}]\) from the SR. The initial events that couple excitation to contraction are displayed in Figure 1B. This process is initiated by depolarization of the cell membrane, which causes opening of the voltage-gated Na\(^+\) and Ca\(^{2+}\) channels. The initial upstroke of the action potential in ventricular cardiac myocytes is caused by sodium influx via the Na\(^+\) channel, whereas the subsequent inward current maintaining the plateau of the action potential is caused primarily by Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channel. Cumulative Na\(^+\) influx via the Na\(^+\) channel also contributes to maintenance of the intracellular sodium concentration in myocytes and thus can influence \([\text{Ca}^{2+}]\) via the Na\(^+\)–Ca\(^{2+}\) exchanger. However, it is Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channel that is considered to be of greatest importance in E–C coupling.\(^22–25\)

An important pharmacological characteristic of Ca\(^{2+}\) channels is that they contain a high-affinity receptor for 1,4-dihydropyridine ligands. These ligands can function either as antagonists or agonists. Antagonists such as nifedipine, when bound to channels, effectively block their activity. Bay K 8644 is a well-known dihydropyridine receptor agonist and increases Ca\(^{2+}\) current carried by cardiac Ca\(^{2+}\) channels.\(^26\) Because dihydropyridine receptors are found in extremely high density in skeletal muscle T tubules,\(^27\) Ca\(^{2+}\) channels have been isolated from this source. Most of our present knowledge of these channels is based on studies of the skeletal muscle channel. The skeletal muscle channel differs functionally from the heart channel in that it is coupled to the SR Ca\(^{2+}\) release channel by a voltage sensor mechanism.\(^28\) However, it is believed that the architecture of the two channels is essentially similar.\(^29\)

The L-type calcium channel is an oligomeric complex of five subunits. These subunits are designated \(\alpha_1\), \(\alpha_2\), \(\beta\), \(\gamma\), and \(\delta\). The \(\alpha_2\) and \(\delta\) are linked by disulfide bonds and are encoded by the same gene.\(^29,30\) As such, they are designated \(\alpha_2/\delta\). The \(\alpha_1\) subunit seems to provide the primary structural and functional basis for the assembled channel. For example, it contains the receptor for at least three classes of channel antagonists, including the dihydropyridines; it contains phosphorylation sites for cyclic AMP (cAMP)-dependent protein kinase; and it is known to contain the pore of the channel. It can transmit modest quantities of current and apparently retains the voltage sensitivity of the native channel. The \(\alpha_1\) subunit from heart is somewhat larger than the skeletal muscle subunit.\(^31\)

The relation between subunit organization and function is now under investigation. It appears that the
FIGURE 1. This page and facing page. Schematic illustration of the sequence of E–C coupling in an adult mammalian ventricular myocyte. The intracellular ion transport events are shown in the main figures; the corresponding changes in membrane potential, cytosolic \([\text{Ca}^{2+}]\), and contractile force (shortening) from an isolated adult rabbit ventricular myocyte loaded with the \(\text{Ca}^{2+}\)-sensitive dye indo-1 stimulated at 1 Hz at 36°C are shown in the insets on the left. Panel A: The processes ongoing in the resting myocyte or at end diastole. Panel B: The events associated with early E–C coupling before a rise in cytosolic \([\text{Ca}^{2+}]\).
Figure 1. Continued. Panel C: Processes associated with the rise in \([\text{Ca}^{2+}]_i\) and contraction. Panel D: The events associated with a fall in \([\text{Ca}^{2+}]_i\) and relaxation. See text for discussion. SR, sarcoplasmic reticulum; SL, sarcolemma.
various subunits of the calcium channel regulate and enhance many of the properties exhibited by the \( \alpha \) subunit. It has recently become possible to express cardiac \( \alpha \) subunits in Xenopus oocytes by injecting the appropriate messenger RNA.\(^{30} \) By expressing the skeletal muscle subunits \( \alpha_1/\beta \) and \( \beta \) alone or in combination, it has become possible to demonstrate interactions between these subunits and \( \alpha_i. \) The current caused by the \( \text{Ca}^{2+} \)-channel agonist Bay K through \( \alpha_i \) alone is small but is enlarged if \( \alpha_i \) is coexpressed with \( \alpha_1/\beta \) and is somewhat reduced by coexpression with \( \beta + \alpha_1/\delta. \)\(^{30} \)

Experiments of this type reveal that the various subunits modulate sensitivity to dihydropyridine agonists, kinetics, and voltage dependence of activation and inactivation. The broad conclusion is that all subunits are required before the channel can fully express its native properties, although it should be emphasized that no combination of subunits yet reported completely mimics the native channel.

It appears that \( \text{Ca}^{2+} \) influx via the \( \text{Na}^+\text{–Ca}^{2+} \) exchanger ("reverse mode") can also occur during initial depolarization (see Figure 1B). First, theoretical considerations (see Equation 3) indicate that with abrupt membrane depolarization positive to the reversal potential for the \( \text{Na}^+\text{–Ca}^{2+} \) exchanger, \( \text{Ca}^{2+} \) influx should occur as the exchange reverses. This process may be further stimulated by subsarcolemmal rises in sodium concentration caused by the sodium influx via the cardiac sodium channel.\(^{32,33} \) However, whether or not this occurs during normal E–C coupling remains controversial.\(^{34} \)

The \( \text{Ca}^{2+} \) that enters the cell early after depolarization via the \( \text{Na}^+\text{–Ca}^{2+} \) channel (and possibly on the \( \text{Na}^+\text{–Ca}^{2+} \) exchanger) can bind to the \( \text{Ca}^{2+} \) release channel of the SR (Figure 1B) and activate this channel,\(^{35} \) causing release of calcium from internal stores within the SR (calcium-induced calcium release)\(^{36} \) (Figure 1C). As the \([\text{Ca}^{2+}]_{\text{SR}}\) concentration subsequently begins to rise as a result of \( \text{Ca}^{2+} \) release from the SR, the reversal potential for the \( \text{Na}^+\text{–Ca}^{2+} \) exchanger becomes greater than the plateau of the membrane potential because of the rise in the \([\text{Ca}^{2+}]_{\text{SR}}\). Thus, after an initial period, which may be as brief as 10 msec,\(^{19} \) the \( \text{Na}^+\text{–Ca}^{2+} \) exchanger begins a \( \text{Na}^+\text{–in/ Ca}^{2+}\text{–out} \) "forward" mode of operation and thus contributes to \( \text{Ca}^{2+} \) influx, as we discuss below.

The \( \text{Ca}^{2+} \) release channel in the SR has been cloned\(^{37} \) and has been shown to be a 4,969-amino acid protein with a molecular mass of 564,711 d. Experiments by Anderson et al\(^{35} \) and Rousseau et al\(^{38} \) have demonstrated that the probability of opening of this channel is markedly increased by exposure to micromolar concentrations of \( \text{Ca}^{2+} \). This channel is also opened by methylxanthines such as caffeine\(^{39} \) and has a high affinity for the plant alkaloid ryanodine. In low concentrations (<10 \( \mu \text{mol/L} \), ryanodine induces an open, low-conductance configuration of the channel; at higher concentrations, ryanodine completely blocks the SR calcium release channel.\(^{40} \) The SR calcium release channel has phosphorylation, ATP-binding, and calmodulin-binding sites. The extent and mechanisms of its regulation are not well understood at present, although calmodulin appears to directly reduce the duration of single-channel opening without having an effect on single-channel conductance.\(^{38} \)

Low concentrations of ryanodine open the SR calcium release channel and thus deplete the SR calcium stores. This effect has been used to examine the extent to which calcium release from SR by the calcium-induced calcium release mechanism is involved in cardiac E–C coupling. The magnitude of the decrease in contractile force induced by exposure to ryanodine ranges from 10% to 90%, depending on the species studied,\(^{41} \) with rat being the most sensitive. However, this may not accurately reflect the contribution of \( \text{Ca}^{2+} \) released from the SR to force development, because in the presence of continued stimulation of myocardial cells, it appears that SR \( \text{Ca}^{2+} \) stores can be maintained even in the presence of ryanodine.\(^{42,43} \) Thus, with continued stimulation of myocytes, ryanodine does not completely deplete the SR. In rested cells exposed to ryanodine,\(^{43} \) the SR becomes completely depleted. Under these conditions, \( \text{Ca}^{2+} \) influx via the \( \text{Ca}^{2+} \) channel (and perhaps on the \( \text{Na}^+\text{–Ca}^{2+} \) exchanger) causes little or no contraction directly. Interpretation of this result is complicated by the possibility that some of this influx of \( \text{Ca}^{2+} \) could be directly taken up by an empty SR and thus prevented from binding to contractile elements. These observations, however, suggest that most of the \( \text{Ca}^{2+} \) causing contraction in mammalian myocytes during normal E–C coupling is \( \text{Ca}^{2+} \) released from the SR. The primary roles of the quantitatively relatively small \( \text{Ca}^{2+} \) influx across the sarcolemma\(^{44} \) are to trigger SR \( \text{Ca}^{2+} \) release in mammalian myocardium and to maintain SR \( \text{Ca}^{2+} \) loading.

There is good evidence that not all the \( \text{Ca}^{2+} \) within the SR is released with each beat\(^{45} \) and that SR \( \text{Ca}^{2+} \) release can be "graded" by the amount of trigger \( \text{Ca}^{2+} \) entering the cell. The evidence indicating a central role of \( \text{Ca}^{2+} \) influx via the \( \text{Ca}^{2+} \) channel in this process has recently been summarized by Fabiato.\(^{46} \) However, a graded mechanism requires that \( \text{Ca}^{2+} \) released from a \( \text{Ca}^{2+} \) release channel does not open adjacent \( \text{Ca}^{2+} \) release channels not initially opened by the trigger \( \text{Ca}^{2+}. \) This might occur if the rate constant of \( \text{Ca}^{2+} \) binding to an inactivating site of the SR \( \text{Ca}^{2+} \) release channel were lower than to an activating site.\(^{47} \) However, studies of the behavior of SR \( \text{Ca}^{2+} \) release channels incorporated into lipid bilayer membranes\(^{35,40} \) have failed to show evidence of \( \text{Ca}^{2+} \) induced inactivation of these channels. It is also possible that diffusion in the \( \text{Ca}^{2+} \) release channel environment is restricted,\(^{33} \) so that \( \text{Ca}^{2+} \) released from an SR release channel might not have easy access to other channels. However, \( \text{Ca}^{2+} \) spontaneously released from a \( \text{Ca}^{2+} \)-overloaded SR may induce adjacent SR \( \text{Ca}^{2+} \) release, resulting in a propagating "wave" of increasing \([\text{Ca}^{2+}]_{\text{SR}}\); and contraction.\(^{48,49} \) A difference in the system "gain" may account for the fact that \( \text{Ca}^{2+} \) released from SR does not activate adjacent SR \( \text{Ca}^{2+} \) release channels under conditions of normal E–C coupling.\(^{25} \) but this issue is still not satisfactorily resolved.

The \( \text{Ca}^{2+} \) that is released from the SR initiates contraction by binding to the contractile proteins (Figure 1C). In the resting state, the interaction of actin and myosin is inhibited by the troponin–tropomyosin complex, which is bound to actin. When \( \text{Ca}^{2+} \) binds to troponin C, a conformational change is induced that results in relief of this inhibition, with cross-bridge interaction and contractile element shortening. This
interaction can be modulated by other contractile element proteins, such as C protein and myosin light chain 2 (see Reference 51 for review). Troponin T can also influence the affinity of troponin C for Ca\(^{2+}\) and thus alter the sensitivity of the contractile elements for Ca\(^{2+}\). The affinity of troponin C for Ca\(^{2+}\) is also decreased by decreased intracellular pH.\(^5\) cAMP-dependent protein kinase–induced phosphorylation of troponin I\(^6\) decreases calcium sensitivity.\(^5\) Thus, changes in the affinity of the contractile elements for Ca\(^{2+}\) as well as the magnitude of the Ca\(^{2+}\) transient achieved during a single beat can regulate force development by a myocyte.

**Relaxation of the Ventricular Myocyte**

The decay of the calcium transient (Figure 1D) occurs because of reuptake of Ca\(^{2+}\) into the SR mediated by the SR calcium ATPase and extrusion of Ca\(^{2+}\) from the myocyte as mentioned above primarily by the Na\(^+-Ca\(^{2+}\) exchange. The SR calcium ATPase calcium transport enzyme is concentrated in the longitudinal component of the SR.\(^5\) The cardiac SR calcium ATPase, SERCA2, is a 100–115-kd protein that has been cloned.\(^5\) The Ca\(^{2+}\) transport characteristics of this enzyme have been well characterized by studies of calcium uptake by SR membrane vesicles, including vesicles from human myocardium.\(^5\) The \(K_C\) for calcium transport is 300–400 nmol/L. Thus, the SR Ca\(^{2+}\) ATPase transport is strongly activated in the range of physiological calcium concentrations that occur during the normal contraction–relaxation cycle.

Regulation of the SR calcium ATPase occurs primarily by phosphorylation of phospholamban. Phospholamban is a 6,080-d protein\(^5\) that binds to and inhibits Ca\(^{2+}\) transport by the SR calcium ATPase. When it is phosphorylated by cAMP-dependent protein kinase, this inhibition is removed, resulting in a greater \(V_{\text{max}}\) and a lower \(K_C\) for calcium transport.\(^5\) Phosphorylation of phospholamban can also occur via action of Ca\(^{2+}\)-calmodulin–dependent and Ca\(^{2+}\)-phospholipid–dependent protein kinases at distinct sites.\(^6\) Dephosphorylation of phospholamban occurs via an SR-associated type 1 phosphatase.\(^6\) After uptake by the SR calcium ATPase in the longitudinal SR, Ca\(^{2+}\) is bound to calsequestrin, which is located primarily in the junctional SR. Calsequestrin is the major Ca\(^{2+}\)-binding protein in cardiac muscle (=50 mol Ca\(^{2+}\) per mol protein). Binding and release of Ca\(^{2+}\) by calsequestrin is thought to be important in E–C coupling, but its exact role is not understood.\(^5\)

Ca\(^{2+}\) extrusion from the cells via the Na\(^+-Ca\(^{2+}\) exchanger also occurs during the declining phase of the Ca\(^{2+}\) transient. At steady state, a balance exists between the amount of Ca\(^{2+}\) entering the cell as the Ca\(^{2+}\) current, \(I_C\), and the amount of Ca\(^{2+}\) extruded by the Na\(^+-Ca\(^{2+}\) exchanger.\(^5\) Measurements of the amounts of current generated by these opposite Ca\(^{2+}\) transport pathways show a 2:1 relation (Figure 2). For each 2+ charge entering the cell as a Ca\(^{2+}\), a 3+ charge leaves the cell as Na\(^+\), transported with a 3 Na\(^+\):1 Ca\(^{2+}\) exchange stoichiometry. As previously mentioned, this electronegative Na\(^+-Ca\(^{2+}\) exchange is sensitive to membrane potential,\(^8\) and therefore the time course with which calcium is extruded is influenced by the trajectory of membrane potential. It has also been established that inward exchange current (corresponding to Ca\(^{2+}\) extrusion) is linearly related to \([\text{Ca}^{2+}]_i\).\(^5\) Thus, as the membrane potential changes during the Ca\(^{2+}\) transient, the trajectory of Na\(^+-Ca\(^{2+}\) exchange current will reflect both the time courses of the Ca\(^{2+}\) transient and the membrane potential.

The striking effect of membrane potential on \([\text{Ca}^{2+}]_i\) can be inferred from work by Bridge et al\(^1\) on voltage-clamped myocytes. Ventricular myocytes in which the SR was disabled with caffeine were unable to relax from a contraction (induced by an imposed voltage clamp) until the membrane potential was restored toward resting value (Figure 3, left panel). Moreover, the rate of relaxation depended on the membrane potential at which relaxation occurred and was also Na\(^+\) dependent (see Figure 2). These experiments are consistent with a significant effect of membrane repolarization on the rate of Ca\(^{2+}\) extrusion via a voltage-sensitive Na\(^+-Ca\(^{2+}\) exchange. There is not a great deal of information on the relation between Na\(^+-Ca\(^{2+}\) exchange and voltage at various \([\text{Ca}^{2+}]_i\) levels. Recent results indicate that at elevated \([\text{Ca}^{2+}]_i\), the relation between exchange current and voltage is steep and is sigmoidal\(^9\) (see Figure 3, right panel). Miura and Kimura\(^6\) have obtained similar results. We may infer from these current–voltage relations that membrane repolarization will have an activating effect on Na\(^+-Ca\(^{2+}\) exchange. It should be appreciated that under certain circumstances, the inactivating effect of a declining Ca\(^{2+}\) could be offset by the activating effect of membrane repolarization if the membrane potential and \([\text{Ca}^{2+}]_i\) transient coincide appropriately. In particular, this is likely to occur if membrane repolarization is rapid during a slowly declining \([\text{Ca}^{2+}]_i\) transient (see Figure 1D).

Competition between Ca\(^{2+}\) extrusion on the Na\(^+-\) Ca\(^{2+}\) exchanger and Ca\(^{2+}\) uptake by the SR is physiologically important because calcium that is taken up by the SR is available (after transport to SR Ca\(^{2+}\) release sites where it is stored, bound to calsequestrin) to enhance the Ca\(^{2+}\) transient during a subsequent beat and thus can contribute to increased force development. Under steady-state conditions, the amount of Ca\(^{2+}\) entering the cell equals that extruded from the cell, and the amount released by the SR equals that sequestered by the SR. If an abrupt change in this balance of fluxes takes place, then the calcium content of the SR will be affected. It is perhaps worth considering, therefore, how competition between the SR Ca\(^{2+}\) pump and the Na\(^+-\) Ca\(^{2+}\) exchange might be regulated by membrane potential. Let us imagine that some intervention abruptly shortens the action potential, so that the steep phase of repolarization occurs earlier during the calcium transient. Because repolarization increases forward exchange earlier during the \([\text{Ca}^{2+}]_i\) transient, more Ca\(^{2+}\) will be extruded than on a beat during which action potential duration was not reduced. Thus, the SR calcium content would be reduced at least by the additional calcium extruded. An action potential that was suddenly prolonged relative to the calcium transient would have the opposite effect, i.e., Ca\(^{2+}\) extrusion would be suppressed by membrane depolarization.

Results reported by Spurgeon et al\(^1\) indicate that in rat ventricular myocytes, initial repolarization does occur early during the calcium transient. Thus, one would expect Na\(^+-Ca\(^{2+}\) exchange to be activated and the resulting inward current to contribute to the plateau of
FIGURE 2. Tracings and graph demonstrating the quantitative relation between the amount of Ca\textsuperscript{2+} entering the cell as the calcium current \(I_{Ca}\) and the amount subsequently extruded by the Na\textsuperscript{+}--Ca\textsuperscript{2+} exchanger, \(I_{Na-Ca}\). In the left panel, an inward calcium current (initial portion of trace A) is elicited by a voltage clamp pulse from \(-40\) to \(0\) mV in the absence of external sodium (Na\textsubscript{0}). This is associated with a contraction, trace D. On repolarization of the voltage clamp to \(-40\) mV (lower trace), there is no relaxation in the absence of Na\textsubscript{0}. However, when Na\textsubscript{0} is abruptly increased to 145 mmol/L (upper trace), there is onset of relaxation (trace E) associated with an inward current caused by electrogenic Na\textsuperscript{+}--Ca\textsuperscript{2+} exchange (trace B and with an expanded scale, trace C). The right panel shows the relation of the integrals of \(I_{Ca}\) and \(I_{Na-Ca}\) from 11 experiments obtained in a similar protocol. The solid lines indicate the expected relations for a stoichiometry of 4 or 3 Na\textsuperscript{+}:1 Ca\textsuperscript{2+}. The results indicate that for the expected stoichiometry of 3:1, the amount of Ca\textsuperscript{2+} entering as \(I_{Ca}\) is balanced by the amount of Ca\textsuperscript{2+} extruded by Na\textsuperscript{+}--Ca\textsuperscript{2+} exchange. Reprinted with permission.\textsuperscript{66}

the rat action potential, as demonstrated by Schouten and Ter Keurs.\textsuperscript{71} Conversely, if the Ca transient were short compared with the cardiac action potential duration, different effects might be predicted. For example, were the SR to pump [Ca\textsuperscript{2+}], down to diastolic levels before significant repolarization had taken place, one might expect a reverse exchange (Ca\textsuperscript{2+} influx) during the late plateau of the action potential. Thus, agents that prolong the cardiac action potential might cause calcium overload by both impairing Ca\textsuperscript{2+} extrusion and

FIGURE 3. Left panel: Tracings showing voltage clamp pulses (top trace) and corresponding shortening records obtained in isolated guinea pig ventricular myocytes. In these experiments, in which the sarcoplasmic reticulum was disabled by exposure to 10 mmol/L caffeine, the extent of relaxation (and presumably decline in [Ca\textsuperscript{2+}]) was strongly dependent on membrane voltage. Right panel: Graph showing the dependence of \(I_{Na-Ca}\) on membrane voltage in clamped guinea pig myocytes in which [Ca\textsuperscript{2+}] was elevated in the absence of Na\textsubscript{0} by rapid pacing during exposure to 1 \(\mu\)mol/L ryanodine. Na\textsubscript{0} was then rapidly applied at different voltages to activate the Na\textsuperscript{+}--Ca\textsuperscript{2+} exchange current, \(I_{Na-Ca}\). Note the strong dependence of \(I_{Na-Ca}\) on membrane voltage over the range of potentials shown in the left panel. Reprinted with permission.\textsuperscript{64}
increasing Ca\textsuperscript{2+} influx via Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange. Calcium overload may cause spontaneous oscillatory release of Ca\textsuperscript{2+} from SR,\textsuperscript{46} resulting in delayed afterdepolarizations and "triggered" arrhythmias in the intact heart. This mechanism may contribute to arrhythmias seen in the context of a prolonged QT interval caused by antiarrhythmic drug therapy and perhaps in the long-QT syndrome.

**Inotropic Drug Effects on Ca\textsuperscript{2+} Homeostasis**

In the context of the above discussion, we may now briefly consider some of the mechanisms of action of inotropic drugs. The most commonly used of these is digitalis. The cardiac glycosides bind to the α-subunit of the Na\textsuperscript{+},K\textsuperscript{+}-ATPase and inhibit the translocation of Na\textsuperscript{+} and K\textsuperscript{+}.\textsuperscript{72} Thus, partial inhibition of the sodium pump in the sarcolemma of a myocyte by cardiac glycosides causes a slight rise in intracellular sodium,\textsuperscript{73,74} and a slight fall in intracellular potassium. An increase in intracellular Na\textsuperscript{+} concentration would be expected to increase the influx of calcium on the Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger initially during the action potential\textsuperscript{25} and to impair extrusion of Ca\textsuperscript{2+} by Na\textsuperscript{+}–Ca\textsuperscript{2+} exchange subsequently during the Ca\textsuperscript{2+} transient. Both these effects would be expected to cause the observed increase in diastolic [Ca\textsuperscript{2+}]\textsubscript{i} and loading of the SR with Ca\textsuperscript{2+},\textsuperscript{76} providing more calcium available for Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release and thus an enhanced calcium transient and a positive inotropic effect. A slight prolongation of membrane action potential observed in some studies during inhibition of the Na\textsuperscript{+} pump\textsuperscript{24} might also be expected to impair calcium extrusion by Na\textsuperscript{+}–Ca\textsuperscript{2+} exchange and contribute to an inotropic effect.

Catecholamines bind to β-receptors in the cardiac cell membrane, activating a guanine nucleotide-binding protein (G protein), G. This in turn causes stimulation of adenylate cyclase and a resulting increase in the production of cAMP, which causes activation of cAMP-dependent protein kinases.\textsuperscript{77} As mentioned above, this kinase causes phosphorylation of the α\textsubscript{1} subunit of the calcium channel, which increases calcium influx, and of phospholamban, which increases calcium uptake by the SR. These effects produce both an increase in the "trigger" Ca\textsuperscript{2+} entering the cell and an increase in releasable SR calcium content. In spite of a marked increase in calcium influx via the calcium channel, the cell is able to maintain its ability to relax (positive lusitropic effect) because of the stimulation of the SR calcium ATPase and shortening of the duration of the action potential. The shortening of the duration of the action potential and the corresponding enhanced extrusion of calcium by the Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger may explain why progressive calcium overload, with elevation of diastolic [Ca\textsuperscript{2+}]], does not usually occur in myocytes stimulated with catecholamines. In fact, end-diastolic [Ca\textsuperscript{2+}] may actually decline in myocytes exposed to isoproterenol.\textsuperscript{79} The increased Ca\textsuperscript{2+} influx per beat can be extruded more effectively by the Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger because of the shorter duration of the action potential, which in turn results from a cAMP-induced increase in I\textsubscript{k} and I\textsubscript{Ca}.\textsuperscript{76,79}

Some inotropic agents, including milrinone and amrinone, exert a positive inotropic effect by inhibiting phosphodiesterase, the enzyme that breaks down cAMP. Cyclic nucleotide phosphodiesterases differ with respect to drug sensitivity and substrate specificity,\textsuperscript{80} and the extent to which both cAMP-dependent protein kinases and phosphodiesterases are physically located adjacent to their particular substrates is currently under investigation.\textsuperscript{81} The phosphodiesterase inhibitors also are potent vasodilators, and thus the beneficial hemodynamic effects of these agents are a result of both positive inotropy and afterload reduction.

Some inotropic agents increase the sensitivity of the contractile elements to calcium. Endothelin\textsuperscript{82,83} and angiotensin II\textsuperscript{84} can exert a potent inotropic effect in cardiac myocytes by inducing an intracellular alkalosis, and this change in intracellular pH sensitizes the contractile elements to calcium. The mechanisms of other agents that appear to sensitize contractile elements to calcium\textsuperscript{84–86} and thus produce an increase in force development without a corresponding increase in the magnitude of the calcium transient are less well understood. These agents may bind to the contractile element tropinin–tropomyosin complex and, by a direct effect on these thin-filament regulatory proteins, alter the calcium sensitivity of the myofilaments. These agents have generated considerable interest because increased Ca\textsuperscript{2+} loading of the SR is not a component of their action, and thus a positive inotropic effect may be achieved without danger of Ca\textsuperscript{2+} overload–induced arrhythmias.

**Summary**

Calcium homeostasis in cardiac myocytes results from the integrated function of transsarcolemmal Ca\textsuperscript{2+} influx and efflux pathways modulated by membrane potential and from intracellular Ca\textsuperscript{2+} uptake and release caused predominantly by SR function. These processes can be importantly altered in different disease states as well as by pharmacological agents, and the resulting changes in systolic and diastolic [Ca\textsuperscript{2+}] can cause clinically significant alterations in contraction and relaxation of the heart. It may be anticipated that a rapid increase in our understanding of the pathophysiology of Ca\textsuperscript{2+} homeostasis in cardiac myocytes will be forthcoming as the powerful new tools of molecular and structural biology are used to investigate the regulation of Ca\textsuperscript{2+} transport systems.

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