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Functional coupling of Ca^{2+} channels and ryanodine receptors in cardiac myocytes

JAMES S. K. SHAM*, LARS CLEEMANN†, AND MARTIN MORAD†‡

*Division of Pulmonary and Critical Care Medicine, The Johns Hopkins University Medical School, Baltimore, MD 21205; and †Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007

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ABSTRACT **In skeletal muscle, dihydropyridine receptors are functionally coupled to ryanodine receptors of the sarcoplasmic reticulum in triadic or diadic junctional complexes. In cardiac muscle direct physical or functional couplings have not been demonstrated. We have tested the hypothesis of functional coupling of L-type Ca^{2+} channels and ryanodine receptors in rat cardiac myocytes by comparing the efficacies of Ca^{2+} in triggering Ca^{2+} release when the ion enters the cell via the Ca^{2+} channels or the Na^+/Ca^{2+} exchanger. Ca^{2+} transported through the Ca^{2+} channels was 20–160 times more effective than Ca^{2+} influx via the Na^+/Ca^{2+} exchanger in gating Ca^{2+} release from the sarcoplasmic reticulum, suggesting privileged communication between Ca^{2+} channels and ryanodine receptors. In support of this hypothesis we found that Ca^{2+} channels were inactivated by Ca^{2+} release from the sarcoplasmic reticulum, even though the myoplasmic Ca^{2+} concentrations were buffered with 10 mM EGTA. The data thus suggest privileged cross signaling between the dihydropyridine and ryanodine receptors such that Ca^{2+} flux through either the Ca^{2+} channel or the ryanodine receptor alters the gating kinetics of the other channel.**

In skeletal muscle, ultrastructural and biophysical evidence suggests that the dihydropyridine (DHP) receptors are physically coupled to the ryanodine receptors of the sarcoplasmic reticulum (SR) (1–4). The intramembrane charge movement associated with the activation of the DHP receptor, rather than the influx of Ca^{2+}, is thought to open the Ca^{2+} release channels (5). In cardiac myocytes, communication between Ca^{2+} channels and ryanodine receptors and release of Ca^{2+} from the SR appear to be mediated by the Ca^{2+}-induced Ca^{2+} release mechanism requiring influx of Ca^{2+} through the Ca^{2+} channels (6–11). For Ca^{2+}-induced Ca^{2+} release to produce a graded response, it is postulated that either larger Ca^{2+} releases must inactivate the Ca^{2+} release channel (7) or the released Ca^{2+} must dissipate rapidly from the vicinity of the ryanodine receptor during random and Ca^{2+}-independent closed intervals of the release channel (35). While the first mechanism requires Ca^{2+} to accumulate in the microenvironment of the ryanodine receptor [a theory supported by Ca^{2+} release in skinned myocytes (7), but not in intact myocytes (12)], the latter emphasizes the intracellular microarchitecture and spatial relationships of sarcolemmal Ca^{2+} channel and SR release channels.

In the present study we test the hypothesis of functional coupling of Ca^{2+} channels and ryanodine receptors by evaluating the efficacies of Ca^{2+} entry via different molecular routes in activating Ca^{2+} release and determine the extent of cross signaling between the Ca^{2+} channels and ryanodine receptors. This type of coupling represents an alternative to the direct physical coupling which was proposed (13), but later rejected (14), on the basis of the effect of ryanodine on the Ca^{2+} current (I_{Ca}) in the absence and presence of Ca^{2+} buffers. We present evidence for discrete, Ca^{2+}-mediated cross signaling between the ryanodine and DHP receptors in rat ventricular myocytes, independent of bulk cytosolic Ca^{2+} concentrations.

MATERIALS AND METHODS

Cell Isolation. Ventricular myocytes were enzymatically isolated from male Wistar rats (150–250 g) as described (15). In brief, rats were anesthetized (sodium pentobarbital, 30 mg/kg), and the excised hearts were perfused retrogradely through the aorta, first for 5 min with Ca^{2+}-free Tyrode's solution and then with Ca^{2+}-free Tyrode's solution containing collagenase (1.4 mg/ml; type A; Boehringer Mannheim) and protease (0.28 mg/ml; type XIV; Sigma). Myocytes were then dispersed in 0.2 mM Ca^{2+} Tyrode's solution and stored at room temperature for 1 hr before use.

Measurements of Membrane Currents and Intracellular Ca^{2+} Transients. Myocytes were voltage clamped in the whole-cell clamp configuration. Membrane currents were recorded with a Dagan Instruments (Minneapolis) amplifier. Intracellular Ca^{2+} transients were simultaneously monitored with Ca^{2+}-sensitive fluorescent dye (0.2 mM fura-2 or 0.1 mM indo-1) introduced into the myocytes through the patch pipettes (6, 16). The myocyte under examination was monitored continuously with a CCD television camera (Dage–MTI, Michigan City, IN) using red light. Ca^{2+} concentrations were calculated with the equation of Grynkiewicz *et al.* (17) by assuming that the K_d values of fura-2 and indo-1 were 224 and 213 nM, respectively (17, 18). Indo-1 fluorescence was calibrated as described (16).

Solutions. The modified Tyrode's solution contained 142 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose, and 0.02 mM tetrodotoxin (TTX) at pH 7.4. The standard internal solution contained 110 mM CsCl, 10 mM NaCl, 5 mM MgATP, 10 mM Hepes, 20 mM Et_4NCl, and 0.01 mM cAMP at pH 7.2. In the experiments in which we studied possible inactivation of I_{Ca} by SR Ca^{2+} release, 0.2 mM cAMP was added to the internal solution to optimize I_{Ca} and Ca^{2+} uptake. The high concentrations of cAMP were not essential for these experiments, because inactivation of I_{Ca} by Ca^{2+} release could be consistently seen in myocytes where no cAMP was included in the patch pipette. In some experiments a solution containing 98 mM CsCl, 10 mM Hepes, 20 mM Et_4NCl, 10 mM EGTA, 5 mM $CaCl_2$, 5 mM MgATP, and 0.2 mM cAMP at pH 7.2 was used to buffer the internal Ca^{2+} activity to about 150 nM (10 mM EGTA is half-saturated with 5 mM Ca^{2+}, and the K_d is about 150 nM). Experiments were performed at room temperature, about 25°C.

Experimental Protocols and Data Analysis. After the myocytes were dialyzed for ≥5 min, Ca^{2+} release induced by I_{Ca} was elicited by depolarizing pulses from −80 mV to −40, −20, or 0 mV in the presence of 0.02 mM TTX to inhibit Na^+

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Abbreviations: DHP, dihydropyridine; SR, sarcoplasmic reticulum; TTX, tetrodotoxin.

‡To whom reprint requests should be addressed at: Department of Pharmacology, Georgetown University Medical Center, 3900 Reservoir Road NW, Washington, DC 20007.

current. I_{Ca} was measured as the peak inward current after leak subtraction, by assuming that the background current had a linear current–voltage (*I–V*) relation with reversal potential at 0 mV. The Ca^{2+} charge entering via Ca^{2+} channels was measured by integrating I_{Ca}. Release mediated by the Na^+/Ca^{2+} exchanger was activated by pulses to +80 mV, at which potential Ca^{2+} influx via the exchanger was favored. $I_{Na/Ca}$ was quantified by blocking the exchanger with 5 mM Ni^{2+}. The contribution of Cl^- current to the measured currents was minimal, since cAMP-dependent and Ca^{2+}-activated Cl^- currents are not expressed in rat ventricular myocytes (19, 20), and the current traces were leak subtracted. The total charge carried by Ca^{2+} (Q_{Ca}) was quantified by integrating I_{Ca} or $-2{\cdot}I_{Na/Ca}$ (assuming in the latter case a stoichiometry of 3 Na^+ to 1 Ca^{2+}). The integration covered a period extending from the beginning of depolarization either to the onset (Q_{onset}) or to the peak (Q_{peak}) of Ca^{2+} release, yielding values considered, respectively, as the quantity of Ca^{2+} required to activate or sustain Ca^{2+} release. The rate of Ca^{2+} release (R) vs. Q_{onset} was approximated by a least-squares fit to the Hill equation, $R/R_{max} = 1/[1+(K_m/Q_{onset})^N]$, where R_{max} is the maximal rate of release, K_m is the half-maximum point, and N is the Hill coefficient.

The inactivation of current through the Ca^{2+} channel (carried by Ca^{2+} or Ba^{2+}) and its relation to Ca^{2+} release were probed by use of 180-ms clamp pulses to 0 mV before and following depletion of the SR with 5 mM caffeine or 10 μM ryanodine. Time constants for inactivation of I_{Ca} were obtained by the least-squares fit of two exponentials with the CLAMPFIT program (PCLAMP software; Axon Instruments, Foster City, CA). All external solutions bathing the myocytes were exchanged rapidly (<200 ms) with a concentration clamp system (9) for 5–10 s to prevent alteration of cytosolic Na^+ and Ca^{2+} concentrations. Data are mean ± SEM.

RESULTS

Efficacies of I_{Ca} and Na^+/Ca^{2+} Exchange in Triggering SR Ca^{2+} Release. To compare the efficacy of Ca^{2+} entry via Ca^{2+} channels and Na^+/Ca^{2+} exchanger in triggering Ca^{2+} release, we measured the Ca^{2+} charge required to initiate release when transported either by the Ca^{2+} channel or by the Na^+/Ca^{2+} exchanger. Fig. 1*A* shows the Ca^{2+} currents, intracellular Ca^{2+} transients, and Q_{Ca} elicited by depolarizing pulses to −40, −20, and 0 mV. At 0 mV, the fully activated Ca^{2+} current triggered Ca^{2+} releases which were both larger and faster than those activated by the smaller Ca^{2+} currents at −40 and −20 mV. Similarly, a larger Q_{onset} was measured at 0 mV than at −40 and −20 mV, consistent with the notion that the activity of the Ca^{2+} release channels is proportional to the activating Ca^{2+} concentration as measured in single-channel studies of the ryanodine receptor (21–23).

Fig. 1*B* illustrates the time course and the magnitude of Ca^{2+} release triggered by activation of the Na^+/Ca^{2+} exchanger at +80 mV. The exchanger-dependent Ca^{2+} release was characterized by a slow rise in intracellular Ca^{2+}, reaching 128 ± 13 nM after 501 ± 46 ms (Table 1), followed by a rapid rise in the intracellular Ca^{2+} activity. The rapid component was abolished by caffeine (24), which depletes SR Ca^{2+} stores, whereas both the slow and fast components of the Ca^{2+} release at +80 mV were suppressed when 5 mM Ni^{2+} was added to inhibit Na^+/Ca^{2+} exchange (Fig. 1*B*; refs. 24 and 25). When the Q_{onset} values of the releases mediated by I_{Ca} and the exchanger are compared, it can be seen that the Ca^{2+} charge required to activate Ca^{2+} release was significantly smaller when Ca^{2+} entered the myocyte via the Ca^{2+} channels at any of the three potentials tested.

Table 1 quantifies the total transmembrane influx of Ca^{2+} at the onset (Q_{onset}) and at the peak (Q_{peak}) of Ca^{2+} transient for Ca^{2+} releases elicited by I_{Ca} and Na^+/Ca^{2+} exchange. The Q_{onset} of I_{Ca}-activated Ca^{2+} release at 0, −20, and −40 mV was

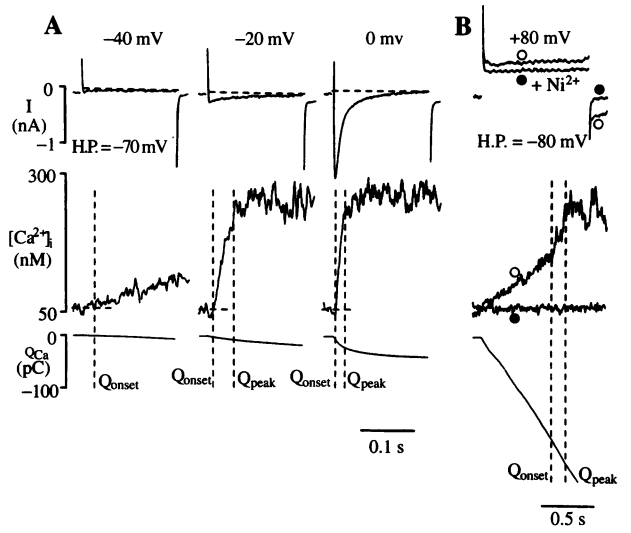

FIG. 1. Comparisons of membrane currents, Ca^{2+} transients, and Ca^{2+} charge (Q_{Ca}) during the activation of Ca^{2+} channels and Na^+/Ca^{2+} exchanger. (*A*) Ca^{2+} channels were activated by 180-ms depolarizing pulses from a holding potential (H.P.) of −70 mV to a test potential of −40, −20, and 0 mV in the presence of 0.02 mM TTX. Q_{Ca} was the integral of the Ca^{2+} current, Q_{onset} was the Q_{Ca} at the onset of the Ca^{2+} release, and Q_{peak} was the Q_{Ca} at the peak of Ca^{2+} release, estimated by the intersection of the maximum slope of rise in Ca^{2+} transient with the peak intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). (*B*) Ca^{2+} influx through the Na^+/Ca^{2+} exchanger was activated by a 1-s depolarizing pulse from a holding potential of −80 mV to +80 mV. Q_{Ca} was 2 times the integral of the Ni^{2+}-sensitive current, assuming 3 Na^+/1 Ca^{2+} stoichiometry. The traces shown in *A* and *B* were obtained from the same cell.

6.0 ± 1.1, 4.0 ± 0.9, and 0.8 ± 0.2 pC, respectively. These values were 20 (at 0 mV) to 160 (at −40 mV) times smaller than Q_{onset} of the exchanger-mediated Ca^{2+} release at +80 mV (131 ± 29 pC). Since the rate of Ca^{2+} release triggered by the exchanger (1.4 ± 0.6 μM/s) was equivalent at best to the lowest rate of I_{Ca}-activated release (3.1 ± 0.9 μM/s at −40 mV), Ca^{2+} entry via the Ca^{2+} channel was at least 160 times more effective in triggering Ca^{2+} release from the SR. The high efficacy of I_{Ca} in activating Ca^{2+} release suggests a privileged pathway of Ca^{2+} transport from Ca^{2+} channels to ryanodine receptors.

Plotting the normalized rate of Ca^{2+} release activated by I_{Ca} at different test potentials versus the quantity of Ca^{2+} charge transported prior to the onset of release (Q_{onset}) gives a sigmoidal relationship (Fig. 2) similar to the open-probability curves of single SR release channels activated by different Ca^{2+} concentrations (21, 22). The rate of Ca^{2+} release plateaus at Q_{onset} of 80–100 nC/μF, suggesting that the local Ca^{2+} concentrations were sufficiently high to cause maximal activation of ryanodine receptors. The curve in Fig. 2 may be calibrated in terms of the local Ca^{2+} concentration at the activation site if it is assumed that the concentration of Ca^{2+}, for comparable rates of release, may be obtained from the fura-2 measurements of intracellular Ca^{2+} just prior to the exchanger-induced release. This is based on the assumption that during the 0.5-s interval leading up to the exchanger-induced release, the Ca^{2+} activity at the activation site may come into equilibrium with bulk cytoplasmic Ca^{2+}. From the sigmoidal curve (or with some approximation from the measurements at −40 mV; Table 1), it is estimated that a Q_{onset} of 4.6 ± 0.8 nC/μF, when entering the myocyte through the Ca^{2+} channel, activates a Ca^{2+} release with the same normalized rate of release (5.3 ± 1.7 s^{-1}) as that induced by the Na^+/Ca^{2+} exchanger when intracellular Ca^{2+} reaches a threshold of 128 nM. If Q_{onset} for the I_{Ca}-induced release were proportional to Ca^{2+} concentration in the immediate vicinity of the ryanodine

Table 1. Ca^{2+} charge carried by I_{Ca} and $I_{Na/Ca}$ at onset and peak of Ca^{2+} transient

Calcium transporter	V_m, mV	I, nA	T_{onset}, ms	Q_{onset}, pC	Q_{peak}, pC	Peak Ca^{2+} release, nM	$R_{release}$, $\mu M/s$
Ca^{2+} channel	−40	−0.13 ± 0.05	13.4 ± 3.0	0.8 ± 0.2	4.4 ± 1.1	218.5 ± 41.2	3.1 ± 0.9
	−20	−0.75 ± 0.20	10.3 ± 0.9	4.0 ± 0.9	10.4 ± 1.8	276.3 ± 31.9	10.0 ± 2.0
	0	−1.24 ± 0.26	8.6 ± 0.8	6.0 ± 1.1	16.1 ± 2.7	285.7 ± 33.4	11.3 ± 1.1
Na^+/Ca^{2+} exchanger	+80	0.23 ± 0.03	501.1 ± 45.7	131 ± 29	184 ± 48	322.5 ± 41.3	1.4 ± 0.6

The I_{Ca}-dependent Ca^{2+} release was elicited by 180-ms depolarizing pulses to −40, −20, and 0 mV, whereas the Na^+/Ca^{2+} exchange-dependent Ca^{2+} release was elicited by a 1-s depolarizing pulse to +80 mV. The holding potential was −70 or −80 mV. T_{onset} was the time from the beginning of depolarization to the onset of Ca^{2+} release. Q_{onset} and Q_{peak} were the amounts of Ca^{2+} charge carried through the transporters at the onset and the peak of Ca^{2+} release, respectively. The rate of release, $R_{release}$, was the maximum slope of rise of Ca^{2+} transients. Data are expressed in mean ± SEM (n = 6). Cell capacitance = 123 ± 14 pF.

receptor, the Ca^{2+} concentration for maximal activation of ryanodine receptors would be about 2.3 μM [128 nM × (85/4.6)] and K_m of about 386 nM.

Inactivation of Ca^{2+} Channel by Release of Ca^{2+} from the SR. Because of the dependence of the inactivation of Ca^{2+} channel on intracellular Ca^{2+} (26–28), the kinetics of inactivation of Ca^{2+} channel may well be the best natural probe for examining the degree of accessibility of the Ca^{2+} channel to the ryanodine receptor (29). When I_{Ca} triggers Ca^{2+} release, the Ca^{2+} channel shows biexponential inactivation [τ = 6.9 ± 0.5 and 38.3 ± 1.8 ms (n = 12)]. Following the depletion of SR Ca^{2+} stores by rapid application of 5 mM caffeine, I_{Ca} inactivated significantly slower (Fig. 3*A*) [τ = 16.4 ± 0.6 and 51.3 ± 2.5 ms (n = 10)]. The rate of inactivation of I_{Ca} [τ = 6.8 ± 2.3 and 38.2 ± 2.5 ms (n = 5)] was also significantly reduced when ryanodine was used to suppress Ca^{2+} release [τ = 14.9 ± 2.3 and 53.4 ± 4.0 ms (n = 5)] from the SR (Fig. 3*B*). Further, 5 mM caffeine in the presence of ryanodine did not produce additional slowing of inactivation [τ = 16.9 ± 3.4 and 55.2 ± 5.5 ms (n = 3); Fig. 3*C*]. Thus, the reduction in the rate of inactivation of I_{Ca} was primarily due to the depletion of SR Ca^{2+} stores and not to other independent effects of caffeine or ryanodine on the inactivation of I_{Ca}. Complete suppression of Ca^{2+}-induced inactivation of the Ca^{2+} channel by the use of Ba^{2+} as charge carrier yielded a slower, monoexponentially decaying current through the Ca^{2+} channel [τ = 72.8 ± 2.4 ms (n = 5)] whose kinetics were not significantly altered by caffeine or ryanodine. These results indicate that both SR Ca^{2+} release and I_{Ca} contribute to the inactivation of the Ca^{2+} channel.

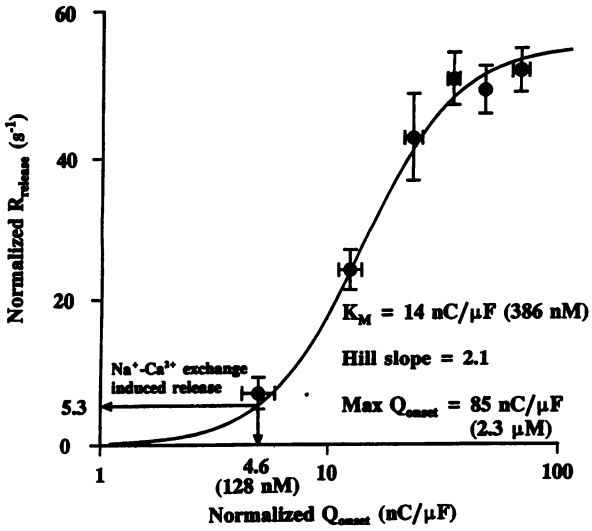

FIG. 2. The relationship of the rate of Ca^{2+} release ($R_{release}$) vs. Q_{onset} at different test potentials. I_{Ca}-dependent Ca^{2+} releases were activated by 180-ms depolarizing pulses to −40, −30, −20, −10, and 0 mV in six different cells. $R_{release}$ was the maximum slope of rise of Ca^{2+} transient and was normalized with respect to the maximum Ca^{2+} release at 0 mV. Q_{onset} was normalized with respect to cell capacitance. Data are expressed as mean ± SEM (n = 5). The sigmoidal curve represents the least-squares fit of the Hill equation, with R_{max} = 55 ± 4 s^{-1}, K_m = 14 ± 2 nC/μF, and N = 2.1 ± 0.5. Arrows indicate the reference point for the Na^+/Ca^{2+}-exchange-dependent Ca^{2+} release, where an intracellular Ca^{2+} concentration of 128 ± 13 nM triggered Ca^{2+} releases with normalized $R_{release}$ of 5.3 ± 1.7 s^{-1}. If we assume that the cytoplasmic Ca^{2+} concentration equaled the Ca^{2+} concentration around the ryanodine receptors, as Ca^{2+} influx via the Na^+/Ca^{2+} exchanger is slow, 128 nM Ca^{2+} activates the ryanodine receptors to the same extent as a Ca^{2+} influx of 4.6 ± 0.8 nC/μF via Ca^{2+} channels. If Q_{onset} is proportional to Ca^{2+} concentration in the junctional space, the K_m and maximum Ca^{2+} concentration for ryanodine receptor activation *in vivo* were 386 nM and 5–10 μM, respectively, and a maximum Q_{onset} of 85 nC/pF at 0 mV was equivalent to a cytoplasmic Ca^{2+} concentration of 2.3 μM.

The effect of release of Ca^{2+} from the SR on the inactivation of I_{Ca} might result either from an overall increase in the cytoplasmic Ca^{2+} concentration or from a local increase in Ca^{2+}, if the two channels were closely associated. To distinguish between these two possibilities, myocytes were dialyzed with 10 mM EGTA and 5 mM Ca^{2+} to buffer the intracellular Ca^{2+} to about 150 nM and to prevent large changes in bulk myoplasmic Ca^{2+} concentration yet allow the SR to load adequately. In these highly Ca^{2+}-buffered myocytes, the rise in cytosolic Ca^{2+} concentration evoked by activation of I_{Ca} was greatly reduced and the Ca^{2+} transients were very brief, lasting 50–100 ms (Fig. 4*A*), and the voltage dependence of Ca^{2+} release was bell shaped (Fig. 4*B*). The buffering of intracellular Ca^{2+} by 10 mM EGTA, however, did not by itself significantly slow the rate of inactivation of I_{Ca} [τ = 4.9 ± 0.3 and 48.9 ± 3.4 ms (n = 9)] compared with control myocytes. Nevertheless, the rate of inactivation of I_{Ca} continued to slow upon depletion of the Ca^{2+} content of the SR by caffeine [τ = 15.9 ± 0.6 and 58.9 ± 4.3 ms (n = 9); Fig. 4*C*], indicating that the buffering of cytosolic Ca^{2+} does not prevent the Ca^{2+} released from the SR from reaching the inactivation site of the DHP receptor. Similarly, replacement of Ca^{2+} with Ba^{2+} [τ = 62.3 ± 4 ms (n = 8); Fig. 4*C*] reduced the rate of inactivation of I_{Ca} as effectively as in the unbuffered myocytes (Fig. 3), suggesting that Ca^{2+} ions entering through the Ca^{2+} channel are not bound by the Ca^{2+} buffer before reaching the inactivation site. These results suggest that the inactivation of I_{Ca} during Ca^{2+} release from the SR does not require an overall increase in cytoplasmic Ca^{2+}, but rather a local increase in Ca^{2+} in the vicinity of the ryanodine receptor.

DISCUSSION

The major conclusion of this study is that L-type Ca^{2+} channels are in close functional association with ryanodine receptors, so that the flux of Ca^{2+} through either channel modifies the activity of the other channel. This cross signaling between the DHP and ryanodine receptors does not seem to include the Na^+/Ca^{2+} exchanger.

Quantitative comparison of the rates of release of Ca^{2+} induced by I_{Ca} and $I_{Na/Ca}$ suggests that I_{Ca} activated a fast Ca^{2+} release compared with the slower and somewhat delayed Ca^{2+} release activated by the exchanger (Fig. 1). For an equivalent rate of Ca^{2+} release, Ca^{2+} entering the myocyte through the Ca^{2+} channel was 160 times more efficient than Ca^{2+} entering via the

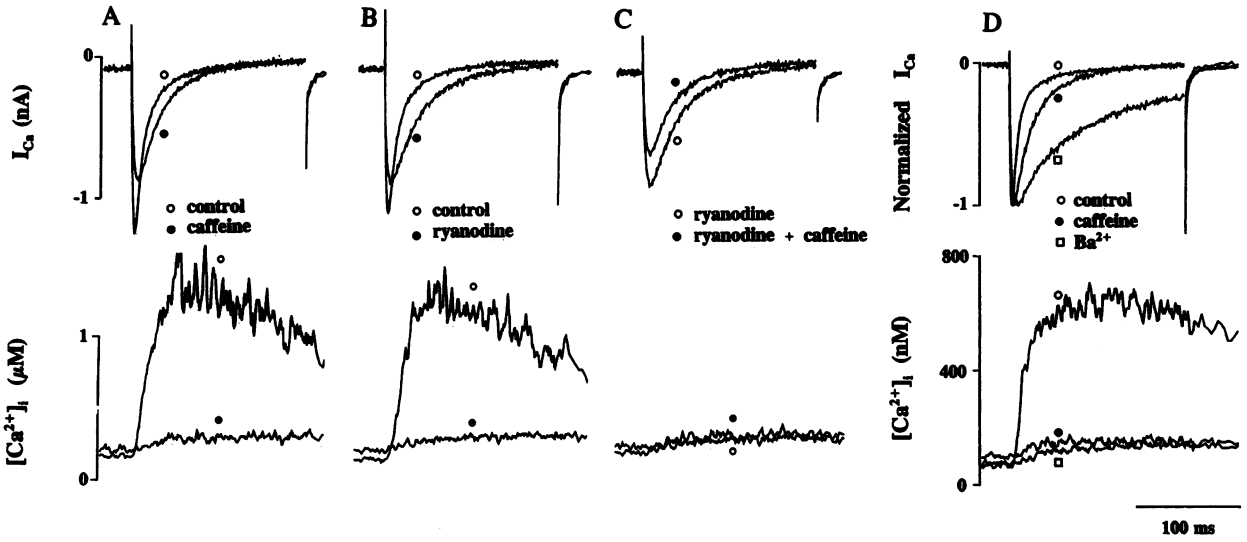

FIG. 3. Effects of SR Ca^{2+} release on I_{Ca} inactivation. (*A–C*) Depletion of SR Ca^{2+} by rapid application of 5 mM caffeine (*A*) or with 10 μM ryanodine (*B*) slowed I_{Ca} inactivation. Caffeine in the presence of ryanodine had no effect on the rate of inactivation (*C*). (*D*) I_{Ca} and Ca^{2+} transients in the absence (○) or presence (●) of caffeine or with 2 mM Ba^{2+} as charge with carrier (□). Traces were from a cell other than the one in *A–C* and were leak subtracted and scaled to the same magnitude for comparison (holding potential, −60 mV; test potential, 0 mV).

Na^+/Ca^{2+} exchanger. The high efficiency of I_{Ca} in triggering Ca^{2+} release suggests close association of DHP and ryanodine receptors. It could be argued that the low efficiency of the exchanger-mediated Ca^{2+} release is related to the lower rate of trans-sarcolemmal Ca^{2+} influx (7). This assertion, however, is not supported by the present findings (Fig. 1; Table 1), as the slower influx of Ca^{2+} through the Ca^{2+} channels at −40 mV triggered a faster Ca^{2+} release than that activated by a faster Ca^{2+} influx via the Na^+/Ca^{2+} exchanger [Q_{onset}/(integration time), 74 ± 25 pA for I_{Ca} at −40 mV and 267 ± 53 pA for $I_{Na/Ca}$].

Since the reported Ca^{2+} sensitivity of the ryanodine receptors varies considerably, depending on experimental conditions such as intracellular Mg^{2+}, ATP, and calmodulin (22, 23, 30), it is uncertain what Ca^{2+} concentrations the ryanodine receptors are exposed to under our experimental conditions. By comparing the rate of release of Ca^{2+} and Q_{onset} (Fig. 2) and

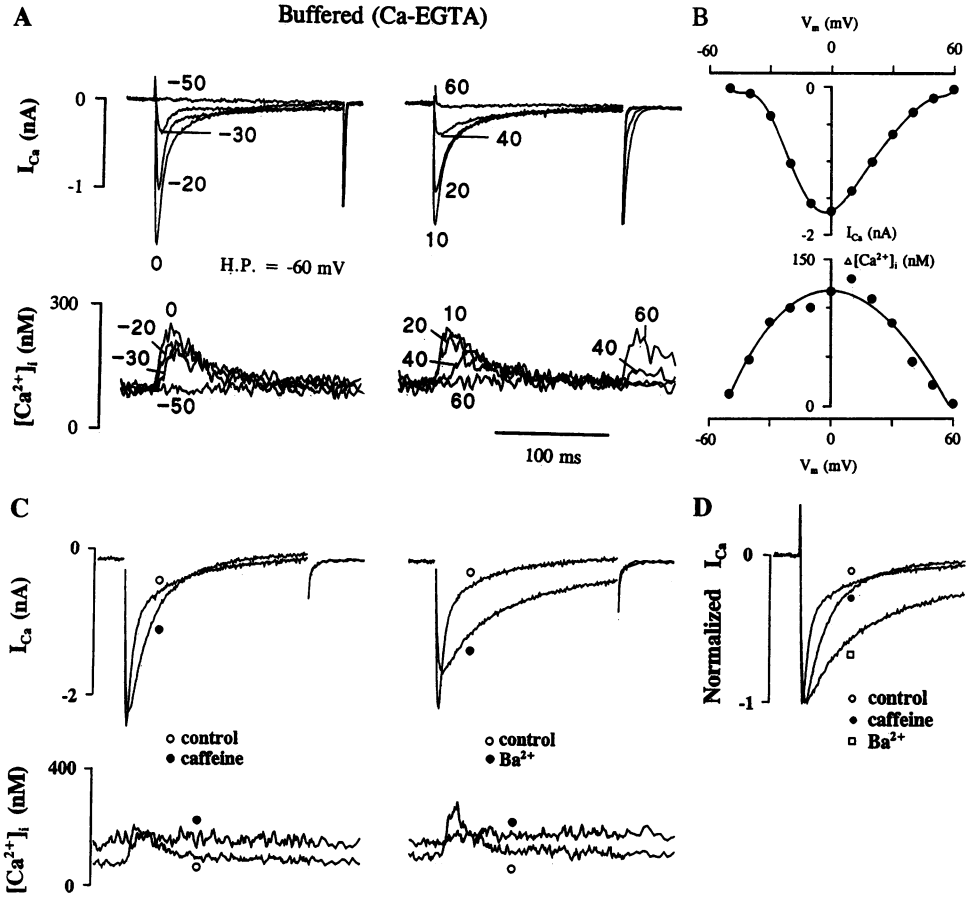

FIG. 4. (*A* and *B*) Voltage dependence of I_{Ca} and Ca^{2+} transients in myocytes dialyzed with 10 mM EGTA/5 mM Ca^{2+}. (*C*) Effect of caffeine and replacement of Ca^{2+} with Ba^{2+} on I_{Ca} inactivation in Ca^{2+}/EGTA-buffered myocytes (test potential, 0 mV). (*D*) Traces were leak subtracted and scaled to the same magnitude for comparison.

assuming that the rise in Ca^{2+} concentration is uniformly distributed in the myoplasm during Ca^{2+} influx via the exchanger, we estimate a K_m of 386 nM with maximal activation occurring at about 3 μM (Fig. 2). These values are remarkably similar to the values reported in skinned myocytes, where activation of release had a threshold at 100–160 nM and was maximal at 3 μM (7, 31). Similarly, in SR vesicles and single-channel studies, K_m and maximal activation were found to range, respectively, from 300 to 700 nM and from 2 to 20 μM, depending on the mode of activation (steady state vs. transient; refs. 22, 23, and 30).

The results showing significant inactivation of I_{Ca} by the release of Ca^{2+} from the SR in highly Ca^{2+}-buffered myocytes provide further support for close association of Ca^{2+} channels and ryanodine receptors. It was somewhat surprising that the release of Ca^{2+} from the SR inactivated the Ca^{2+} channels to a similar extent as Ca^{2+} passing through the sarcolemmal Ca^{2+} channels (Fig. 3). Theoretical considerations indicate that the local $[Ca^{2+}]$ at the inner mouth of the Ca^{2+} channel during the peak of I_{Ca} could reach as high as 0.5 mM (32). Although the Ca^{2+} inactivation site of the Ca^{2+} channel may reside at some distance from the direct permeation path (26), the Ca^{2+} concentration in the vicinity of the channel (e.g., about 30 μM at 5 nm from the pore) (32) should still be well above the physiological range of global cytoplasmic Ca^{2+} concentration. The observed inactivation of the Ca^{2+} channel by the released Ca^{2+} therefore suggests that the inactivation site of the Ca^{2+} channel is within the Ca^{2+}-accessible domain of the ryanodine receptors. This consideration places the Ca^{2+} channels and ryanodine receptors within perhaps 10–20 nm of each other (32). This scheme is further supported by finding that effective buffering of the global cytoplasmic Ca^{2+} concentration with 10 mM EGTA had no significant effect on the Ca^{2+}-dependent inactivation of Ca^{2+} channels caused either by I_{Ca} or by Ca^{2+} release from the SR (Fig. 4). Most likely both the proximity of the ryanodine receptor and the slow kinetics of EGTA (33) contribute to the persistence of Ca^{2+}-dependent inactivation of I_{Ca} in myocytes with high content of EGTA. These interactions provide a negative feedback mechanism capable of controlling Ca^{2+} influx and Ca^{2+} release from the SR on a millisecond time scale. The requirements of high Ca^{2+} and fast response time for such functional interactions tend to undermine "common pool" models, which consider changes of Ca^{2+} concentration in the myoplasm as a whole (34), and favor "local control" models, which consider dynamic changes of Ca^{2+} in well-defined spaces or "domains" (32, 35, 36) as controlling the Ca^{2+} release from the SR. In this model, Ca^{2+} release from SR is controlled by the unitary current rather than the macrosopic I_{Ca} (37). This could explain why Ca^{2+} release is activated by Ca^{2+}-induced Ca^{2+} release (6, 7, 9), even though the voltage dependence of Ca^{2+} transients may depart from that of I_{Ca} (11, 38).

Junctional complexes as the site for close coupling between Ca^{2+} channels and ryanodine receptors is consistent with the finding that these receptors are concentrated in the transverse tubules and terminal cisternae, respectively (39–41). Since ligand binding studies show that the number of ryanodine receptors is 3–9 times higher than that of L-type Ca^{2+} channels (42, 43), one Ca^{2+} channel may be coupled to a cluster of ryanodine receptors as proposed in the "cluster bomb" model (35) or even to a larger number of "sleepy" ryanodine receptors. Irrespective of the exact stoichiometry, the functional coupling of the two types of channel is essential for efficient signaling of Ca^{2+} release in rat cardiac myocytes because only I_{Ca}, and not the Na^+/Ca^{2+} exchanger, triggers the fast, graded Ca^{2+} releases (24, 44). It is likely that a somewhat different spatial relation may exist between Na^+/Ca^{2+} exchangers and ryanodine receptors in guinea pig ventricular myocytes, in which exchanger has been implicated in fast Ca^{2+} release (45, 46).

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1. Block, B. A., Imagawa, T., Campbell, K. P. & Franzini-Armstrong, C. (1988) *J. Cell Biol.* **107,** 2587–2600.
2. Rios, E. & Brum, G. (1987) *Nature (London)* **325,** 717–720.
3. Tanabe, T., Beam, K. G., Powell, J. A. & Numa, S. (1988) *Nature (London)* **336,** 134–139.
4. Tanabe, T., Beam, K. G., Adams, B. A., Niidome, T. & Numa, S. (1990) *Nature (London)* **346,** 567–569.
5. Schneider, M. F. & Chandler, W. K. (1973) *Nature (London)* **242,** 244–246.
6. Cleemann, L. & Morad, M. (1991) *J. Physiol. (London)* **432,** 283–312.
7. Fabiato, A. (1985) *J. Gen. Physiol.* **85,** 247–289.
8. Morad, M. & Cleemann, L. (1987) *J. Mol. Cell. Cardiol.* **19,** 527–553.
9. Nabauer, M., Callewaert, G., Cleemann, L. & Morad, M. (1989) *Science* **244,** 800–803.
10. Beuckelmann, D. J. & Wier, W. G. (1988) *J. Physiol. (London)* **405,** 233–255.
11. Cannell, M. B., Berlin, J. R. & Lederer, W. J. (1987) *Science* **238,** 1419–1423.
12. Nabauer, M. & Morad, M. (1990) *Am. J. Physiol.* **258,** C189–C193.
13. Cohen, N. M. & Lederer, W. J. (1988) *J. Physiol. (London)* **406,** 115–146.
14. Balke, C. & Wier, W. G. (1991) *Circ. Res.* **68,** 897–902.
15. Mitra, R. & Morad, M. (1985) *Am J. Physiol.* **249,** H1056–H1060.
16. Callewaert, G., Lipp, P., Pott, L. & Carmeliet, E. (1991) *Cell Calcium* **12,** 269–277.
17. Grynkiewicz, G., Poenie, M. & Tsien, R. Y. (1985) *J. Biol. Chem.* **260,** 3440–3450.
18. Benham, C. D. (1989) *J. Physiol. (London)* **415,** 143–158.
19. Callewaert, G., Cleemann, L. & Morad, M. (1989) *Am. J. Physiol.* **257,** C147–C152.
20. Dukes, I. D., Cleemann, L. & Morad, M. (1990) *J. Pharmacol. Exp. Ther.* **254,** 560–569.
21. Gyorke, S. & Fill, M. (1993) *Science* **260,** 807–809.
22. Rousseau, E. & Meissner, G. (1989) *Am. J. Physiol.* **256,** H328–H333.
23. Smith, J. S., Rousseau, E. & Meissner, G. (1989) *Circ. Res.* **64,** 352–359.
24. Sham, J. S. K., Cleemann, L. & Morad, M. (1992) *Science* **255,** 850–853.
25. Kimura, J., Miyamae, S. & Noma, A. (1987) *J. Physiol. (London)* **384,** 199–222.
26. Imredy, J. P. & Yue, D. T. (1992) *Neuron* **9,** 197–207.
27. Kokubun, S. & Irisawa, H. (1984) *Jpn. J. Physiol.* **34,** 599–611.
28. Morad, M., Davis, N. W., Kaplan, J. H. & Lux, H. D. (1988) *Science* **241,** 842–844.
29. Gyorke, S. & Palade, P. (1993) *Am J. Physiol.* **33,** C1505–C1512.
30. Meissner, G. & Henderson, J. S. (1987) *J. Biol. Chem.* **262,** 3065–3073.
31. Fabiato, A. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41,** 2238–2244.
32. Simon, S. M. & Llinas, R. R. (1985) *Biophys. J.* **48,** 485–498.
33. Stern, M. D. (1992) *Cell Calcium* **13,** 183–192.
34. Earm, Y. E. & Noble, D. (1990) *Proc. R. Soc. London B* **240,** 83–96.
35. Stern, M. D. (1992) *Biophys. J.* **63,** 497–517.
36. Chad, J. E. & Eckert, R. (1984) *Biophys. J.* **45,** 993–999.
37. Wier, W. G., Egan, T. M., Lopez-Lopez, J. R. & Balke, C. W. (1994) *J. Physiol. (London)* **474,** 463–471.
38. DuBell, W. J. & Houser, S. R. (1989) *Am. J. Physiol.* **257,** H746–H759.
39. Brandt, N. & Bassett, A. (1986) *Arch. Biochem. Biophys.* **244,** 872–875.
40. Inui, M., Saito, A. & Fleischer, S. (1987) *J. Biol. Chem.* **262,** 15637–15642.
41. Inui, M., Wang, S., Saito, A. & Fleischer, S. (1988) *J. Biol. Chem.* **263,** 10843–10850.
42. Bers, D. M. & Stiffel, V. M. (1993) *Am. J. Physiol.* **264,** C1587–C1593.
43. Wibo, M., Bravo, G. & Godfraind, T. (1991) *Circ. Res.* **68,** 662–673.
44. Bouchard, R. A., Clark, R. B. & Clark, W. R. (1993) *J. Physiol. (London)* **472,** 391–413.
45. Leblanc, N. & Hume, J. R. (1990) *Science* **248,** 372–376.
46. Kohmoto, O., Levi, A. J. & Bridge, J. H. B. (1994) *Circ. Res.* **74,** 550–554.