Microstructure-Based Monte Carlo Simulation of Ca$^{2+}$ Dynamics Evoking Cardiac Calcium Channel Inactivation

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Abstract: Ca$^{2+}$ dynamics underlying cardiac excitation-contraction coupling are essential for heart functions. In this study, we constructed microstructure-based models of Ca$^{2+}$ dynamics to simulate Ca$^{2+}$ influx through individual L-type calcium channels (LCCs), an effective Ca$^{2+}$ diffusion within the cytoplasmic space and in the dyadic space, and the experimentally observed calcium-dependent inactivation (CDI) of the LCCs induced by local and global Ca$^{2+}$ sensing. The models consisted of LCCs with distal and proximal Ca$^{2+}$ (Calmodulin-Ca$^{2+}$ complex) binding sites. In one model, the intracellular space was organelle-free cytoplasmic space, and the other was with a dyadic space including sarcoplasmic reticulum membrane. The Ca$^{2+}$ dynamics and CDI of the LCCs in the model with and without the dyadic space were then simulated using the Monte Carlo method. We first showed that an appropriate set of parameter values of the models with effectively extra-slow Ca$^{2+}$ diffusion enabled the models to reproduce major features of the CDI process induced by the local and global sensing of Ca$^{2+}$ near LCCs as measured with single and two spatially separated LCCs by Imredy and Yue (Neuron. 1992;9:197-207). The effective slow Ca$^{2+}$ diffusion might be due to association and dissociation of Ca$^{2+}$ and Calmodulin (CaM). We then examined how the local and global CDIs were affected by the presence of the dyadic space. The results suggested that in microstructure modeling of Ca$^{2+}$ dynamics in cardiac myocytes, the effective Ca$^{2+}$ diffusion under CaM-Ca$^{2+}$ interaction, the nanodomain structure of LCCs for detailed CDI, and the geometry of subcellular space for modeling dyadic space should be considered.

Key words: calcium, L-type calcium channel, dyadic space, Monte Carlo simulation.

Ca$^{2+}$ plays indispensable roles in the contraction of the heart and muscles [1–5]. The cytoplasmic Ca$^{2+}$ concentration in cardiac myocytes is kept extremely low at around 0.1 µM. A rise of intracellular Ca$^{2+}$ concentration is usually caused by Ca$^{2+}$ influx from the extracellular space and/or Ca$^{2+}$ release from intracellular organelles. Ca$^{2+}$ serves as the link between excitation and contraction. The L-type calcium channel (LCC) on the sarcolemma opens in response to membrane depolarization, allowing Ca$^{2+}$ influx into the dyadic space, the narrow space in the cytoplasm between the surface/T-tubule and sarcoplasmic reticulum (SR) membranes. When Ca$^{2+}$ influx from LCC diffuses within the dyadic space and reaches ryanodine receptors (RyRs) on the SR surface, it triggers Ca$^{2+}$ release from the SR via these RyRs, which causes further elevation of the cytoplasmic Ca$^{2+}$ concentration. This is known as Ca$^{2+}$-induced Ca$^{2+}$-release (CICR) [6, 7], leading to Ca$^{2+}$ binding to troponin C for muscle fiber contraction. Defective excitation and contraction coupling in cardiac myocytes occur in various heart diseases [8–11], suggesting that the quantity and the dynamics of Ca$^{2+}$ in the microenvironment of the dyadic space is under tight control. Intracellular Ca$^{2+}$ regulates the amount of Ca$^{2+}$ influx across the sarcolemma through calcium-dependent inactivation (CDI) of LCC [12]. This mechanism is important in the regulation of cardiac excitation and contraction coupling [13, 14], especially under β-adrenergic stimulation, which is known to be highly arrhythmogenic [15–19].

A need for a microstructure-based simulation of Ca$^{2+}$ dynamics has arisen recently because both the time and spatial resolutions of experimental studies have improved. Until now, ventricular myocytes have been modeled by compartmentalizing cytoplasmic into the main...
space and several cytoplasmic subspaces including SR [20–23]. In these models, Ca2+ in each space is lumped and represented by its concentration, and movement of Ca2+ between compartments is represented as macroscopic Ca2+ current. More complex models have used Markov chain representations of LCC and RyRs [24, 25]. However, even these models [24, 25] do not treat Ca2+ at the microscopic level. Modeling with compartmentalized intracellular spaces with lumped Ca2+ has a serious problem when the models are used to interpret experimental data of intracellular Ca2+ dynamics with high spatiotemporal resolutions. For example, a peak Ca2+ concentration in the dyadic space during an action potential is from 100 to 1,000 µM, suggesting that only 10 to 100 free calcium ions are present within a single dyadic space [26]. When CICR is blocked, the number of Ca2+ in the dyadic space could be even less. With such a small number of Ca2+, it is no longer acceptable to assume that the behavior of Ca2+ could be treated as a mass and that Ca2+ signaling in the dyadic space should be analyzed as a stochastic process. The stochastic properties of Ca2+ in the dyadic space may play important roles in the excitation and contraction coupling. At this level, the geometry of the dyadic space and interactions among LCCs should also be considered.

In this study, we develop microstructure-based models of Ca2+ dynamics using the Monte Carlo method to simulate Ca2+ influx through individual LCCs and diffusion within the dyadic space to induce CDI. The Monte Carlo method has begun to be used to examine Ca2+ dynamics in the dyadic space. For example, Koh et al. [27] and Tanskanen et al. [26] developed models of stochastic molecular signaling between LCCs and RyR. LCC-RyR signaling in them was influenced by both the stochastic dynamics of Ca2+ in the dyad as well as by the shape and relative positioning of the different proteins in the dyad. Here we construct microstructure-based models whose geometry is similar to what Koh et al. [27] and Tanskanen et al. [26] presented. Our model used in the current study is much simpler than the one by Tanskanen et al. [26] because it does not include calmodulin (CaM) or a CICR mechanism. Instead, we concentrate on gaining insights into how nanodomain properties of LCC-CaM-Ca2+ interactions and the microstructure of the dyadic space might contribute to Ca2+ signaling. To this end, we try to construct a model that can reproduce the experimental results of Imredy and Yue [28], who measured Ca2+ influx from single LCC and from two spatially separated adjacent LCCs in isolated cardiac myocytes. They showed that the dynamic properties of the CDI of the LCC with and without the presence of an adjacent LCC were different, suggesting that a local inhibition exists between neighboring LCCs. That is, Ca2+ influx through one channel contributes to the inactivation of an adjacent LCC without an elevation of bulk intracellular Ca2+ concentration. As a mechanism underlying such local and global Ca2+ sensing by the CaM-Ca2+ complex for the CDI of LCCs, Tadross et al. [29] proposed that the C lobe of CaM senses local Ca2+ as a result of Ca2+ influx from the host LCC, and the N lobe senses global and small changes arising from distant Ca2+ sources.

The local inhibition between LCCs was shown to appear within 50–200 ms after the depolarizing voltage clamp stimulus, leading to the hypothesis that Ca2+ accumulation in the vicinity of the LCC, to which Ca2+ influx through one LCC and Ca2+ diffused from the adjacent LCC contribute, is responsible for the local inhibition [28]. However, if the diffusion constant of Ca2+ within the cytoplasmic space is as large as that in the pure water, there may be no chance for Ca2+ to accumulate in the vicinity of the LCC, since 50–200 ms is long enough for the Ca2+ to diffuse away from the vicinity of the adjacent LCC. This suggests that CaM-Ca2+ interaction and microstructure-dependent slow Ca2+ diffusion within the dyadic space might be involved as an underlying mechanism of the local inhibition.

The results of this study suggest that the effectively slow Ca2+ diffusion under CaM-Ca2+ interaction, the nanodomain structure of LCCs for detailed CDI processes, and the restricted narrow space of dyad could play an important role in controlling Ca2+ dynamics in the cytoplasmic and dyadic spaces. A similar process may be involved when the Ca2+ released from the SR through RyRs controls Ca2+ influx through LCCs, and it will influence local Ca2+ signaling in the dyadic space, including CICR, which will be dealt with in the next step of this modeling study.

**METHODS**

**Geometry of the cytoplasmic space, LCCs, and junctional SR (JSR).** In this study, two different geometrical conditions were examined. The first consisted of the cell membrane and a cytoplasmic space with no subcellular organelles that would interfere with the free diffusion of the Ca2+ ions (Fig. 1a). The cytoplasmic space was represented as a square 80 nm deep and with 140 nm sides. Note that it was confirmed that the enlargement of the size of the cytoplasmic space did not change the results shown in this paper. The membrane contained either one LCC situated near the center of the membrane patch (Fig. 1a, left) or two LCCs placed at a distance of 30 nm (Fig. 1a, right). The second model included a dyadic space (Fig. 1b). The geometry of the dyadic space was essentially the same as that presented by Tanskanen et al. [26]. The surface membrane contained one or two LCCs within an area of 200 × 200 nm. The distance between the surface membrane and JSR was 15 nm. Arrays of RyRs were placed on the JSR membrane. Each RyR was assumed to be a rectangular parallelepiped with a base of 27 × 27 nm and a height of 12 nm. Therefore the distance between
Modeling of Ca²⁺ Dynamics

The motion of individual Ca²⁺ ions in the cytoplasmic space was modeled to follow Brownian dynamics with an “effective” diffusion constant $D$ m²/s, which is the main parameter varied to reproduce the experimental observation. The term effective resulted because the diffusion of Ca²⁺ near the LCCs could be much slower than diffusion in the pure water. This might be due to the presence of the calcium buffer, mainly CaM buffers. The value of $D$ required for reproducing the effects of local Ca²⁺ diffusion on CDI in the experiments of Imredy and Yue [28] was obtained by trial and error.

The time step of temporal discretization ($\Delta t_{\text{ion}}$) was set to be 100 ns. The cytoplasmic and dyadic spaces were discretized with cubes having sides of $2\sqrt{D\Delta t_{\text{ion}}/\pi}$ (0.0111 nm). We assumed that a Ca²⁺ in one cube isotropically diffuses at every time step to one of the six neighboring cubes. Interactions between Ca²⁺ and membrane surface charges were not considered in the present study. The cell and JSR membranes acted as diffusion barriers. Ca²⁺ that reached the boundary of the $200 \times 200$ nm square was eliminated.

L-type calcium channel model. A model of LCC was developed based on the Markov chain model of the LCC proposed by Faber et al. [25]. We hypothesized that there should be two Ca²⁺ (more precisely CaM-Ca²⁺ complex) binding sites responsible for the CDI of LCCs for reproduction of the experimental results by Imredy and Yue [28]. One is the proximal site, sensitive to and controlled directly by the Ca²⁺ influx through the individual LCC under consideration. The other is the distal site, sensitive to and controlled by Ca²⁺ diffused in the cytoplasm from the host and adjacent LCCs into the vicinity of the host LCC under consideration. The proximal and distal sites, respectively, might correspond to the binding site of the LCC with C and N lobes of the CaM where the C and N lobes of the CaM buffer.
lobe acts as a local Ca\(^{2+}\) sensor and the N lobe as a global Ca\(^{2+}\) sensor [29]. The distal site is sensitive to the presence of the Ca\(^{2+}\) buffer BAPTA, and the proximal site is not. We extended the Markov representation of the LCC developed by Faber et al. [25] to include two modes of CDI, Mode-Ca-p for CDI at the proximal binding site and Mode-Ca-d for CDI at the distal binding site (Fig. 1c). Channel inactivation due to the CDI was represented by shifts from the voltage-gating mode (Mode-V) to either Mode-Ca-p or Mode-Ca-d. Mode-V has four closed states C\(_{0}\), C\(_{1}\), C\(_{2}\), and C\(_{3}\), a single open state O, and two states representing fast (I\(_{Vf}\)) and slow (I\(_{Vs}\)) voltage depending inactivation. In Faber et al. [25], Ca\(^{2+}\) influx through LCC accumulates in and diffusively leaks from the subspace with Ca\(^{2+}\) concentration \([\text{Ca}^{2+}]_{\text{SS}}\) in mM, which determined the transition rate \(\delta = 4/(1 + 1/[\text{Ca}^{2+}]_{\text{SS}})\) from any state in Mode-V to the corresponding state in Mode-Ca. Here we defined two subspaces around each LCC. The first represents the proximal space close to the pore with a small volume with a radius \(R_p = 0.5\) nm. The Ca\(^{2+}\) concentration of this space, \([\text{Ca}^{2+}]_{\text{SP}}\) in mM, determines the transition rate modeled as \(\delta_0 = 2S_p/(1 + 1/[\text{Ca}^{2+}]_{\text{SS}})\) from any state in Mode-V to the corresponding state in Mode-Ca-p. This process represents the CDI induced by CaM-Ca\(^{2+}\) binding to the site in the proximal region of the LCC protein. Because of the small volume assigned to this proximal subspace, a single Ca\(^{2+}\) was the equivalent to \(\sim 10^4\) mM \([\text{Ca}^{2+}]_{\text{SP}}\) and therefore a value of 100,000 was used as a denominator to match the scale of Ca\(^{2+}\) concentration with the model by Faber et al. [25]. The coefficient \(S_p\) modifies the rate constant \(\delta_0\) and its value was also determined by trial and error as parameter \(D\) so that the simulated CDI could reproduce the experimental result [28]. The second subspace surrounds the proximal subspace and is referred to as the distal subspace. It is the area between the proximal subspace and the circle of radius \(R_d\), and this was set as 27 nm. Ca\(^{2+}\) concentration in the distal subspace, \([\text{Ca}^{2+}]_{\text{SD}}\) in mM, determines the transition rate \(\delta_0 = 2S_d/(1 + 1/[\text{Ca}^{2+}]_{\text{SS}})\) from any state of Mode-V to the corresponding state in Mode-Ca-d. This corresponds to the CDI induced by CaM-Ca\(^{2+}\) binding at the distal region of the LCC. The parameter \(S_d\) modifies the rate constant \(\delta_0\) and was also determined by trial and error. The transition between states in the model was calculated with a time step \(\Delta t = 1\) ms. This is ten times longer than the time step used for simulating the motion of Ca\(^{2+}\), and this was to speed up the calculation. When LCC was open (state O), at any time step a single Ca\(^{2+}\) would enter the cytoplasmic space with a probability of 0.1. This corresponded to a single channel current through LCC of \(-0.3\) pA.

**Conditional open probability analysis.** Conditional open probability analysis methods [28, 30] were used to analyze the dynamics of the LCC model in response to a 200 ms voltage-clamp step from \(-80\) mV to \(+15\) mV. The conditional open probability, \(P_{\text{OO}}(\tau, t)\), is defined as the probability that an LCC is open at time \(\tau\), conditioned upon known opening time \(t\). Since the LCC model has only one open state, after ensemble averaging of multiple openings at specific values of \(t\), the decay of \(P_{\text{OO}}(\tau, t)\) reflects the rate constants for channel inactivation. If the functional shape of \(P_{\text{OO}}(\tau, t)\) depends on the condition time \(t\), it means that the rate constants for inactivation change as time elapses, and this is due to changes in Ca\(^{2+}\) concentrations from the onset of the voltage clamp (\(t = 0\)), responsible for CDI. We used time ranges from 8 ms to 12 ms for calculating \(P_{\text{OO}}(\tau, 10)\), from 35 to 65 ms for \(P_{\text{OO}}(\tau, 50)\), and from 150 ms to 190 ms for \(P_{\text{OO}}(\tau, 170)\), as did Imredy and Yue [28]. Calculated probabilities with multiple single channel trials were averaged to obtain \(P_{\text{OO}}(\tau, t)\).

**Effects of local Ca\(^{2+}\) diffusion and inhibitory coupling between LCCs.** To examine the role of local Ca\(^{2+}\) diffusion and the presence of inhibitory coupling between adjacent LCCs, Ca\(^{2+}\) dynamics were simulated with two LCCs. The effect of the local Ca\(^{2+}\) diffusion was revealed by suppressing the Ca\(^{2+}\) diffusion. To this end, the simulations were performed with Ca\(^{2+}\) concentration in the distal subspace \([\text{Ca}^{2+}]_{\text{SD}}\) set to 0 mM. This condition of simulation corresponds to the experimental condition with BAPTA in Imredy and Yue [28] by which global (diffusing) Ca\(^{2+}\) is buffered and cannot contribute to CDI. Under this condition, the transition rate from Mode-V to Mode-Ca-d was zero, and CDI occurred only through the transition from Mode-V to Mode-Ca-p, i.e., by the local Ca\(^{2+}\) influx from the host LCC.

**Simulation environment.** The models used in this study are constructed on an *in silico* platform [31] (See also [32]). The platform provides a systematic support of model developments of physiological functions using agent-based models, ordinary differential equations, and hybrids of these types of modeling. The agent-based models often involve geometries of biological entities such as cells and subcellular organelles. Each agent may change its state, spatial position, and posture with time according to its own rules. This *in silico* platform was validated, for example, by confirming the reproduction ability for a similar Monte Carlo simulation on Ca\(^{2+}\) dynamics in the dyadic space performed by Koh et al. [27] (Kawazu et al. [33]). Our simulations of the Ca\(^{2+}\) dynamics in the heart cell employed an agent-based model that includes LCCs, individual Ca\(^{2+}\) ions, surface membrane, and JSR membrane surface with RyRs as the agents.

The agent-based models were simulated on PC clusters with 32 nodes of dual Xeon 3.2G processors for calculating sample paths with different seeds of random variables generated by the Mersenne Twister pseudorandom number generator.
RESULTS

For the local diffusion of Ca\(^{2+}\) from an adjacent LCC to influence the conditional open probability of an LCC at \(T\) ms after onset of the voltage-clamp step, the distance between LCCs (\(L\) nm) and \(\sqrt{D\tau}\) must be of the same order. The difference of \(P_{\text{OO}}(\tau, t)\) shape after 100 ms in Imredy and Yue [28] implies that \(T\) should be of the order of 100 ms and \(D \sim 10^{-15} \text{ m}^2/\text{s}\), which is \(10^{-5}\) smaller than that of Ca\(^{2+}\) in pure water. Starting from this initial guess of \(D\) value and using the model without the dyadic space, we determined the effective diffusion constant \(D\), the sensitivities of the proximal and distal CaM-Ca\(^{2+}\) binding sites of LCC, as \(D = 9.65 \times 10^{-16} \text{ m}^2/\text{s}\), \(S_p = 6 \times 10^{-2}\), and \(S_d = 4\) by trial-and-error comparison of \(P_{\text{OO}}(\tau, t)\) generated in our simulations with the data of Imredy and Yue [28].

These parameter values were then used for the model with the dyadic space to examine the influence of the presence of the SR on the CDI process.

Ca\(^{2+}\) dynamics beneath a simple membrane with one and two LCCs

Using the obtained values of \(D, S_p,\) and \(S_d\), we simulated Ca\(^{2+}\) dynamics beneath a simple membrane and its influence on CDI. Figure 2 shows the spatiotemporal dynamics of Ca\(^{2+}\) diffusion in a model with two LCCs responding to a voltage step from –80 mV to +15 mV. Ca\(^{2+}\) entering via one LCC diffused and reached the distal area of the other LCC around 100 ms after the onset of the voltage clamp step. Figure 2 can be compared with Fig. S9 of Tadross et al. [29].

Figure 3 shows the profiles of Ca\(^{2+}\) currents through one LCC, the Ca\(^{2+}\) concentration in the proximal and distal regions of the LCC, and the corresponding \(P_{\text{OO}}(\tau, t)\). In single and double LCC models, the Ca\(^{2+}\) currents through the LCC initially increased and then decayed after reaching a peak (Fig. 3a). Just after the onset of the voltage step, Ca\(^{2+}\) within the proximal subspace was the dominant source of Ca\(^{2+}\) for CDI of the target LCC (Fig. 3b). However, despite the high [Ca\(^{2+}\)]\(_{\text{SS-P}}\), the transition rate from Mode-V to Mode-Ca-p is severely limited by the low sensitivity of the proximal CaM-Ca\(^{2+}\) binding site; as a consequence, the LCC remains in Mode-V. As time elapsed, Ca\(^{2+}\) from the target LCC diffused into the distal subspace (Fig. 2), and [Ca\(^{2+}\)]\(_{\text{SS-D}}\) gradually increased and enhanced the transition from Mode-V to Mode-Ca-d. In the model with two LCCs, Ca\(^{2+}\) flowing into the cytoplasmic space via the adjacent LCC eventually reached the distal subspace of the target LCC, leading to a further increase of its [Ca\(^{2+}\)]\(_{\text{SS-D}}\). Simulating the effect of BAPTA by setting [Ca\(^{2+}\)]\(_{\text{SS-D}}\) to zero prevented the CDI at the distal site, and the decay of the Ca\(^{2+}\) current and the Ca\(^{2+}\) concentration were slow.

Figure 3c examines the conditional open probabilities of the LCC. In the one channel model, the conditional open probabilities at time intervals of \(t = 10, 50,\) and 170 ms showed almost the same decay (Fig. 3c, one channel). However, this was not so for the two channel model (Fig. 3c, two channels), where the decay exhibited differential time courses with slow decay at 10 ms, which then accelerated at later periods. This time-dependent alteration
of $P_{oo}(\tau, t)$ decay qualitatively reproduced the results of Imredy and Yue [28]. Figure 3c (+BAPTA) shows that for two LCCs with $[Ca^{2+}]_{3{s-d}}$ fixed at zero (representing BAPTA buffering of Ca$^{2+}$), no Ca$^{2+}$ could affect the distal binding site, and thus the time-dependent alteration of $P_{oo}(\tau, t)$ of the target LCC became slower (Fig. 3c, +BAPTA). In this model, therefore, local coupling between neighboring LCCs was mediated by the distal Ca$^{2+}$-binding site.

Dyadic space Ca$^{2+}$ simulations with one and two LCCs

We next examined the effects of the dyadic space on the Ca$^{2+}$ dynamics and inactivation properties of LCC. Figure 4 shows the Ca$^{2+}$ current profile of the target LCC, Ca$^{2+}$ concentrations of the proximal and distal subspaces, and the corresponding $P_{oo}(\tau, t)$ for the dyadic space model with two channels. The Ca$^{2+}$ current decays faster than the simple membrane model (Fig. 4a). This is due to the smaller volume of the dyadic space, which results in a higher Ca$^{2+}$ concentration and thus a more rapid onset of CDI via Mode-Ca-d (Fig. 4b). As expected, fixing $[Ca^{2+}]_{3{s-d}}$ at zero slowed the decay of Ca$^{2+}$ current and that of the proximal concentration. The conditional open probability functions still showed time-dependent alteration, though the differences were less pronounced than those seen with the simple membrane model with two LCCs (Fig. 4c, control). Figure 4c (+BAPTA) shows Ca$^{2+}$ dynamics...
in the dyadic space model with \([\text{Ca}^{2+}]_{SS-D}\) fixed at zero. Here, as under equivalent conditions in the simple membrane model, the time-dependent alteration of \(P_{oo}(t, t)\) disappeared, and the decay of the calcium current was slow. In conclusion, the simulation suggests that the narrow dyadic space geometry is able to influence interactions between adjacent LCCs.

**DISCUSSION**

The model of cardiac LCC with two CaM-Ca\(^{2+}\) binding sites for CDI and the Ca\(^{2+}\) diffusion within the cytoplasmic space was constructed. We showed that it could reproduce the local inhibition between two adjacent LCCs observed in guinea pig ventricular myocytes by Imredy and Yue [28]. The SR membrane was then included in the model to form a sarcolemmal dyadic space. We proceeded to show that the Ca\(^{2+}\) current decayed faster in the model with the dyadic space than in the simple membrane model without the dyadic space, in which the influence of the local inhibition between adjacent LCCs remained, though it was smaller than in the model without the dyadic space.

In our model, it was necessary for the reproduction of the local inhibition between adjacent LCCs to set the effective diffusion constant of Ca\(^{2+}\) in the dyadic space to be \(10^{-5}\) less than that in the pure water. The mechanisms responsible for the dramatic decrease of the effective diffusion of Ca\(^{2+}\) in the dyadic space could be due to the presence of the calcium buffer, mainly the CaM buffers. The overall average calcium affinity to CaM is relatively low, about 10 \(\mu\)M, under physiological conditions [34]. With such a low affinity, CaM will mainly be in the free calcium state (apocaldulin, apoCaM) at the intracellular concentration of calcium of about 0.1 \(\mu\)M. Upon the opening of LCC and with the substantial local increase
in Ca\(^{2+}\) concentration to about 10 \(\mu\)M, the CaM would be expected to buffer a sizable amount of Ca\(^{2+}\). However, setting a diffusion constant of Ca\(^{2+}\)-CaM complex at about 0.1 times the diffusion constant of the free calcium [35] would not dramatically change the overall effective diffusion of the free calcium ion within the dyadic space. It has been estimated that the local concentration of CaM near the channel can be as high as 2.5 nM, leading to about 25 CaM molecules near the channel [36]. Given the small dimension of the dyadic space, the CaM dimensions of about 4.5 \(\times\) 4.5 \(\times\) 6.5 nm [37], as well as considerations on the average distance between channels within the dyadic space, leads to a picture of a dyadic space crowded and saturated with CaM molecules. Because the local concentration of CaM near the channel is different from the background intracellular CaM concentration, the local concentration of CaM near the channel can be as high as 2.5 nM, leading to about 25 CaM molecules near the channel [36]. Given the small dimension of the dyadic space, the CaM dimensions of about 4.5 \(\times\) 4.5 \(\times\) 6.5 nm [37], as well as considerations on the average distance between channels within the dyadic space, leads to a picture of a dyadic space crowded and saturated with CaM molecules. Because the local concentration of CaM near the channel is different from the background intracellular CaM concentration ranging from 5 nM to 6 \(\mu\)M [34, 36], it has been estimated that at Ca\(^{2+}\) concentration smaller than 0.1 \(\mu\)M, the RyRs can bind with high affinity \((K_D = 5–25 \text{ nM})\) as many as 16 CaMs and 4 CaMs for Ca\(^{2+}\) concentration in the range of 100 \(\mu\)M. It has also been suggested that few CaMs can bind the LCC itself [36]. The affinity of CaM for Ca\(^{2+}\) is low; however, upon the binding of CaM to its many target proteins, the affinity of the complex CaM target for Ca\(^{2+}\) can increase as much as a 1,000-fold, to about 10 nM [39]. These considerations of a high concentration of immobile CaM-target buffers with the associated high increase in the affinity for calcium validate the so-called excess buffer approximation [40] in which the effective diffusion of free Ca\(^{2+}\) under the boundary condition of a weak Ca\(^{2+}\) source can be estimated as

\[
D = \frac{D_C}{1 + [B]/K_D}
\]

where \(D\) is the effective diffusion constant of Ca\(^{2+}\), \(D_C\) the diffusion constant of free Ca\(^{2+}\) \((D_C = 7 \times 10^{-10} \text{ m}^2/\text{s})\), \(K_D\) the dissociation constant (assuming the affinity of the order \(K_D = 10 \text{ nM}\)), and \([B]\) the bulk equilibrium buffer concentration, i.e., CaM-target (assuming \([B] = 2.5 \text{ mM}\)), leading to an effective diffusion constant of free Ca\(^{2+}\) of \(D = 2.8 \times 10^{-10} \text{ m}^2/\text{s}\), which is close to the one used for the simulations in this study. A further decrease in the effective diffusion can be supplied by the adsorption of Ca\(^{2+}\) to the phospholipids present in the bilayer membrane. It has been theoretically estimated that the effective diffusion constant of Ca\(^{2+}\) in the retinal rods can be reduced by a factor of 7-31 because of the adsorption dynamics [41].

The geometric arrangement of membrane proteins such as LCC and RyR in the dyadic space should also influence Ca\(^{2+}\)-dynamics. According to Tanskanen et al. [26], an LCC is about 19 nm high and 14.5 nm wide, and it projects about 2 nm from the surface membrane into the dyadic space, where it is separated from the surface of an RyR by only 3 nm. In this study, the Ca\(^{2+}\)-dynamics in the dyadic space model showed an accelerated decay of Ca\(^{2+}\) current and a concentration compared to the simple single-membrane formulation. Further studies are needed to examine the effects of the detailed morphological features of dyadic proteins on Ca\(^{2+}\)-dynamics. The current model also needs to include Ca\(^{2+}\) release from SR via RyRs and Ca\(^{2+}\) extrusion systems, such as the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) and the Ca\(^{2+}\)-pump on the cell membrane. Nevertheless, it has been shown that Ca\(^{2+}\) released from the SR dominates CDI initially, and Ca\(^{2+}\) entry via LCC then dominates because of Ca\(^{2+}\) reuptake by SR, which leads to a decrease in SR-dependent contribution to CDI [25, 42]. In response to depolarizing voltage-clamp stimulus, LCC-dependent CDI becomes dominant about 50 ms after the onset, and reaches about 80% contribution in CDI. In other words, RyRs open quickly, and then they are almost fully inactivated. Thus the time window in which SR-dependent Ca\(^{2+}\) contributes to CDI might be limited to the first 50 ms. This suggests that for the local inhibition between LCCs at 50–200 ms after the depolarizing voltage-clamp stimulus shown by Imredy and Yue [28], the Ca\(^{2+}\) influx from LCC might dominate CDI even with SR and CICR conditions. The transition rates \(\delta_0\) and \(\delta_0\) from the open to the inactivated state via CICR would reflect the sensitivities of the proximal and distal CaM-Ca\(^{2+}\) binding site of LCC, respectively. If the same Ca\(^{2+}\) concentrations of \([\text{Ca}^{2+}]_{3S,d}\) and \([\text{Ca}^{2+}]_{3S,D}\) were assumed, \(\delta_0\) was set about 10,000 times larger than \(\delta_0\) for the distal CaM-Ca\(^{2+}\) binding. This seemingly does not agree with the experimental data for the properties of CaM-Ca\(^{2+}\) binding to the cardiac Ca\(^{2+}\) channel, Ca1.2 [43], where the N lobe of CaM responsible for the global Ca\(^{2+}\) sensor is considered to be insensitive to the rapid dynamics of Ca\(^{2+}\) spikes; thus it does not directly induce CDI in response to high-amplitude Ca\(^{2+}\) spikes from the host LCC. This discrepancy might be partially because the spatial location of our distal site of LCC and that of the LCC binding site with N lobe of CaM might not be quantitatively the same. That is, the distal site of our model was modeled to occupy a more distant area than the location of the N lobe of CaM, and \([\text{Ca}^{2+}]_{3S,D}\) did not increase much compared with the high amplitude of \([\text{Ca}^{2+}]_{3S,P}\) in response to Ca\(^{2+}\) influx from LCC. Tadross et al. [29] proposed a model of LCC that can explain both local and global Ca\(^{2+}\)-sensitive CDIs. They modeled association and dissociation of apoCaM and CaM-Ca\(^{2+}\) complex with LCC and suggested that rapid Ca\(^{2+}\) release from CaM combined with greater affinity of LCC for apoCaM compared with its affinity for CaM-Ca\(^{2+}\) complex can induce the global Ca\(^{2+}\)-dependent CDI. Nevertheless, their model might also require a slow increase of the global Ca\(^{2+}\) concentration in response to Ca\(^{2+}\) influx from LCC, which requires an effectively slow Ca\(^{2+}\) diffusion possibly.
because of the presence of CaM buffers. Further studies are needed to resolve these differences between experimental data and in silico simulation.

The reproduction of the local inhibitory feedback control of LCC by CDI suggests that it will influence \( \text{Ca}^{2+} \) release from the SR. The study could therefore be a key step in linking excitation and contraction in cardiomyocytes, which is critical in various pathological conditions where \( \text{Ca}^{2+} \) leak and release from overloaded or pathological SR states are the basis for weakened contraction and various ventricular arrhythmias. The development of microstructure-based modeling of \( \text{Ca}^{2+} \)-dynamics in the dyadic space is thus of importance to link the molecular events to cardiac excitation and contraction. This study is an initial step for modeling the stochastic behavior of \( \text{Ca}^{2+} \) influx through an individual LCC and its effects on a neighboring channel. Further developments will include more complex interactions between \( \text{Ca}^{2+} \) and other elements in the dyadic space, which will eventually lead to a quantitative understanding of the roles of dyadic \( \text{Ca}^{2+} \) dynamics under various conditions.

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**APPENDIX**

The kinetic transition rates used in the Markov models of LCC (Fig. 1c) are as follows:

\[
\begin{align*}
\alpha &= 0.925\exp(V_m/30) \\
\beta &= 0.39\exp(-V_m/40) \\
\gamma &= 4\alpha \\
\omega &= 3\alpha \\
\omega_s &= 2\alpha \\
\omega_f &= \alpha \\
\kappa &= 0.7(1 - \exp(-V_m/280)) \\
\nu &= 0.005\exp(-V_m/40) \\
\phi &= 0.02\exp(V_m/500) \\
\lambda &= 0.03\exp(3V_m/300) \\
\lambda_s &= 0.001\exp(V_m/500) \\
\lambda_f &= (\beta\alpha\omega_f)/(\omega\alpha_s) \\
\lambda_s &= (\lambda_f\delta)/(\omega\alpha_s) \\
\delta &= \phi_0 \\
\delta_0 &= 2\phi_0/(1 + [(\text{Ca}^{2+})_{\text{SR}}/100000]) \\
\delta_0 &= 2\phi_0/(1 + [(\text{Ca}^{2+})_{\text{SR}}/100000]) \\
\theta &= 0.01
\end{align*}
\]

where \( V_m \) represents the membrane potential. \( \delta_f \) and \( \delta_0 \) represent the sensitivities or affinities of the hypothetical \( \text{Ca}^{2+} \) binding sites at the proximal and distal regimes of LCC, respectively.

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