Three-dimensional simulation of calcium waves and contraction in cardiomyocytes using the finite element method

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Simulation studies have also been performed to integrate the experimental findings and provide a mechanistic explanation for them (1, 10, 16, 19, 29, 31). Basically, all of these models assumed that Ca2+ diffusion from one release site to another and CICR were the basic mechanisms, although various modifications were made by incorporating novel experimental findings, such as stochastic opening of ryanodine receptors (RyR) (16, 19) and anisotropy in diffusion (31). In addition, to reproduce the evolution of the characteristic wave pattern, including the spiral wave, simulations have been extended to two-dimensional (2-D) space (10, 16, 31). However, there are virtually no 3-D models that can be used for detailed analyses. Indeed, a recent study using a novel confocal microscope clearly demonstrated the 3-D nature of Ca2+ wave propagation (15), thus necessitating the development of a competitive 3-D model.

Another important step in excitation-contraction coupling that is not taken into consideration by current simulation models is myocyte contraction. Initially identified as a focal contraction (7), a Ca2+ wave definitely changes the distance between adjacent Ca2+ release sites as it propagates longitudinally along a myocyte. Furthermore, force development may change the affinity of troponin C (TnC) for Ca2+, a major buffering system in the cytoplasm (13). Both of these aspects could potentially modulate Ca2+ wave propagation but have not yet been investigated fully.

In the present study, we have developed a 3-D simulator of Ca2+ wave propagation and contraction in cardiac myocytes in which the sarcolemma, myofibril, and Z line with Ca2+ release sites were modeled as separate structures using the finite element method. The wave front in 3-D space can be visualized in an arbitrary 2-D plane, facilitating detailed comparisons with the results obtained in earlier experimental and model studies. Furthermore, the effect of contraction on Ca2+ waves can be evaluated by simulation for the first time.

MATERIALS AND METHODS

CICR and Ca2+ diffusion model. The basic principle of the present simulation model was similar to those of previous reports (1, 10, 31) and is illustrated schematically in Fig. 1. In each sarcomere, Ca2+ release channels are located at the Z lines. Ca2+ released from the junctional SR (JSR) through a release channel (I_{Ca}) is buffered by calmodulin (CaM) in the cytosol and TnC on the thin filament or sequestered by the nonjunctional SR (NSR) (I_{NSR}) and translocated to the JSR to replenish it (I_{NSR}). The Ca2+ leak current (I_{leak}) was also

CARDIAC MUSCLE CONTRACTION is regulated by rhythmic changes in intracellular Ca2+ concentration ([Ca2+]i). Under normal conditions, transsarcolemmal Ca2+ influx activated by membrane depolarization triggers synchronous Ca2+ release from the sarcoplasmic reticulum (SR) to bring about a uniform rise in [Ca2+]i ([Ca2+]i-induced Ca2+ release, or CICR) (3). SR Ca2+ release can occur spontaneously without membrane depolarization to cause a local elevation of [Ca2+]i that propagates throughout the cell in a wavelike pattern under certain conditions (8, 9, 30). Besides their importance in basic physiology, Ca2+ waves also have clinical relevance because a focal increase in [Ca2+]i could activate a transient inward current and membrane depolarization, thus constituting a potentially arrhythmogenic event (2, 6). Accordingly, several studies have attempted to clarify the characteristics and underlying mechanisms of Ca2+ waves using single-cell (8, 15), multicellular (24), and whole heart (17) preparations.

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modeled. The remaining free Ca\textsuperscript{2+} diffuses into the sarcomere space, and if the [Ca\textsuperscript{2+}] at the adjacent Z line exceeds a certain threshold, regenerative Ca\textsuperscript{2+} release takes place. In this analysis, we adopted a deterministic rule for triggering Ca\textsuperscript{2+} release (22), instead of a stochastic model (16, 19), for the following reasons: 1) although a stochastic model may be a prerequisite for analyzing the evolution of Ca\textsuperscript{2+} from a Ca\textsuperscript{2+} spark, the present analysis exclusively studied the wave propagation; and 2) implementation of a stochastic process heavily increases the computational burden. We admit that this model is not necessarily the best, but it should represent a reasonable starting point. For the same reasons, transsarcolemmal ion exchange processes were eliminated.

The mathematical formulation for [Ca\textsuperscript{2+}] dynamics is represented by the reaction-diffusion equation described below for the y-axis coordinate in the longitudinal direction and the x- and z-axis coordinates for the transverse directions (Fig. 1):

\[
\frac{d[Ca^{2+}]}{dt} = \nabla \cdot (D_i \nabla [Ca^{2+}]) + f([Ca^{2+}]) \tag{1}
\]

where \( dt \) is development over time, \( D_i \) is a diagonal matrix describing the diffusivity of Ca\textsuperscript{2+}, and \( f([Ca^{2+}]) \) describes the kinetics of Ca\textsuperscript{2+} transport into and out of the cytoplasm. In this analysis, the diffusion coefficient for Ca\textsuperscript{2+} (diagonal elements of \( D_i \)) was set to 1.0 m\textsuperscript{2}/ms for the longitudinal (y-axis) direction and 0.5 m\textsuperscript{2}/ms for the transverse (x- and z-axis) directions on the basis of previous experimental studies (16, 27). Modifications were made depending on the location to yield the following equations. At the nodes where NSR is facing cytoplasm (NSR node in Fig. 1A, top):

\[
\frac{d[Ca^{2+}]}{dt} = \nabla \cdot (D_i \nabla [Ca^{2+}]) + (I_{up} - I_{rel}) V_{NSRnode} V_{myonode} - \frac{d[[CaM - Ca^{2+}]]}{dt} - \frac{d[[TnC - Ca^{2+}]]}{dt} \tag{2}
\]

where \( \text{CaM} \) is calcium-binding protein, \( \text{CaM} \), \( \text{TnC} \), and \( \text{Ca}^{2+} \) are rate constants governing the transition between the states; right: physical entities of the states. TCa\textsuperscript{*} and T\textsuperscript{*} are force-bearing states.
Table 1. Mechanical properties of myocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Shell Element, Sarcolemma</th>
<th>Truss Element</th>
<th>Solid Element</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus, kPa</td>
<td>10</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Poisson ratio</td>
<td>0.45</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Thickness, μm</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>1</td>
<td>0.01</td>
<td>1</td>
</tr>
</tbody>
</table>

At the nodes where JSR is facing cytoplasm (JSR node in Fig. 1A, top):

$$\frac{d([\text{Ca}^{2+}])}{dt} = \nabla \cdot (D \nabla [\text{Ca}^{2+}]) + I_{\text{tot}} \nabla V_{\text{JSRnode}}$$

$$\frac{d([\text{Ca}^{2+}] - [\text{Ca}^{2+}])}{dt} - \frac{d([\text{Ca}^{2+}] - [\text{Ca}^{2+}])}{dt}$$

where (TnC – Ca²⁺) is the Ca²⁺ buffered by TnC and (CaM – Ca²⁺) is the Ca²⁺ buffered by CaM. At nodes corresponding to the nucleus, permeability to Ca²⁺ diffusion was altered to examine its effect. We assumed that the nuclear membrane does not act as a diffusion barrier (4).

Muscle contraction model. To relate the local [Ca²⁺], to cross-bridge kinetics and force generation, we adopted the theoretical formalism proposed by Negroni and Lasclo (25). Briefly, the TnC state (regardless of whether bound to Ca²⁺) and the cross-bridge state (attached or detached) are combined into four states as shown in Fig. 1B: 1) TnC is not bound to Ca²⁺, and the cross bridge is attached (TnC*); 2) TnC is not bound to Ca²⁺, but the cross bridge remains attached (T*); 3) TnC is bound to Ca²⁺, but the cross bridge is detached (TCa*); and 4) TnC is not bound to Ca²⁺, and the cross bridge is detached (T). The transitions between these four states are governed by the evolution equations described in the Appendix. In the analysis examining the effect of the affinity of TnC for Ca²⁺, we changed the rate constant for Ca²⁺ binding to TnC (Y1). Of the four states, TCa* and T* contribute to force generation such that the active force per unit length (Fb) is as follows:

$$F_b = A \cdot ([\text{TCa}^*] + [\text{T}^*]) \cdot h$$

where A is a constant and h is the cross-bridge elongation. The sarcomere shortening concomitant with the detachment of a certain proportion of cross bridges further decreases the number of attached cross bridges through two additional paths whereby TCa* and T* decrease depending on the velocity of the shortening. This mechanism is known as shortening-induced deactivation (13).

The passive property is characterized by the force developed by the parallel elastic component (Fp):

$$F_p = K \cdot (L - L_0)^3$$

where K is a constant, L is the length of a half-sarcomere (L), and L₀ is the unstressed length of L.

The total muscle force (F) can be expressed as the sum of the active and passive forces as follows:

$$F = F_b + F_p$$

Cell geometry and finite element modeling. We assumed the geometry of the cell to be a cylinder with a diameter of 16 μm and a height of 104 μm. The Z lines, each of which is represented by a truss element network, are spaced at 2-μm intervals. The myofibrils were modeled by 113 vertical truss elements within a sarcomere, each of which had a diameter of 1 μm. They occupied 47% of the cross section. These values were estimated from Figs. 1 and 2 in Lipp and Niggli (21) and Fig. 2 in Ishida et al. (15). The sarcomere including the cytoskeleton was represented by mixed interpolation of tensorial component shell elements (11), and the cytoplasm was represented by hexahedral bilinear solid elements. The nucleus had a diameter of 5 μm and a height of 10 μm (15). Its center was located 15 μm from the end in the longitudinal direction and 4 μm from the center in the cross section. We also estimated these values from data in the literature (15, 21). There were 15,168 solid elements, 16,884 truss elements, and 5,248 shell elements, and the total number of degrees of freedom was 64,401. As a constitutive law for these finite elements, we assumed an isotropic St. Venant’s hyperelastic model, in which the strain energy function is calculated as follows (5):

$$W = \frac{1}{2} \left( \frac{E_v}{(1 + v)(1 - 2v)} \right) (trE)^2 + 2 \left( \frac{E}{2(1 + v)} \right) I : E$$

where E is the Green-Lagrange strain tensor, E is the Young’s modulus, and v is the Poisson’s ratio. The colon denotes the scalar products of two second-order tensors (23). Differentiation of the above equation with respect to E gives the second Piola-Kirchhoff stress tensor as follows:

$$S = \left[ \left( \frac{E_v}{(1 + v)(1 - 2v)} \right) I \otimes I + 2 \left( \frac{E}{2(1 + v)} \right) I \right] : E = \mathbf{C} : \mathbf{E}$$

where I is the second-order unit tensor, and (I ⊗ I) and I are the fourth-order tensors that operate on E as (I ⊗ I) : E = (trE)I and I : E = E, respectively. C is the fourth-order elasticity tensor that results in a constant due to the quadratic form of W, and S is the second Piola-Kirchhoff stress. These equations were defined using the coordinate system shown in Fig. 1. Because of the lack of numeric data on the material properties of the subcellular components, these values were adjusted to reproduce the cardiac muscle properties determined at the tissue level (20). The model-adjusted material properties used in this simulation are summarized in Tables 1–4.

Table 2. Cell geometry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Cell length</td>
<td>104 μm</td>
</tr>
<tr>
<td>r</td>
<td>Cell radius</td>
<td>8 μm</td>
</tr>
<tr>
<td>V_cell</td>
<td>Cell volume</td>
<td>20.9 × 10⁻⁶ μl</td>
</tr>
<tr>
<td>V_myo</td>
<td>Cytoplasm volume</td>
<td>V_cell × 0.47 = 9.83 × 10⁻⁶ μl</td>
</tr>
<tr>
<td>V_mito</td>
<td>Mitochondria volume</td>
<td>V_cell × 0.36 = 7.53 × 10⁻⁶ μl</td>
</tr>
<tr>
<td>V_SR</td>
<td>SR volume</td>
<td>V_cell × 0.035 = 0.732 × 10⁻⁶ μl</td>
</tr>
<tr>
<td>V_NSRR</td>
<td>NSR volume</td>
<td>V_cell × 0.0315 = 0.659 × 10⁻⁶ μl</td>
</tr>
<tr>
<td>V_JSR</td>
<td>JCSR volume</td>
<td>V_cell × 0.0035 = 0.0732 × 10⁻⁶ μl</td>
</tr>
<tr>
<td>V_other</td>
<td>Other volume</td>
<td>V_cell × 0.115 = 2.405 × 10⁻⁶ μl</td>
</tr>
</tbody>
</table>

SR, sarcoplasmic reticulum; JSR, junctional SR; NSR, nonjunctional SR; V_myond, VSRnode, V_SRnode, V_JSR, and V_SHnode (FE node numbers corresponding to NSR = 11,744) (μl); V_myond, V_SRnode, V_JSR, and V_SHnode (FE node numbers corresponding to JSR = 5,986) (μl); V_myond, V_SHnode (FE node numbers corresponding to cytoplasm = 17,730) (μl).
Table 3. Initial conditions of stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Contraction and Ca2+ Wave Collision</th>
<th>Cell Contraction with 3-D Ca2+ Wave Propagation</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca2+]i</td>
<td>0.00014 mmol/l</td>
<td>0.000115 mmol/l</td>
</tr>
<tr>
<td>[Ca2+]NSR</td>
<td>1.889 mmol/l</td>
<td>1.661 mmol/l</td>
</tr>
<tr>
<td>[Ca2+]JSR</td>
<td>1.842 mmol/l</td>
<td>1.628 mmol/l</td>
</tr>
<tr>
<td>[CSQN − Ca2+]</td>
<td>6.972 mmol/l</td>
<td>6.705 mmol/l</td>
</tr>
<tr>
<td>[CaM</td>
<td>0.002769 mmol/l</td>
<td>0.002304 mmol/l</td>
</tr>
<tr>
<td>[TCa]</td>
<td>0.2006 × 10−2 mmol/l</td>
<td>0.1678 × 10−2 mmol/l</td>
</tr>
<tr>
<td>[TCa*]</td>
<td>0.8131 × 10−2 mmol/l</td>
<td>0.6029 × 10−2 mmol/l</td>
</tr>
<tr>
<td>[T]</td>
<td>0.4638 × 10−2 mmol/l</td>
<td>0.3884 × 10−2 mmol/l</td>
</tr>
</tbody>
</table>

3-D, three-dimensional. See text for additional definitions.

modeled at the nodes on the cross-sectioned planes corresponding to the Z lines (Fig. 1A, top). Ca2+ was sequestered or released on the basis of Eqs. 2 and 3, and the diffusion was simulated using an element method, in which a time step of Δt = 0.01 ms was used. The Ca2+ concentration thus computed was applied to Negroni and Lascano’s model (25) to evaluate the contraction force. Next, the total muscle force was calculated using Eqs. 4–6 such that the new internal force of the truss element was determined for the finite element deformation analysis of the cell model. The resultant deformation of the myofibril was returned to the finite element model of the Ca2+ diffusion analysis and Negroni and Lascano’s model. Excitation-contraction coupling was thus realized. Excitation-contraction coupling analyses were performed for every 200 steps of the Ca2+ diffusion analysis.

Experiments. Although originally recognized as spontaneous myofilament oscillation within cells (7), sarcomere dynamics during Ca2+ waves are not well understood. We monitored the sarcomere length in isolated rat cardiomyocytes during Ca2+ waves. Hearts were removed from adult male Wistar rats (200–300 g) that were under pentobarbital sodium anesthesia (50 mg/kg), and the left ventricular myocytes were isolated using enzymatic dissociation as described previously (35). Myocytes were suspended in 1.8 mmol/l Ca2+ HEPES-Tyrode solution (in mmol/l: 137 NaCl, 5.4 KCl, 1.8 CaCl2, 0.5 MgCl2, 0.33 NaH2PO4, 5 HEPES, and 5 glucose, adjusted to pH 7.4 with NaOH at 22°C), transferred to a silicone-coated (Sigma; Sigma) glass chamber, and viewed using an inverted microscope (×40 objective, IX71; Olympus). During spontaneous Ca2+ waves, we recorded the sarcomere length in the middle region of the sarcomere using online Fourier analysis of digitized myocyte images (SarcLen; IonOptix). From simultaneously recorded myocyte images, we measured the cell length using NIH Image software (National Institutes of Health, Bethesda, MD). To visualize Ca2+ waves, myocytes were loaded with fluo-3 by incubation in the 1.8 mmol/l Ca2+ HEPES-Tyrode solution containing 5 mol/l fluo-3 AM (Molecular Probes, Eugene, OR) for 45 min at room temperature. Myocytes were observed using a confocal microscope (CSU22; Yokogawa) equipped with a charge-coupled device camera (EVM285SPD; Texas Instrument).

RESULTS

Simulation of normal excitation and Ca2+ waves. Simulated cell shortening and color-coded [Ca2+]i, during normal excitation are shown as time-lapse images in Fig. 2. In this case, normal excitation was simulated by homogeneously raising the [Ca2+]i, above the threshold (0.2 μmol/l; Refs. 18, 19) at time 0. Ca2+ release was evoked uniformly and instantaneously (0.1 s) along the whole cell length, such that the cell contracted quickly (0.1–0.3 s). When a localized Ca2+ spark occurred (simulated as Ca2+ release from a single release site; 0.05 s), it evolved into a Ca2+ wave and spread in opposite directions at equal velocity (Fig. 3). However, in this case, the Ca2+ wave

Table 4. Model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kms,up</td>
<td>Half-saturation concentration of L&lt;sub&gt;up&lt;/sub&gt;</td>
<td>0.00092 mmol/l</td>
</tr>
<tr>
<td>L&lt;sub&gt;up&lt;/sub&gt;</td>
<td>Maximum current through the L&lt;sub&gt;up&lt;/sub&gt; channel</td>
<td>0.00875 mmol/l/μms</td>
</tr>
<tr>
<td>Kn,muscle</td>
<td>Half-saturation concentration of I&lt;sub&gt;muscle&lt;/sub&gt;</td>
<td>0.00092 mmol/l</td>
</tr>
<tr>
<td>I&lt;sub&gt;muscle&lt;/sub&gt;</td>
<td>Maximum current through the I&lt;sub&gt;muscle&lt;/sub&gt; channel</td>
<td>0.0015 mmol/l/μms</td>
</tr>
<tr>
<td>[Ca2+]JSR</td>
<td>Maximum Ca2+ buffered in the JSR</td>
<td>15 mmol/l</td>
</tr>
<tr>
<td>τ&lt;sub&gt;off&lt;/sub&gt;</td>
<td>Time constant of Ca2+ transfer from the JSR to the NSR</td>
<td>180 ms</td>
</tr>
<tr>
<td>τ&lt;sub&gt;on&lt;/sub&gt;</td>
<td>Time constant of Ca2+ release from the JSR to the cytoplasm due to CICR</td>
<td>21/μms</td>
</tr>
<tr>
<td>τ&lt;sub&gt;on&lt;/sub&gt;</td>
<td>Time constant</td>
<td>10 ms</td>
</tr>
<tr>
<td>τ&lt;sub&gt;off&lt;/sub&gt;</td>
<td>Time constant</td>
<td>10 ms</td>
</tr>
<tr>
<td>[CSQN]</td>
<td>Maximum Ca2+ buffered by CSQN</td>
<td>10 mmol/l</td>
</tr>
<tr>
<td>Km,CSQN</td>
<td>Equilibrium constant for buffering by CSQN</td>
<td>0.8 mmol/l</td>
</tr>
<tr>
<td>Y1</td>
<td>Rate parameter of Ca2+ binding to TnC</td>
<td>78 l/mmol/μms</td>
</tr>
<tr>
<td>Y2</td>
<td>Rate parameter of cross-bridge attachment to a thin filament site associated with TCa</td>
<td>0.104 l/μms</td>
</tr>
<tr>
<td>Y3</td>
<td>Rate parameter of Ca2+ release from TCa*</td>
<td>0.36 l/μms</td>
</tr>
<tr>
<td>Y4</td>
<td>Rate parameter of T&lt;sup&gt;*&lt;/sup&gt; detachment</td>
<td>0.2 l/μms</td>
</tr>
<tr>
<td>Diag (D&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>Diagonal matrices describing the diffusivity</td>
<td>(0.5–1.0, 0.5) μm&lt;sup&gt;2&lt;/sup&gt;/μms</td>
</tr>
<tr>
<td>Z1</td>
<td>Rate parameter of Ca2+ unbinding from TCa</td>
<td>0.36 l/μms</td>
</tr>
<tr>
<td>Z2</td>
<td>Rate parameter of TCa&lt;sup&gt;*&lt;/sup&gt; detachment</td>
<td>0.0013 l/μms</td>
</tr>
<tr>
<td>Z3</td>
<td>Rate parameter of Ca2+ rebinding to T&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.120 l/mmol/μms</td>
</tr>
<tr>
<td>Y4</td>
<td>Parameter determining the influence of dX/dt on Q&lt;sub&gt;el&lt;/sub&gt; and Q&lt;sub&gt;ex&lt;/sub&gt;</td>
<td>9.000 ms/μm&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>[T]</td>
<td>Total TnC</td>
<td>0.07 mmol/l</td>
</tr>
<tr>
<td>R</td>
<td>Exponential parameter characterizing the L dependence of [TCa]&lt;sub&gt;off&lt;/sub&gt;</td>
<td>20 l/μm&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>L&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Optimal L for maximum [TCa]&lt;sub&gt;off&lt;/sub&gt;</td>
<td>1.17 μm</td>
</tr>
<tr>
<td>[CaM]</td>
<td>Maximum Ca2+ buffered by CaM</td>
<td>0.05 mmol/l</td>
</tr>
<tr>
<td>K&lt;sub&gt;CaM&lt;/sub&gt;</td>
<td>Equilibrium constant for buffering by CaM</td>
<td>0.000238 mmol/l</td>
</tr>
<tr>
<td>K</td>
<td>Proportional parameter of the parallel elastic component of F&lt;sub&gt;p&lt;/sub&gt;</td>
<td>10,000 mN/mm&lt;sup&gt;2&lt;/sup&gt;/μm&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>a0</td>
<td>Unstressed L characterizing F&lt;sub&gt;p&lt;/sub&gt;</td>
<td>0.97 μm</td>
</tr>
<tr>
<td>h&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Cross-bridge elongation at steady-state L</td>
<td>0.005 μm</td>
</tr>
<tr>
<td>A</td>
<td>Stiffness of the equivalent cross-bridge elastic element referred to concentrations</td>
<td>1,800 mN4/mm&lt;sup&gt;2&lt;/sup&gt;/μmol/μmol</td>
</tr>
<tr>
<td>B</td>
<td>Proportionality parameter for dX/dt</td>
<td>1.2 l/μms</td>
</tr>
</tbody>
</table>
could induce only weak contraction (0.1–0.3 s), consistent with our experimental observations (Figs. 2B and 3B) and those in another report (24). We also simulated a case in which two independent Ca\[^{2+}\]/H\(^{11001}\) waves collided (Fig. 4). Waves were initiated at both ends of the cell and propagated toward the center. Because the propagation velocity was the same, the two waves collided in the middle (0.4 s) and then disappeared (0.6 s). This simulation also reproduced our experimental findings (Fig. 4B) and those described in a previous report (8).

Wave propagation velocity. In this study, the Ca\[^{2+}\]/H\(^{11001}\) wave propagation velocity was 140 \(\mu\text{m/s}\) under the control conditions (resting [Ca\[^{2+}\]]\(_i\) = 0.13 \(\mu\text{mol/l}\), [Ca\[^{2+}\]]\(_\text{JSR}\) = 1.86 mmol/l). Because previous experimental studies (24) suggested a dependence of the propagation velocity on Ca\[^{2+}\] loading of the cell, we evaluated the effect of the SR Ca\[^{2+}\] content on the propagation velocity to investigate whether they had a linear relationship with each other (Fig. 5A). The dependence was confirmed when either the myocyte was allowed to shorten (solid line) or its length was fixed (broken line). We changed the Ca\[^{2+}\] content of the JSR because Miura et al. (24), in the relevant experiment, estimated SR Ca\[^{2+}\] loading on the basis of the released Ca\[^{2+}\], which presumably was stored in the JSR. We examined the effect of NSR Ca\[^{2+}\] store to find a similar result with JSR Ca\[^{2+}\] store (data not shown). We think this is
because the Ca\(^{2+}\) content of NSR and JSR are closely related. The difference in velocity between the two conditions is discussed below. When we changed the affinity of TnC for Ca\(^{2+}\) by altering the rate constant for Ca\(^{2+}\) binding with TnC (Y1 in Fig. 1B), the velocity decreased as the affinity increased (Fig. 5B). These results are consistent with previous experimental findings (8, 24) and therefore validate the basic assumption of the current simulation model.

**Effect of contraction on Ca\(^{2+}\) waves.** In all of the analyses described above, the myocyte was allowed to shorten in the unloaded condition. Under physiological conditions, however, each myocyte is connected to adjacent myocytes and thus under constraint. Accordingly, we simulated a case in which the myocyte length was held constant under load (isometric condition). In Fig. 6, the propagations of both the Ca\(^{2+}\) (left column) and contraction (sarcomere strain pattern; right column) waves are color coded and shown for the unloaded (Fig. 6A) and isometric (Fig. 6B) conditions with time on the x-axis and longitudinal location on the y-axis. The Ca\(^{2+}\) waves propagated smoothly under both conditions, but the unloaded Ca\(^{2+}\) wave reached the end of the cell earlier because of cell shortening. On the other hand, the contraction (sarcomere shortening) wave exhibited different patterns of propagation depending on the mode of contraction. In contrast to the very smooth propagation in the unloaded condition (Fig. 6A, right), isometric contraction produced a heterogeneous progression of sarcomere length distribution (Fig. 6B, right). Such heterogeneity in the strain distribution cannot be explained by the
distribution or the stress equilibrium on the basis of the static stress-strain relationship. In Negroni and Lascano’s contraction model (25), shortening deactivation is implemented by the force deficit terms $Q_{d1}$ and $Q_{d2}$, formulated as follows (see also the APPENDIX):

$$Q_{d1} = Y_d \cdot \left(\frac{dX}{dt}\right)^2 \cdot [T^*]$$  \hspace{1cm} (9)

$$Q_{d2} = Y_d \cdot \left(\frac{dX}{dt}\right)^2 \cdot [TCa^*]$$  \hspace{1cm} (10)

where $dX/dt$ is the strain rate. We hypothesized that the complex pattern of contraction was induced by the strain rate, depending on the heterogeneity of the stress-strain relationship. In other words, in the proximity of active shortening (blue region), the sarcomere becomes softer because of the high $(dX/dt)^2$ value and local stretching, thus leading to the complex strain pattern in Fig. 6B. To test this hypothesis, we repeated the simulation under the isometric condition without the force deficit terms $Q_{d1}$ and $Q_{d2}$ and compared the result with the original calculation (Fig. 6C). The results clearly revealed that

Fig. 4. A: time-lapse images of a 3-D simulation showing the collision and disappearance of 2 independent Ca$^{2+}$ waves. B: collision of the Ca$^{2+}$ waves observed in the experiment.
the complex pattern disappeared, thus supporting our hypothesis. A similar tendency was observed experimentally in isolated cardiomyocytes. During a Ca$^{2+}$ wave, each myocyte exhibited prolonged shortening to a small extent (<20 μm) (Fig. 7A, A and B, broken lines). However, because the intensity of the cell attachment to the glass surface varied considerably, thereby applying different loads to the contracting cell, the shortening length of each myocyte differed significantly. In a myocyte that shortened by nearly 20 μm (Fig. 7A, broken line), the sarcomere shortened smoothly by ~0.2 μm (Fig. 7A, solid line). On the other hand, in a myocyte shortened to a lesser extent (<10 μm), the sarcomere stretched first before it shortened (Fig. 7B, solid line). In the simulation, heterogeneity in the sarcomere strain had an influence on the propagation velocity of the Ca$^{2+}$ wave. Although the color-coded presentations appeared the same (Fig. 6, left), close examination of the isometric Ca$^{2+}$ wave revealed local heterogeneity in the propagation velocity (Fig. 8A, broken line), in clear contrast to that of unloaded shortening (Fig. 8A, solid line). However, when averaged over its entire course, the propagation velocity of the Ca$^{2+}$ wave was slightly faster in the isometric condition (Fig. 8B, thick solid line).

**Evolution of spiral waves in 3-D space.** We examined the effect of the nucleus on Ca$^{2+}$ wave propagation by initiating Ca$^{2+}$ sparks in the proximity of the nucleus. Each nucleus was treated not only as a region lacking a releasable Ca$^{2+}$ pool but also as a buffering pool (21). In the short-axis slice, the Ca$^{2+}$ wave spread around the nucleus, creating a spiral wave (Fig. 9A, top). In the longitudinal plane (middle and bottom), however, the evolution of the wave differed significantly depending on the depth of the slice. As shown in Fig. 9 (middle), the spiral wave was observed only in the slice including the nucleus. This result is consistent with the experimental observations in Ishida et al. (15), thus substantiating the importance of 3-D simulation. The propagation velocity of a spiral wave was 76 μm/s and thus slower than a planar wave. When the buffering power of the nucleus was excluded, formation of a spiral wave was not evident (data not shown) and the propagation velocity became slightly faster (81 μm/s).

**DISCUSSION**

**Assumption of rapid Ca$^{2+}$ buffering.** The role of buffering in Ca$^{2+}$ waves has been studied in detail by Keizer and colleagues (28, 33). On the basis of a simulation model, they showed that assumption of rapid buffering is valid if the equilibration time of the buffer is much smaller than the time required for Ca$^{2+}$ diffusion across a region of the size of a typical gradient. Quantitatively, their criteria are expressed as follows:

$$\tau = \frac{1}{(k^- + k^+)[Ca^{2+}] + [B]} \ll \frac{L^2}{D_c}$$

where $\tau$ is the equilibration time constant for the buffer, $k^-$ and $k^+$ are the rate constants for the binding and dissociation between Ca$^{2+}$ and the buffer, respectively, [B] is the concentration of the buffer, $L$ is the characteristic length of the [Ca$^{2+}$] profile, and $D_c$ is the diffusion constant of Ca$^{2+}$. Calculating $\tau$ for CaM with the values used in our study ([Ca$^{2+}$] = 1 μmol/l, [CaM − Ca] = 50 μmol/l) and those in the previous report by Smith et al. (28), the $\tau$ value for CaM is ~0.00004. This value is much smaller than the $L^2/D_c$ value calculated in the present study ($L^2/D_c$; 0.001, $L = 1$ μm, and $D_c = 1,000$ μm$^2$/s), thus indicating that the assumption of rapid buffering can be applied to the present simulation.

In this simulation, we treated CaM as stationary for the sake of simplicity. According to Smith et al. (28), however, the existence of a mobile buffer reduces the effective diffusion coefficient for Ca$^{2+}$ ($D_{eff}$) as follows:

$$D_{eff} = \beta(D_c + \gamma_m D_m)$$

where $\beta$ is the differential fraction of free to bound Ca$^{2+}$ (<1), and

$$\gamma = \frac{K_m [B_m]}{(K_m + [Ca^{2+}])^2}$$

where $K_m$ is the dissociation constant of the mobile buffer, $[B_m]_T$ is the total concentration of the mobile buffer, and $D_m$ is the diffusion constant for the mobile buffer. Again, calculating $\gamma_m D_m$ for CaM, the result is ~330 μm/s for the conditions used in this simulation ($K_m = 2.4 \mu$mol/l, $[B_m]_T = 50 \mu$mol/l, $[Ca^{2+}] = 1 \mu$mol/l, $D_m = 32 \mu$m/s). This would amount to ~33% of the $D_c$ used in this study, thus leading to an underestimation of the propagation velocity.
Comparisons with previous studies. Studies of Ca$^{2+}$ waves have been promoted by a number of breakthroughs in experimental technique, such as the application of Ca$^{2+}$ indicators (26, 32), laser confocal microscopy, and digital image processing (8, 15, 24, 34). Along with these developments, simulation studies also have made a significant contribution to the understanding of this complex phenomenon involving various intracellular dynamics (1, 10, 16, 19, 29). Most of these studies are based on a mechanistic model in which the diffusion of Ca$^{2+}$ released by Ca$^{2+}$ sparks induces a regenerative Ca$^{2+}$ release from the adjacent SR (8). Our simulation model also adopted this conceptual framework and used the mathematical formulation of SR Ca$^{2+}$ uptake, relocation, release, and intracellular binding proposed by Luo and Rudy (22). Although transsarcolemmal ion flux was ignored, our model could successfully reproduce the bidirectional propagation and disappearance after collision of Ca$^{2+}$ waves together with the changes in cell length (Figs. 2–4). Furthermore, dependence of the Ca$^{2+}$ wave propagation velocity on intracellular Ca$^{2+}$ loading and affinity binding was also confirmed (24).

On the other hand, we did not incorporate the stochastic nature of Ca$^{2+}$ sparks (16, 19), owing to limitations in com-
puter power. In this sense, our simulator is ineffective for studying the evolution process of Ca$_2^+$ waves from partial Ca$_2^+$ sparks but can be used to investigate the coupling of a Ca$_2^+$ wave with local sarcomere contraction and complete simulation in 3-D space, which were not achieved by previous simulators.

Effect of contraction on Ca$_2^+$ waves. Using the cardiac contraction model of Negroni and Lascano (25), we coupled [Ca$_2^+$], with contraction to evaluate its influence on Ca$_2^+$ waves. To our knowledge, this is the first attempt to couple these aspects of cardiomyocytes using full 3-D simulation. Currently, it requires ~37 h to complete the computation for a single contraction (2 s) using a CPU running at 3.2 GHz with 2-GB memory. Although the limitations in computational power forced us to adopt this relatively simple model, the analysis revealed interesting findings. As expected on the basis of experimental observations, there was significant internal shortening and stretching along the course of Ca$_2^+$ wave propagation while the cell length was kept constant (Fig. 6B, right). These changes in sarcomere length can influence a Ca$_2^+$ wave in at least two ways. First, strain applied to the thin filament increases the affinity of TnC for Ca$_2^+$ (14) and thus potentially slows the propagation. This property was incorporated into our model as a decrease in Ca$_2^+$ from TnC as the sarcomere shortened. Second, sarcomere shortening can decrease the diffusion distance to accelerate the propagation. Through these mechanisms, isometric contraction is expected to slow the propagation. In our analysis, however, isometric contraction exhibited a complex velocity pattern (Fig. 7A). To clarify these apparently contradictory results, further studies are required in both the experimental and simulation fields.

Nucleus and Ca$_2^+$ waves. Although 3-D simulations of Ca$_2^+$ waves have been reported (16, 31), most have been simple extrapolations of 2-D simulations. Considering the axisymmetric and repetitive structure of cardiomyocytes, this type of simplification may be validated. However, a recent report by Ishida et al. (15) showing the evolution of spiral waves in 3-D space highlights the necessity for complete 3-D simulation, especially in the presence of the nucleus as an obstacle to propagation. The role of the nucleus in the generation of spiral waves was investigated by Dupont et al. (10), but their 2-D simulation could not provide an answer to the vertical heterogeneity in wave propagation reported by Ishida et al. (15). Although our simulation model successfully reproduced the previously reported generation, velocity, and vertical heterogeneity of spiral waves (15, 21), we could not model the oscillatory waves emanating from the spiral waves (21). Generation of repetitive waves may require a different model for CICR. Because recent studies have revealed spreading of Ca$_2^+$ waves over trabeculae (24) and the whole heart (17), oscillatory waves may be an origin of fatal arrhythmia. Further studies are required to clarify the conditions for generating oscillatory waves.

Study limitations. As stated repeatedly, we excluded the transsarcolemmal ion flux from the analysis because of limi-
Simulations in computer power. It has been shown that spontaneous Ca²⁺ release can cause membrane depolarization that sometimes induces an action potential (6). To analyze this potentially arrhythmogenic event, more comprehensive models that include ion channels and exchangers are needed.

Because of a lack of experimental data, the nucleus was treated as a region lacking a Ca²⁺ release store and a site for Ca²⁺ buffering. However, judging by the complex wave pattern around the nucleus reported in the literature (15, 21), the nucleus may have another role in cytosolic Ca²⁺ regulation. Further experimental evaluation of not only this aspect but also anisotropy in diffusion (12, 31) and Ca²⁺ buffering will surely contribute to the development of a complete model of cardiomyocytes.

Summary. To simulate Ca²⁺ wave propagation in cardiomyocytes, a finite element simulation program incorporating Ca²⁺ diffusion and excitation-contraction coupling mechanisms was developed. The results clearly indicate the 3-D nature of this phenomenon, the modulatory effect of contraction, and the role of the nucleus in the evolution of complex wave patterns.

APPENDIX: FORMULATION OF THE MODEL

1) Ca²⁺ dynamics

At the NSR node:

\[
\frac{d[Ca^{2+}]_{NSR}}{dt} = \nabla \cdot (D_{NSR} \nabla [Ca^{2+}]) + (I_{leak} - I_{up}) \frac{V_{NSR,node}}{V_{myonode}};
\]

Ca²⁺ balance in the cytoplasm

\[
\frac{d[Ca^{2+}]_{JSR}}{dt} = (I_{leak} - I_{up}) \frac{V_{JSR,node}}{V_{myonode}}; \quad \text{Ca}^{2+} \text{ balance in the JSR}
\]

\[
I_{leak} = \frac{I_{up}}{[Ca^{2+}]_{NSR}} [Ca^{2+}]_{JSR} + K_{Ca} \text{ : Ca}^{2+} \text{ leakage from the NSR}
\]

\[
I_{up} = \frac{(I_{Ca} - I_{Ca})}{\tau_{Ca}}; \quad \text{Ca}^{2+} \text{ uptake by the NSR}
\]

\[
I_{Ca} = \frac{[Ca^{2+}]_{JSR}}{\tau_{Ca}} \cdot \nabla \cdot (D_{Ca} \nabla [Ca^{2+}]) + \frac{V_{Ca}}{V_{myonode}}; \quad \text{Ca}^{2+} \text{ balance in the cytoplasm}
\]

At the JSR node:

\[
\frac{d[Ca^{2+}]_{JSR}}{dt} = \frac{V_{JSR,node}}{V_{myonode}} \nabla \cdot (D_{Ca} \nabla [Ca^{2+}]) + I_{relCICR} \nabla \cdot (V_{myonode} - V_{myonode}; \quad \text{Ca}^{2+} \text{ balance in the JSR}
\]

\[
[Ca^{2+}]_{JSR,total} = [Ca^{2+}]_{JSR} + [CSQN - Ca^{2+}]; \quad \text{Total Ca}^{2+} \text{ in the JSR}
\]

\[
[CSQN - Ca^{2+}] = \frac{[CSQN] \cdot [Ca^{2+}]_{JSR}}{([Ca^{2+}]_{JSR} + K_{Ca,CSQN})}; \quad \text{Ca}^{2+} \text{ buffering in the JSR}
\]

\[
I_{relCICR} = \frac{G_{relCICR} \cdot ([Ca^{2+}]_{JSR} - [Ca^{2+}])}{\nabla \cdot (D_{Ca} \nabla [Ca^{2+}]) + \frac{V_{Ca}}{V_{myonode}}}; \quad \text{Ca}^{2+} \text{ balance in the JSR}
\]

\[
G_{relCICR} = 0 \quad \text{when } [Ca^{2+}] < \text{ the threshold level}
\]

\[
G_{relCICR} = \frac{1 - \exp(-t/\tau_m)}{\tau_m} \cdot \exp\left(-\frac{t}{\tau_{off}}\right)
\]

when \( [Ca^{2+}] \geq \text{ the threshold level; } t = 0 \text{ at the onset of CICR} \)

For both the NSR and JSR nodes:

\[
[Ca^{2+}]_{total} = [Ca^{2+}]_i + [TnC - Ca^{2+}] + [CaM - Ca^{2+}]; \quad \text{Total Ca}^{2+} \text{ in the cytoplasm}
\]
2) \( \text{Ca}^{2+} \) buffering in the cytoplasm

\[ [\text{CaM} - \text{Ca}^{2+}] = \frac{[\text{CaM}] \cdot [\text{Ca}^{2+}]}{([\text{Ca}^{2+}]) + K_{\text{CaM}}} \]

(Fast buffering was assumed; see text for details.)

\( \text{TnC} \):

\[ [\text{TN} - \text{Ca}^{2+}] = [\text{TCa}] + [\text{TCa}^*] \]

Total amount of TnC bound to Ca

\[ \frac{d[\text{TCa}]}{dt} = Q_a - Q_6 \\
\frac{d[\text{TCa}^*]}{dt} = Q_6 - Q_a - Q_{62} \\
\frac{d[\text{T}^*]}{dt} = Q_d - Q_a - Q_{61} \]

\[ [\text{T}] = [\text{T}] - [\text{TCa}] - [\text{TCa}^*] - [\text{T}^*] \]

\[ Q_a = Y_t \cdot [\text{Ca}^{2+}] \cdot [\text{T}] - Z_t \cdot [\text{TCa}] \]

\[ Q_6 = Y_s \cdot [\text{Ca}^{2+}]_d - Z_s \cdot [\text{TCa}] \]

\[ [\text{TCa}]_d = [\text{TCa}] \cdot \exp(-R(L - L_d)^3) \]

\[ Q_d = Y_d \cdot \frac{dX}{dt} \cdot [\text{T}^*] \]

\[ Q_{62} = Y_t \cdot \frac{dX}{dt} \cdot [\text{TCa}^*] \]

At nodes including the nucleus:

\[ \frac{d[\text{Ca}^{2+}]}{dt}_{\text{nucleus}} = -I_{\text{nucleus}} \cdot \frac{[\text{Ca}^{2+}]}{([\text{Ca}^{2+}] + K_{\text{Ca}^{2+}})} \]

\[ I_{\text{nucleus}} = I_{\text{nucleus}} \cdot [\text{Ca}^{2+}] + \frac{Q_{62}}{} \cdot \text{Ca}^{2+} \text{ uptake by the nucleus} \]

3) Mechanical properties of myofilaments

\[ F = F_b + F_p \]

\[ F_p = K \cdot (L - L_0)^3 \]

\[ \frac{dX}{dt} = B \cdot (h - h_0) \]

\[ F_b = A \cdot ([\text{TCa}^*] + [\text{T}^*]) \cdot h \]

\[ h = L - X \]

Definitions of variables: [\( \text{Ca}^{2+} \)], free \( \text{Ca}^{2+} \) concentration in the cytoplasm (mmol/l); [\( \text{Ca}^{2+} \)] \(_{\text{total}} \), total cytoplasmic \( \text{Ca}^{2+} \) concentration (mmol/l); [\( \text{CaM} - \text{Ca}^{2+} \)], CaM-buffered \( \text{Ca}^{2+} \) concentration (mmol/l); [\( \text{TN} - \text{Ca}^{2+} \)], TN-buffered \( \text{Ca}^{2+} \) concentration (mmol/l); [\( \text{TCa} \)], total \( \text{Ca}^{2+} \) concentration in the cytoplasm (mmol/l); [\( \text{TCa}^* \)], NSR \( \text{Ca}^{2+} \) concentration (mmol/l); [\( \text{TCa}^* \)] \(_{\text{NSR}} \), NSR \( \text{Ca}^{2+} \) concentration (mmol/l); [\( \text{TCa}^* \)] \(_{\text{JSR}} \), JSR \( \text{Ca}^{2+} \) concentration (mmol/l); [\( \text{TCa}^* \)] \(_{\text{tot}} \), total JSR \( \text{Ca}^{2+} \) concentration including buffered \( \text{Ca}^{2+} \) (mmol/l); [\( \text{TCa}^* \)] \(_{\text{total}} \), total \( \text{Ca}^{2+} \) concentration including buffered \( \text{Ca}^{2+} \) (mmol/l); [\( \text{CNOS} - \text{Ca}^{2+} \)], casquestrin-buffered \( \text{Ca}^{2+} \) concentration (mmol/l); [\( \text{I}_{\text{I}} \)], \( \text{Ca}^{2+} \) uptake from the cytoplasm to the NSR (mmol/l); \( \text{I}_{\text{leak}} \), \( \text{Ca}^{2+} \) leakage from the NSR to the cytoplasm (mmol/l); [\( \text{I}_{\text{CICR}} \)], \( \text{Ca}^{2+} \) release from the JSR to the cytoplasm due to CICR (mmol/l); \( \text{I}_{\text{nucleus}} \), \( \text{Ca}^{2+} \) uptake from the cytoplasm to the nucleus (mmol/l/m); [\( \text{TCa}^* \)], thin filament site with TnC bound to \( \text{Ca}^{2+} \) (mmol/l); [\( \text{TCa}^* \)] \(_{\text{nsr}} \), effective [\( \text{TCa}^* \)] (mmol/l); [\( \text{TCa} \)], thin filament site with an attached cross bridge (mmol/l); [\( \text{T} \)], thin filament site with free TnC (mmol/l); [\( \text{T}^* \)], thin filament site with an attached cross bridge and without \( \text{Ca}^{2+} \) bound to TnC (force generator) (mmol/l); \( Q_s \), net rate of \( \text{Ca}^{2+} \) binding to T (mmol/l/ms); \( Q_{6} \), net rate of cross-bridge attachment; \( Q_6 \), net rate of \( \text{Ca}^{2+} \) release from \( \text{TCa}^* \) (mmol/l/ms); \( Q_s \), rate of \( \text{T}^* \) detachment to give \( \text{Ca}^{2+} \) and T (mmol/l/ms); \( Q_{61} \), additional rate of \( \text{T}^* \) detachment during filament sliding (mmol/l/ms); \( F_{6} \), equivalent cross-bridge force normalized with respect to the muscle cross-sectional area (kpA); \( F_{6} \), elastic force in parallel with \( F_{6} \) normalized with respect to the muscle cross-sectional area (kpA); \( F \), force developed by a muscle unit normalized with respect to the muscle cross-sectional area (kpA); \( K_{\text{nuc}} \), half-concentration saturation of \( \text{I}_{\text{I}} \); \( L \), sarcomere length (\( \mu \)m); \( X \), length composed of half of the thick filament and the free portion of the thin filament (\( \mu \)m); \( \text{VNSR}_{\text{node}} \) and \( \text{VJSR}_{\text{node}} \), NSR and VSR volume for each FE node, respectively; \( \text{V}_{\text{myo node}} \), cytoplasm volume for each FE node.

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