Spontaneous Ca\textsuperscript{2+} sparks and Ca\textsuperscript{2+} homeostasis in a minimal model of permeabilized ventricular myocytes

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INTRACELLULAR Ca\textsuperscript{2+} is a ubiquitous biological signal that serves diverse functions in many cell types. In individual cells, information can be conveyed by both “global,” or cell-wide, changes in [Ca\textsuperscript{2+}] and by “local,” subcellular Ca\textsuperscript{2+} signals. Local signals are frequently form the building blocks from which global signals are built (32), and, conversely, changes in bulk [Ca\textsuperscript{2+}] in the myoplasm or SR can influence the frequency, amplitude, and kinetics of local events. In ventricular myocytes, for instance, propagating Ca\textsuperscript{2+} waves emerge when spontaneous Ca\textsuperscript{2+} sparks trigger additional sparks in a regenerative fashion (5). Similarly, changes in channel gating at the level of individual RyRs can immediately affect the production of local events and, over time, influence bulk myoplasmic and SR [Ca\textsuperscript{2+}]. An increase in RyR opening will cause a gradual decrease in SR [Ca\textsuperscript{2+}] (46), whereas inhibition of RyR opening, over time, will lead to elevated SR [Ca\textsuperscript{2+}] (21).

In heart cells, changes in Ca\textsuperscript{2+} signaling due to altered RyR activity are currently receiving considerable attention because of close links to disease (13, 48). In particular, catecholaminergic polymorphic ventricular tachycardia (CPVT), an inherited disorder associated with a dramatic increase in arrhythmia risk, results from mutations in either RyRs or calsequestrin, a SR Ca\textsuperscript{2+} buffer protein that associates with and modulates RyRs (20, 33). Experiments in vitro have shown that CPVT-causing mutations usually increase the open probability of the RyR, resulting in a hyperactive or “leaky” channel (12, 28, 31). Studies (1, 36) have also suggested that leaky RyRs are characteristic of several experimental heart failure models. Thus, a quantitative understanding of how changes in RyR gating influence local and global Ca\textsuperscript{2+} responses can provide insights into disease pathophysiology and can potentially suggest novel therapies.

The difference in spatial scales between local and global Ca\textsuperscript{2+} signals, however, creates significant challenges for the development of mechanistic mathematical models. In particular, gating of RyRs depends on both myoplasmic and SR [Ca\textsuperscript{2+}] (30), and concentrations within clusters during local events can be dramatically different from the bulk concentrations. In addition, because of the relatively small number of RyRs responsible for Ca\textsuperscript{2+} sparks (6), the stochastic gating of these channels must be considered when simulating local events. Previous studies (9, 27, 42, 44) have used Monte Carlo simulation methods to investigate the stochastic triggering of Ca\textsuperscript{2+} sparks, but these have generally treated myoplasmic and bulk SR [Ca\textsuperscript{2+}] as fixed boundary conditions. Conversely, modeling studies (4, 10, 40) focusing on cellular Ca\textsuperscript{2+} transients have usually used representations of SR Ca\textsuperscript{2+} release that do not account for the stochastic nature of the local events. Attempting to simulate Ca\textsuperscript{2+} signaling at both spatial scales simultaneously is a daunting prospect because the stochastic behavior of thousands of local events must be considered to determine the effects on the bulk concentrations. As a result, only a few studies (16, 17, 49, 50) have attempted to capture both phenomena.

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In this report, we introduce a computationally efficient minimal model of the coupling between local and global Ca\textsuperscript{2+} signals in permeabilized ventricular myocytes. The model accounts for the random generation and termination of spontaneous Ca\textsuperscript{2+} sparks, the resulting changes in myoplasmic and SR [Ca\textsuperscript{2+}], and the feedback of these changes on spark frequency. We make few assumptions about the factors influencing RyR gating, which allows us to distinguish between those results that are inherent to the coupling between local and global signals and those that are specific to the RyR gating scheme. To validate the model, we considered experiments recently performed by Zima et al. (52), who showed that tetracaine, a RyR inhibitor, caused an initial suppression of Ca\textsuperscript{2+} sparks followed by an increase in SR [Ca\textsuperscript{2+}] and a partial recovery of spark frequency. Surprisingly, these authors found that prolonged exposure to tetracaine led to an increase in Ca\textsuperscript{2+} spark duration (see Fig. 1C in Ref. 52). The simulations presented here recapitulate these experimental results, suggesting that the observed increase in spark duration directly results from the interplay between RyR inhibition and the resulting changes in SR [Ca\textsuperscript{2+}]. More broadly, this model provides a powerful yet minimal framework for understanding how mutations, posttranslational modifications, or drugs can alter diastolic SR Ca\textsuperscript{2+} release in ventricular myocytes.

**METHODS**

**Model formulation.** The minimal whole cell model of local and global Ca\textsuperscript{2+} responses developed here takes into account stochastic Ca\textsuperscript{2+}-release site dynamics as well as the balance of release and reuptake fluxes leading to Ca\textsuperscript{2+} homeostasis in quiescent ventricular myocytes (Fig. 1). The model assumes that RyR Ca\textsuperscript{2+} channels are clustered on the ER/SR membrane in release sites composed of 10–30 channels. All channels in a given release site experience the same local [Ca\textsuperscript{2+}] (myoplasmic and SR), but these “domain” [Ca\textsuperscript{2+}] are heterogeneous throughout the cell, i.e., different release sites experience different domain [Ca\textsuperscript{2+}]. Similar to previous work by Hinch and colleagues (16, 25, 26), we assume that when the number of open channels in a Ca\textsuperscript{2+}-release site changes, the local [Ca\textsuperscript{2+}] rapidly equilibrates in a manner that balances the fluxes into and out of the spatially restricted domains. In our model formulation, a large number of stochastically gating Ca\textsuperscript{2+}-release sites are coupled to the bulk myoplasmic and SR [Ca\textsuperscript{2+}] in a manner that allows spontaneous Ca\textsuperscript{2+} sparks to change the balance of Ca\textsuperscript{2+} release, or “leak,” and reuptake by sarco(endo)plasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA) pumps. The bulk myoplasmic and SR [Ca\textsuperscript{2+}] determine the relationship between the number of open channels in a Ca\textsuperscript{2+}-release site and the resulting domain [Ca\textsuperscript{2+}], and, consequently, changes in these bulk concentrations influence the dynamics of spontaneous sparks. This minimal yet realistic representation of bidirectional coupling between local and global aspects of Ca\textsuperscript{2+} handling is a novel aspect of our model formulation that has not been emphasized in previous work.

**Ca\textsuperscript{2+}-release site model.** In our model formulation, Ca\textsuperscript{2+}-release sites are composed of \(N\) coupled Markov chains representing individual RyRs. For simplicity, we used a RyR model with two states, closed (C) and open (O), but the model formulation can be generalized for channel models with more states (18, 19). Each RyR opens at a rate that depends on the local myoplasmic domain (i.e., the diadic subspace) [Ca\textsuperscript{2+}], denoted by \(c_{\text{myo}}^{d}\), and closes with a Ca\textsuperscript{2+}-independent transition rate:

\[
R = k_{\text{oc}} c_{\text{myo}}^{d}\rightleftharpoons k_{\text{co}} O
\]

where we assume cooperative Ca\textsuperscript{2+} binding, \(k_{\text{co}} c_{\text{myo}}^{d}\) transition rates (in units of reciprocal time), and \(k_{\text{oc}}\) is an association rate constant (with units of concentration\(^{-2}\) time\(^{-1}\)).

We assume that the local [Ca\textsuperscript{2+}] experienced by the Ca\textsuperscript{2+}-regulatory site of each channel depends only on the number of open channels at the Ca\textsuperscript{2+} release site [\(N_{\text{oc}}\)] and the bulk Ca\textsuperscript{2+} concentrations \(c_{\text{myo}}\) and \(c_{\text{SR}}\), as described below. Because the channels are identical and indistinguishable, the state space for the \(N\) channel Ca\textsuperscript{2+}-release site includes \(N + 1\) states \((0 \leq N_{\text{oc}} \leq N)\), as follows:

\[
\begin{align}
0 & \rightleftharpoons k_{\text{oc}} (c_{\text{myo}}^{d})^{N_{\text{oc}}} k_{\text{co}} (c_{\text{myo}}^{d})^{N - N_{\text{oc}}} \\
1 & \rightleftharpoons 2k_{\text{oc}} (c_{\text{myo}}^{d})^{N_{\text{oc} + 1}} k_{\text{co}} (c_{\text{myo}}^{d})^{N - N_{\text{oc}} - 1} \\
& \vdots \\
N - 1 & \rightleftharpoons N_{\text{oc}} k_{\text{co}} (c_{\text{myo}}^{d})^{N_{oc} - 1} k_{\text{oc}} (c_{\text{myo}}^{d})^{N - N_{oc} - 2} \\
N & \rightleftharpoons N_{\text{oc}} k_{\text{co}} (c_{\text{myo}}^{d})^{N_{oc} - 1} k_{\text{oc}} (c_{\text{myo}}^{d})^{N - N_{oc} - 1}
\end{align}
\]

where the states \((0, 1, \ldots, N - 1, N)\) indicate the possible numbers of open channels and \(c_{\text{myo}}\) is the local myoplasmic domain [Ca\textsuperscript{2+}] that applies when there are \(n\) open channels. The infinitesimal generator matrix, denoted by \(Q = (q_{ij})\), that corresponds to Eq. 2 is tridiagonal:

\[
Q = \begin{pmatrix}
0 & \cdots & k_{\text{oc}} (c_{\text{myo}}^{d})^{N_{oc}} & 0 & \cdots & 0 \\
\vdots & \ddots & \vdots & \ddots & \vdots & \vdots \\
0 & \cdots & 0 & \cdots & (N - 1) k_{\text{oc}} & (c_{\text{myo}}^{d})^{N_{oc} + 1} \\
0 & 0 & 0 & \cdots & 0 & 0 \\
0 & 0 & 0 & \cdots & 0 & 0 \\
\end{pmatrix}
\]
where \( q_i \) is the transition rate from state \( i \) to state \( j \) and the diamonds (\( \diamond \)) indicate a diagonal entry leading to a row sum of zero.

Once the local \( [Ca^{2+}] \) (\( c^{\text{myo}}_{\text{myo}} \)) that apply for any given number of open channels are specified, all of the statistical properties of the \( Ca^{2+} \)-release site model can be determined using Eq. 3. In particular, the time evolution of the probability distribution of the number of open channels in the release site can be found by solving the following ordinary differential equation (ODE) initial value problem:

\[
\frac{d\pi}{dt} = \pi Q
\]  

(4)

where the row vector \( \pi = (\pi_0, \pi_1, \ldots, \pi_N) \), \( \pi(t) \) is the probability of finding a release site in state \( i \), and \( \pi(0) \) is the initial condition. The numerical solution of Eq. 4 can also be interpreted as providing the probability distribution for the state of a release site randomly sampled at time \( t \) from a large population that was initially prepared with a distribution of states given by \( \pi(0) \).

Myoplasmic and SR domain \( Ca^{2+} \). As shown schematically in Fig. 1, the domain \( [Ca^{2+}] \) for each release site (\( c^{\text{myo}}_{\text{myo}} \) and \( c^{\text{sr}}_{\text{sr}} \)) is coupled to the bulk compartments via the myoplasmic and SR fluxes (\( J_{\text{myo}} \) and \( J_{\text{sr}} \)) and coupled to one another through the release flux (\( J_{\text{rel}} \)). We assume these fluxes take the following form:

\[
J^{\text{rel}}_{\text{myo}} = v_{\text{rel}} Y_{\text{rel}} (c^{\text{d},n}_{\text{myo}} - c^{\text{n},n}_{\text{myo}})
\]

(5)

\[
J^{\text{myo}}_{\text{myo}} = v_{\text{myo}} (c^{\text{d},n}_{\text{myo}} - c^{\text{n},n}_{\text{myo}})
\]

(6)

\[
J^{\text{sr}}_{\text{sr}} = v_{\text{sr}} (c^{\text{d},n}_{\text{sr}} - c^{\text{n},n}_{\text{sr}})
\]

(7)

where \( c^{\text{myo}}_{\text{myo}} \) and \( c^{\text{sr}}_{\text{sr}} \) are the bulk myoplasmic and SR concentrations, \( \gamma_{n} = n/N \) indicates the fraction of channels at any given \( Ca^{2+} \)-release site that are open, and \( v_{\text{rel}} \) is the maximum release rate. Because the parameter \( v_{\text{myo}} \) is related to the exponential time constant for the decay of elevated myoplasmic domain \( [Ca^{2+}] \) to the myoplasmic bulk \( [Ca^{2+}] \) when an open release site closes, we refer to \( v_{\text{myo}} \) as the rate of myoplasmic domain collapse (37). Similarly, the parameter \( v_{\text{sr}} \) is the rate of luminal domain recovery that determines the exponential time constant for the relaxation of depleted junctional SR \( [Ca^{2+}] \) as this compartment is refilled via \( Ca^{2+} \) translocation from the bulk SR (27).

Recall that Eqs. 2 and 3 require the specification of the local \( [Ca^{2+}] \) that applies for any given number \( n \) of open channels (\( c^{\text{myo}}_{\text{myo}} \)). Assuming the dynamics of domain \( Ca^{2+} \) are fast compared with the gating of RyRs, these domain \( [Ca^{2+}] \) are found by balancing the fluxes \( J^{\text{rel}}_{\text{myo}} = J^{\text{myo}}_{\text{myo}} \) and \( J^{\text{sr}}_{\text{sr}} = J^{\text{sr}}_{\text{sr}} \). Solving these equations simultaneously for \( c^{\text{d},n}_{\text{myo}} \) and \( c^{\text{d},n}_{\text{sr}} \) yields the following:

\[
c^{\text{d},n}_{\text{myo}} = \frac{v_{\text{myo}}}{v_{\text{myo}} + v_{\text{sr}}} c^{\text{n},n}_{\text{myo}} + \frac{v_{\text{sr}}}{v_{\text{myo}} + v_{\text{sr}}} c^{\text{n},n}_{\text{sr}}
\]

(8)

\[
c^{\text{d},n}_{\text{sr}} = \frac{v_{\text{myo}}}{v_{\text{myo}} + v_{\text{sr}}} c^{\text{n},n}_{\text{myo}} + \frac{v_{\text{sr}}}{v_{\text{myo}} + v_{\text{sr}}} c^{\text{n},n}_{\text{sr}}
\]

(9)

where \( v_{\text{myo}} = \frac{\gamma_{n} v_{\text{rel}}}{\gamma_{n} v_{\text{rel}} + 1} \) and \( v_{\text{sr}} = \frac{\gamma_{n} v_{\text{rel}}}{\gamma_{n} v_{\text{rel}} + 1} \) and \( \gamma_{n} = n/N \).

The solid circles in Fig. 2 show the myoplasmic and SR domain concentrations given by Eqs. 8 and 9 as functions of the number of open channels (\( N_{o} = n \)). Note that as the number of open channels increases, \( c^{\text{d},n}_{\text{myo}} \) increases and \( c^{\text{d},n}_{\text{sr}} \) decreases, but always \( c^{\text{d},n}_{\text{myo}} < c^{\text{d},n}_{\text{sr}} \). The open circles in Fig. 2 show how an increase in the bulk SR \( [Ca^{2+}] \) (\( c^{\text{sr}}_{\text{sr}} \)) influences the relationship between the number of open channels in a \( Ca^{2+} \)-release site and the resulting cytosolic \( [Ca^{2+}] \) and luminal \( [Ca^{2+}] \) domain \( [Ca^{2+}] \). In particular, note that an increase in the bulk SR \( [Ca^{2+}] \) (\( c^{\text{sr}}_{\text{sr}} \)) leads to an increase in cytosolic domain \( [Ca^{2+}] \) (\( c^{\text{d},n}_{\text{myo}} \)) provided one or more RyRs are open (\( n \geq 1 \) in Eq. 8).
(see Supplemental Material). Instead, our model formulation is based on the following numerical solution of concentration balance equations for the total myoplasmic ($c_{\text{myo}}$) and SR ($c_{\text{sr}}$) [Ca$^{2+}$]:

$$\frac{dc_{\text{myo}}}{dt} = J_{\text{rel}}^{T} + J_{\text{leak}} - J_{\text{pump}} + J_{\text{pm}}$$

(15)

$$\frac{dc_{\text{sr}}}{\lambda} = \left( -J_{\text{rel}}^{T} - J_{\text{leak}} + J_{\text{pump}} \right)$$

(16)

where the total release flux $J_{\text{rel}}^{T}$ is given by the following:

$$J_{\text{rel}}^{T} = \sum_{n=0}^{N} \pi_{n} \gamma_{n} \bar{\gamma}^{T} \left( \frac{d_{n}}{V_{\text{myo}}} - \frac{d_{n}}{V_{\text{sr}}} \right)$$

(17)

where $\gamma_{n} = n/N$, $c_{\text{myo}}^{d}$ and $c_{\text{sr}}^{d}$ are given by Eqs. 8–10, $\pi_{n}$ is the probability that a randomly sampled release site has $n$ open channels, and, as mentioned above, $\pi = (\pi_{0}, \pi_{1}, \ldots, \pi_{N})$ is found by integrating Eq. 4. The total myoplasmic ($c_{\text{myo}}$) and SR ($c_{\text{sr}}$) [Ca$^{2+}$] that solve Eqs. 15 and 16 are the following sums of the bulk and domain concentrations weighted by effective volume ratios:

$$\bar{c}_{\text{myo}} = c_{\text{myo}} + \Lambda_{\text{myo}}c_{\text{myo}}^{d}$$

(18)

$$\bar{c}_{\text{sr}} = c_{\text{sr}} + \Lambda_{\text{sr}}c_{\text{sr}}^{d}$$

(19)

In these definitions, $c_{\text{myo}}^{d}$ and $c_{\text{sr}}^{d}$ are the average myoplasmic and SR domain [Ca$^{2+}$] that would be obtained upon randomly sampling release sites from within the cell, that is:

$$c_{\text{myo}}^{d} = \sum_{n=0}^{N} \pi_{n} c_{\text{myo},n}^{d,n}$$

(20)

$$c_{\text{sr}}^{d} = \sum_{n=0}^{N} \pi_{n} c_{\text{sr},n}^{d,n}$$

(21)

The effective volume ratios that appear in Eqs. 18 and 19 are given by

$$\Lambda_{\text{myo}} = \frac{V_{\text{myo}}}{V_{\text{myo}} + V_{\text{sr}}}, \quad \Lambda_{\text{sr}} = \frac{V_{\text{sr}}}{V_{\text{myo}} + V_{\text{sr}}}$$

(22)

where $V_{\text{myo}}$ and $V_{\text{sr}}$ are the effective myoplasmic and SR volumes and $V_{\text{myo}}^{d}$ and $V_{\text{sr}}^{d}$ are the effective volumes of the aggregated myoplasmic and SR domains, respectively.

Because the experimental observations that are of primary relevance for this report involve permeabilized cells (52), the plasma membrane flux ($J_{\text{pm}}$) was chosen to be as follows:

$$J_{\text{pm}} = k_{\text{pm}} (c_{\text{ext}} - c_{\text{myo}})$$

(23)

where $k_{\text{pm}}$ was chosen to be large enough to “clamp” the bulk myoplasmic [Ca$^{2+}$] ($c_{\text{myo}}$) at the level of the extracellular bath ($c_{\text{ext}} = 0.1 \mu M$ in the standard parameter set). Even so, the total myoplasmic [Ca$^{2+}$] ($c_{\text{myo}}$, Eq. 18) that solves Eq. 15 is not fixed, because this concentration includes Ca$^{2+}$ in the myoplasmic domains. The fluxes between the bulk myoplasm and bulk SR that occur in Eqs. 15 and 16 include Ca$^{2+}$ reuptake by SERCA pumps ($J_{\text{pump}}$):

$$J_{\text{pump}} = \frac{V_{\text{pump}}}{k_{\text{pump}}^{2}} c_{\text{myo}}$$

(24)

and a passive leakage flux ($J_{\text{leak}}$):

$$J_{\text{leak}} = c_{\text{sr}} - c_{\text{myo}}$$

(25)

This minimal model of the relationship between Ca$^{2+}$ sparks and Ca$^{2+}$ homeostasis was implemented in Matlab (The Mathworks) running on a 1.67-GHz Power PC with 1-GB memory. The model ODEs are stiff and were integrated using Matlab’s built-in function ode15s using an adaptive time step and relative and absolute tolerances of $10^{-3}$ and $10^{-6}$.

Summary and significance of the model. Although minimal in nature, the whole cell model of local and global Ca$^{2+}$ signaling that is the focus of this report accounts for the changes in myoplasmic and SR [Ca$^{2+}$] mediated by the balance of stochastic release and reuptake by the SR and the feedback of myoplasmic and SR [Ca$^{2+}$] on spark frequency. As discussed in the Introduction, previously published models of Ca$^{2+}$ signaling in cardiac myocytes that include the stochastic release of SR Ca$^{2+}$ either have not included bidirectional coupling between local Ca$^{2+}$ release and global Ca$^{2+}$ homeostasis or, because of the computational challenge of the required Monte Carlo simulations, have not emphasized the phenomenon. To our knowledge, this is the first systematic modeling study of the relationship between RyR kinetics, spontaneous and stochastic release of SR Ca$^{2+}$, and the resulting balance of bulk [Ca$^{2+}$] in permeabilized ventricular myocytes. It is also the first model of Ca$^{2+}$ sparks and homeostasis that bypasses Monte Carlo simulation by assuming both a large number of Ca$^{2+}$-release sites and rapid Ca$^{2+}$-domain dynamics, resulting in a minimal formulation that facilitates parameter studies.

The minimal whole cell model of local and global Ca$^{2+}$ signaling that is the focus of this report includes $N + 3$ ODEs and several algebraic relations. Two ODEs are concentration balance equations for the total myoplasmic ($c_{\text{myo}}$) and SR ($c_{\text{sr}}$) [Ca$^{2+}$] (Eqs. 15 and 16). The additional $N + 1$ ODEs (Eqs. 4) account for the dynamics of a large number of Ca$^{2+}$-release sites, each composed of $N$ two-state RyRs (Eqs. 1 and 2). Algebraic relations include the fluxes (Eq. 17 and Eqs. 22–24) that appear in the concentration balance equations as well as the assumed relationship between myoplasmic ($c_{\text{myo}}^{d}$) and SR ($c_{\text{sr}}^{d}$) domain [Ca$^{2+}$] and the number of open channels ($N$). Note that the fluxes $J_{\text{rel}}, J_{\text{pump}},$ and $J_{\text{leak}}$ are functions of $c_{\text{myo}}$ and $c_{\text{sr}}$, which are functions of $c_{\text{myo}}$, $c_{\text{sr}}$, and $\pi$. The algebraic relationship between these quantities is found by inverting Eqs. 18 and 19 after substitution of Eqs. 8, 9, 20, and 21 (see Supplemental Material).

Although our model formulation assumes a large population of Ca$^{2+}$-release sites, we do not have to specify a precise number. To see this, note that the domain concentrations $c_{\text{myo}}^{d}$ and $c_{\text{sr}}^{d}$ do not depend on the number of release sites in the cell ($M$) when the rate constants are defined by $N_{\text{rel}} = \gamma_{\text{rel}}^{T}/M$, $N_{\text{myo}} = \gamma_{\text{myo}}^{T}/M$, and $N_{\text{sr}} = \gamma_{\text{sr}}^{T}/M$ (Eqs. 8 and 9). Because the rate $v_{\text{rel}}^{T}$ that appears in Eq. 17 does not correspond to release through one release site but rather the entire population, it is convenient to specify $c_{\text{myo}}^{d}$ and $c_{\text{sr}}^{d}$ using Eqs. 8–10 with the replacement of $v_{\text{rel}}^{T}$, $v_{\text{myo}}^{T}$, and $v_{\text{sr}}^{T}$ for $v_{\text{rel}}^{T}$, $v_{\text{myo}}$, and $v_{\text{sr}}$.

The minimal model of local and global Ca$^{2+}$ signaling includes 14 parameters, far fewer than most mathematical models of Ca$^{2+}$ handling in cardiac myocytes (see the Supplemental Table in the Supplemental Material). Some parameters [such as the effective volume ratios $\Lambda_{\text{myo}}$, $\Lambda_{\text{sr}}$, and $\Lambda_{\text{myo}}^{d}$, and the SERCA pump maximum rate ($v_{\text{pump}}$) and dissociation constant ($k_{\text{pump}}$)] were either chosen to be consistent with previous work (49, 50) or do not require extensive consideration because model responses to changes in these parameters are obvious and intuitive. Because the ventricular myocyte is assumed to be permeabilized, the precise value of the parameter $k_{\text{pm}}$ is unimportant so long as there is rapid equilibration of bulk myoplasmic Ca$^{2+}$ with the extracellular [Ca$^{2+}$] ($c_{\text{ext}}$). The assumed number of RyRs in each release site ($N = 10$) was chosen to be consistent with estimates of the number of channels activated during a Ca$^{2+}$ spark (7, 15, 43). This is a smaller number of RyRs than previously reported in electron microscopic studies performed a decade ago (11, 34) but is consistent with more recent estimates based on superresolution optical techniques and three-dimensional electron tomography (2, 23). The most important of the model parameters [the kinetic parameters for the stochastic gating of the two-state RyR ($k_{\text{on}}$ and $k_{\text{off}}$) and the rate constants for Ca$^{2+}$ release $v_{\text{rel}}^{T}$, myoplasmic domain collapse $v_{\text{myo}}$, and luminal domain recovery $v_{\text{sr}}^{T}$] are more difficult to constrain, and, consequently, these parameters are the focus of numerous sensitivity studies (see below).

1 Supplemental Material for this article is available online at the American Journal of Physiology-Heart and Circulatory Physiology website.
Ca\textsuperscript{2+} SPARKS AND HOMEOSTASIS IN PERMEABILIZED MYOCYTES

The following aspects of the model behavior suggest that our standard parameter set is physiologically realistic. At 100 nM cytosolic [Ca\textsuperscript{2+}] (c\text{myo}), the average duration of a spontaneous Ca\textsuperscript{2+} release event is on the order of 20 ms, similar to the observed rise time of Ca\textsuperscript{2+} sparks (6, 7). The Ca\textsuperscript{2+} spark rate with c\text{myo} = 100 nM is 0.043 sparks/s per release site (~1 spark every 23 s). Assuming 20,000 release sites in a ventricular myocyte, this corresponds to 860 sparks/s per cell, that is, 86 sparks/s in a fast two-dimensional confocal frame scan that samples 10% of the cell volume. This value is consistent with an experimental study (3) performed in intact cells that reported spontaneous spark rates of 1–4 × 10\textsuperscript{-5} μM\textsuperscript{-2}s\textsuperscript{-1}, which corresponds to 30–120 sparks/s assuming a cross-sectional area of 100 × 30 μm. Consistent with experiment, an increase in myoplasmic [Ca\textsuperscript{2+}] in the model leads to an increase in the spontaneous Ca\textsuperscript{2+} spark rate.

RESULTS

RyR open probability and spontaneous Ca\textsuperscript{2+} sparks. The minimal model of local and global Ca\textsuperscript{2+} signaling that is the focus of this report simulates stochastic Ca\textsuperscript{2+} release by clusters of RyRs and the resulting whole cell Ca\textsuperscript{2+} homeostasis in quiescent ventricular myocytes. The modeling formalism (described in Summary and significance of the model) was chosen to be as simple as possible while still provide mechanistic insights into the perturbation of SR Ca\textsuperscript{2+} leak that results from pharmacological agents, mutations, or posttranslational modifications of the RyR, as may occur in disease states. For example, tetracaine, a potent local anesthetic that allosterically blocks Ca\textsuperscript{2+}-release channels, reduces the open probability of RyRs in planar lipid bilayer experiments (52) by increasing the mean closed dwell time of channels (21). Because the mean closed time of the two-state RyR model is given by τ\text{c} = 1/k\text{co}(c\text{myo})\text{d} (Eq. 1), we simulated the application of tetracaine to permeabilized ventricular myocytes by decreasing the rate constant k\text{co}, which influences the stochastic dynamics of the Ca\textsuperscript{2+} release sites (Eq. 2) in the minimal whole cell model. We wanted to understand how the simulated application of tetracaine influences the dynamics of Ca\textsuperscript{2+} sparks and homeostasis in the permeabilized ventricular myocyte model.

Figure 3 shows a summary of 60 numerical calculations of the stationary dynamics of the minimal whole cell model performed using different values of the RyR Ca\textsuperscript{2+} activation rate constant k\text{co}. The circles show the result of two particular simulations: one corresponding to the standard parameter values (k\text{co} = 4.5 μM\textsuperscript{-2}s\textsuperscript{-1}; solid circles) and the other corresponding to the simulated application of tetracaine (k\text{co} = 0.5 μM\textsuperscript{-2}s\textsuperscript{-1}; open circles). In the latter case, the value of k\text{co} was chosen so that the single channel probability (P\text{open}) given by the following:

\[ P_{\text{open}} = \frac{(c_{\text{myo}}^d)^2}{(c_{\text{myo}}^d)^2 + K^2} \text{ where } K^2 = \frac{k_{\text{co}}}{k_{\text{co}}} \]  

(25)
decreased by 88% upon the action of tetracaine, consistent with experiments in which 0.7 mM tetracaine was applied (52).

Figure 3A shows that the simulated application of tetracaine led to increased bulk SR [Ca\textsuperscript{2+}] in the whole cell model (c\text{sr} = 342 to 1,112 μM; compare solid and open circles). As expected, steady-state bulk SR [Ca\textsuperscript{2+}] increased as RyR open probability decreased, due to a decrease in the total release flux (Eq. 17). Because SERCA pump flux (Eq. 23) is independent of bulk SR [Ca\textsuperscript{2+}], maximum bulk SR [Ca\textsuperscript{2+}] (c\text{sr}) asymptotically approaches 2.5 mM when association rate constant k\text{co} is very small. When a nonspecific passive leak was not included in the model, c\text{sr} increased further (not shown). Results similar to those shown in Fig. 3A can be obtained without a passive leak by extending the SERCA pump model to include both forward and reverse modes (39).

Figure 3B shows that during the simulated application of tetracaine, the fraction of open channels in a randomly sampled release site (f\text{co}) was reduced by 79%, less than the reduction in the single channel open probability given by Eq. 25 (88%). This result indicates that elevated bulk SR [Ca\textsuperscript{2+}] and the interaction between RyRs combine to attenuate the decrease in channel activity occurring during the simulated application of tetracaine. That is, increased SR [Ca\textsuperscript{2+}] increases the driving force during stochastic Ca\textsuperscript{2+}-release events and elevates myoplasmic domain [Ca\textsuperscript{2+}] (compare solid and open circles in Fig. 2B).

The fraction of open channels can be calculated as f\text{co} = E[N\text{O}]/N, where E[N\text{O}] is the expected value of the number of open channels in a randomly sampled release site:

\[ E[N_\text{O}] = \sum_{n=0}^{N} n \pi_n \]  

(26)

Figure 3, C and D, shows the frequency and duration of spontaneous Ca\textsuperscript{2+} sparks occurring in the whole cell model as a function of the parameter k\text{co}. The presence or absence of Ca\textsuperscript{2+} sparks is assessed by calculating the following Ca\textsuperscript{2+} spark score:

\[ \text{Score} = \frac{1}{N} \text{Var}[N_\text{O}] \]  

(27)

where
The Ca$^{2+}$ spark score takes values from 0 to 1, and a score of 0.2 or higher indicates robust sparks (38). The solid lines in Fig. 3, C and D, show that Ca$^{2+}$ sparks were observed when the RyR Ca$^{2+}$ activation rate constant ($k_{o}$) was between 0.04 and 322 $\mu$M$^{-2}$s$^{-1}$, a range spanning four orders of magnitude.

With spark initiation defined as a release site reaching a threshold number of open channels ($N_{o}$ = 4 to 5 transition) and spark termination defined as all channels closing ($N_{o}$ = 1 to 0 transition), spark frequency and mean duration were calculated using the matrix analytic method described in Ref. 18. The solid and open circles in Fig. 3, C and D, show that the simulated application of tetracaine decreased Ca$^{2+}$ spark frequency but increased mean Ca$^{2+}$ spark duration, consistent with experimental observations (52). While bulk SR [Ca$^{2+}$] ($c_{o}$) and spark frequency are monotone functions of RyR open probability (decreasing and increasing, respectively), mean spark duration is a biphasic function of RyR open probability (first increasing and then decreasing).

**Spark frequency and duration upon the application of tetracaine.** Figure 4A, left, shows representative Ca$^{2+}$-release events exhibited by a Ca$^{2+}$-release site in the standard whole cell simulation. Spontaneous Ca$^{2+}$ sparks were simulated using Gillespie’s method (14). The parameters used correspond to the solid circles shown in Fig. 3 ($k_{o}$ = 4.5 $\mu$M$^{-2}$s$^{-1}$) and resulted in robust Ca$^{2+}$ sparks (score = 0.51) for the bulk concentrations ($c_{myo}$ and $c_{o}$) of the equilibrated whole cell model. Note the high frequency of spontaneous release events, including five sparks ($N_{o}$ = 5) and a large number of smaller release events. These small release events, termed “Ca$^{2+}$ quarks” (32), would not be detectable with standard confocal microscopy and would therefore contribute to “invisible” SR Ca$^{2+}$ leak (43). Figure 4A, right, shows an expanded version of the first spark (asterisk in Fig. 4A, left), which has a duration (17.5 ms) close to the mean spark duration with these standard parameters (18.3 ms; solid circle, Fig. 3D).

Figure 4B shows representative Ca$^{2+}$-release events during the simulated addition of tetracaine. The parameters used correspond to the open circles shown in Fig. 3 ($k_{o}$ = 0.5 $\mu$M$^{-2}$s$^{-1}$). While robust Ca$^{2+}$ sparks were observed (score = 0.51), the simulated application of tetracaine significantly reduced spark frequency; one spark and three quarks were observed. While the mean spark duration was 26.8 ms (open circle, Fig. 3D), Fig. 4B, right, shows an expanded version of the observed spark, which was over 90 ms in duration. Consistent with experimental observations (52), such long-duration sparks are not infrequent during the simulated addition of tetracaine, despite the fact that they almost never occurred with the standard parameter set (see below).

To confirm that this decreased spark frequency and increased mean spark duration are due to overloading of bulk SR [Ca$^{2+}$], Fig. 4C shows a control simulation using the single channel RyR parameters shown in Fig. 4B ($k_{o}$ = 0.5 $\mu$M$^{-2}$s$^{-1}$) with bulk SR [Ca$^{2+}$] “clamped” at the value shown in Fig. 4A ($c_{a}$ = 342 $\mu$M). The resulting simulation showed only a few release events, none with more than three channels open (score = 0.11). We conclude that the overloading of SR [Ca$^{2+}$] that occurs when RyR open probability is decreased is required for the presence of prolonged sparks in the whole cell model.

As mentioned above, the simulated addition of tetracaine resulted in Ca$^{2+}$ sparks whose duration tended to be longer than that observed with the standard parameters (compare Fig. 4, A, right, and B, right). To further quantify this effect, Fig. 5A shows the numerically calculated distribution of spark durations for the standard (solid line) and tetracaine (dashed line) parameter sets. While the mode of these distributions was nearly identical (standard: 4.8 ms and tetracaine: 4.6 ms), in the case of tetracaine the distribution extended further to the right, consistent with the higher probability of long sparks (Fig. 4B, right). Integrating the results shown in Fig. 5A led to cumulative probability distributions (Fig. 5B) that showed that 21.5% of the sparks in the tetracaine simulations but only 9.4% of the sparks in the standard simulations were longer than 40 ms (compare solid and open circles).

**Magnitude of Ca$^{2+}$ release due to spontaneous sparks.** As discussed above, the SR [Ca$^{2+}$] overload induced by tetracaine led to higher myoplasmic domain [Ca$^{2+}$] (compare open and solid circles in Fig. 2A), higher SR domain [Ca$^{2+}$] (Fig. 2B), and higher release flux ($J_{rel}$, Eq. 17) for any given number of open channels. The resulting changes in the dynamics of Ca$^{2+}$-mediated coupling of RyRs led to a decrease in spark frequency and an increase in spark duration (Fig. 3, C and D). Nevertheless, the solid and open circles in Fig. 5C show that the application of tetracaine decreases the aggregate release flux in the whole cell model. In fact, the solid line in Fig. 5C shows that the aggregate release flux is a monotone increasing function of RyR open probability, despite the fact that the SR [Ca$^{2+}$] is monotone decreasing (Fig. 3A).

During experimental observations of spontaneous Ca$^{2+}$ release, small-amplitude events may not be detectable. Thus, it is of interest to dissect the aggregate release flux of the whole cell...
model to determine the fraction of spontaneous release that occurs via release sites that have few open channels. The dotted and dashed lines in Fig. 5C show that the release flux mediated by release sites with one or two open channels is a monotone increasing function of the RyR Ca$^{2+}$-activation rate constant $k_{co}$. Figure 5D shows aggregate flux $J_{rel}^T$ jointly distributed with the number of open channels for both the tetracaine and standard parameter sets. Both distributions were bimodal; a peak was observed at $N_0 = 1$ as well as $N_0 = 6$ or 7. Tetracaine suppressed the proportion of Ca$^{2+}$ released through sites with seven or fewer open channels ($N_0 \leq 7$), whereas release mediated by sites with eight or more open channels ($N_0 \geq 8$) increased slightly in the presence of tetracaine. These observations are consistent with the increased probability of long-duration Ca$^{2+}$ sparks observed upon the application of tetracaine (Fig. 5A).

Because the detectability of sparks recorded with fluorescent dyes is primarily determined by spark amplitude (i.e., integrated Ca$^{2+}$ release) (8, 41), Fig. 6A shows a summary of Monte Carlo simulations analyzing how spark amplitude and duration are jointly influenced by the simulated application of tetracaine (compare solid and open circles). Here, spark events were defined as beginning with a $N_0 = 0 \rightarrow 1$ transition, whereas spark amplitude was the integrated stochastic release flux (Eq. 5) before spark termination via a $N_0 = 1 \rightarrow 0$ transition. Figure 5, B and C, shows the cumulative probability distributions of spark amplitude and duration, respectively. Sparks had both larger amplitude and extended duration when tetracaine was applied (consistent with Fig. 5A). Nevertheless, the decrease in spark frequency in the presence of tetracaine led to an overall decrease in the aggregate release flux discussed above (Fig. 5C).

Figure 6D shows the percentage of undetected spark events (and the “hidden” Ca$^{2+}$-release flux mediated by undetected sparks) as a function of a detection threshold on spark amplitude. The vertical dashed lines in Fig. 6, B and D, indicate a sensitive detection threshold equivalent to the amount of Ca$^{2+}$ released by an average quark; in the standard simulation, this is a single channel release event with a duration of 1.1 ms. With this sensitive detection threshold, 36% of release events are not observed; most of these release events are quarks, brief single channel openings through which <1% of the stochastic Ca$^{2+}$ release occurs. Because the simulated application of tetracaine led to increased SR load and greater release flux for any given number of open RyRs (Fig. 2), the tetracaine condition led to fewer hidden events (15%) and decreased hidden release (<0.1%). The solid and dashed lines in Fig. 6D show that the percentage of hidden release events and hidden release flux were both increasing functions of detection threshold. For the range of possible detection thresholds shown, the percentage of hidden events decreased by two- to threefold upon the application of tetracaine.

Transient effects upon the application of tetracaine. While the above simulations focused on steady-state dynamics of the whole cell model, Fig. 7 shows transient effects upon bulk SR [Ca$^{2+}$] ($c_{SR}$), mean spark duration, and spark frequency that occurred during the simulated application and washout of tetracaine. Consistent with experimental observations (21, 52), the initial application of tetracaine caused spark frequency to decrease; the mean spark duration during this phase (50 ms) was much shorter than the baseline value (18.3 ms). However, this reduced spontaneous Ca$^{2+}$ release caused a slow increase in SR load that ultimately increased the mean spark duration to 26.8 ms, consistent with the steady-state results (Fig. 3D).

Figure 7 also shows that upon the simulated washout of tetracaine (right arrow), there was a transient increase in spark frequency (maximum of ~4 times the baseline value) and a rapid depletion of SR [Ca$^{2+}$] from elevated to baseline values. For a short period of time, the mean spark duration was quite large (see asterisk); however, the value attained is not relevant because it is greater than duration of the phase itself (400 ms). Shortly after this burst of spark activity, the mean spark duration returned to baseline.

Model parameters and Ca$^{2+}$ homeostasis. Figure 8 shows a summary of 2,500 calculations of the stationary dynamics of the whole cell model as a function of $v_{myo}^T$ and $v_{myo}^F$. These parameters control the rate of “diffusion” or translocation of Ca$^{2+}$ between the different cellular subspaces represented in the minimal model, for example, from the individual myoplasmic domains (diadic subspaces) to the cytoplasm and from the bulk SR to the luminal domains (network to junctional SR). These domain rate constants also influence the strength of the Ca$^{2+}$-mediated coupling between RyRs and the extent of SR domain depletion during sparks (recall Eqs. 8–10). Because $v_{myo}^T$ and $v_{myo}^F$ are difficult to constrain via experiments, they are good choices for a parameter study designed to determine their effect on experimentally observable quantities such as steady-state bulk SR [Ca$^{2+}$] and mean spark frequency and duration.

Figure 8A shows that bulk SR [Ca$^{2+}$] is an increasing function of rate of myoplasmic domain collapse ($v_{myo}^T$) and a decreasing function of the rate of SR domain recovery ($v_{myo}^F$).
When $v_{myo}^T$ is very large or $v_{sr}^T$ is very small, RyRs become decoupled such that most openings fail to trigger neighboring channels, and the reduced leak causes an increase in bulk SR $[Ca^{2+}]$. Figure 8, B and D, shows that the fraction of open channels and mean spark duration were much more sensitive to $v_{myo}^T$ than to $v_{sr}^T$. When $v_{myo}^T$ is small, sparks are extremely long because subspace $[Ca^{2+}]$ remains elevated after RyRs close (cf. Refs. 27 and 37). Fast SR refilling alone is not sufficient to induce long-duration $Ca^{2+}$ sparks of the sort observed upon the simulated application of tetracaine (cf. Fig. 4).

Robust $Ca^{2+}$ sparks were observed in the whole cell model even when the domain rate constants were ranged over several orders of magnitude (Fig. 9). This robust $Ca^{2+}$ spark behavior is a consequence of the homeostatic changes in bulk SR $[Ca^{2+}]$ accounted for in our model formulation; when these simulations were repeated with bulk myoplasmic and SR $Ca^{2+}$ concentrations fixed at baseline values ($c_{myo} = 0.1 \mu M$ and $c_{sr} = 342 \mu M$), the range of domain rate constants leading to $Ca^{2+}$ sparks was considerably smaller (Fig. 9).

Figure 10 shows stationary dynamics of the whole cell model as a function of bulk myoplasmic $[Ca^{2+}]$ ($c_{myo}$) and maximum rate of the SERCA pump ($v_{pump}$), two parameters that can be easily manipulated in experiments. Bulk SR $[Ca^{2+}]$ was (as expected) an increasing function of $v_{pump}$; however, the SR $Ca^{2+}$ load was a biphasic function of $c_{myo}$ (Fig. 10A). The

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Fig. 6. A: Monte Carlo sampling of blip/spark duration and amplitude (integrated $Ca^{2+}$ release) for the standard (solid circles) and tetracaine-based (open circles) parameter sets. Each blip or spark was initiated by a $N_0 = 0 \rightarrow 1$ transition (cf. Fig. 5, where $N_0 = 4 \rightarrow 5$ was interpreted as a spark initiation event). B and C: cumulative probability distribution of spark amplitude (B) and duration (C) for the standard (solid line) and tetracaine-based (dashed line) parameter sets. The amplitude of release is expressed in units of femtocoulombs under the assumption of a total myoplasmic volume of $2 \times 10^{-5} \mu l$ and $2 \times 10^4$ release sites per cell (see Supplemental Material). D: percentage of “hidden” (undetected) spark events and percentage of $Ca^{2+}$ release flux mediated by hidden sparks as a function of the spark amplitude detection threshold.

Fig. 7. Transient effects upon bulk SR $[Ca^{2+}]$ ($c_{sr}$), mean spark duration, and spark frequency during the simulated application and washout of tetracaine (arrows). The $Ca^{2+}$ activation rate constant was decreased from $k_{co} = 4.5$ to $0.5 \mu M^{-2} \cdot s^{-1}$ (left arrow) and later restored to its original value (right arrow). *The mean spark duration was not applicable at the indicated time (see text).

Fig. 8. Effect of myoplasmic domain collapse ($v_{myo}^T$) and SR domain recovery ($v_{sr}^T$) on bulk SR $[Ca^{2+}]$ (A), fraction of open channels (B), spark frequency (C), and mean spark duration (D) in the minimal whole cell model. *Standard parameters. White indicates that mean spark duration was not calculated because the spark score was <0.2.
fraction of open channels and mean spark duration were both increasing functions of SERCA pump activity (Fig. 10, B and D). Spark frequency was not sensitive to SERCA activity but was a rapidly increasing function of bulk myoplasmic \([Ca^{2+}]\) (Fig. 10C). Spark duration was a biphasic function of cmyo (Fig. 10D), consistent with the biphasic effect of this parameter on SR load (Fig. 10A). Bulk myoplasmic \([Ca^{2+}]\) (cmyo) significantly changed the functional dependence of the stationary dynamics of the whole cell model on the RyR \(Ca^{2+}\) activation rate constant (\(k_{co}\)). On the other hand, when the application of tetracaine was simulated by comparing \(k_{co}\) values that correspond to a decrease in RyR activity from \(f_0 = 9.6 \times 10^{-4}\) to \(2.0 \times 10^{-4}\) (Table 1), we observed increased SR load, decreased spark frequency, and increased spark duration for cmyo in the range of 0.1–0.5 \(\mu M\).

### RyR inhibition mechanism and spark duration

We modeled the action of tetracaine as a decrease in the \(Ca^{2+}\) activation rate constant \(k_{co}\), which reduces the open probability (Eq. 25) of the RyR model (Eq. 1) by increasing the mean closed dwell time, \(\tau_C = 1/k_{oc}(c_{myo})^2\). However, the open probability of the RyR can also be reduced by increasing the rate constant \(k_{oc}\), thereby decreasing the mean open dwell time (\(\tau_O = 1/k_{oc}\)). Such a change would be analogous to the pharmacological action of the antiarrhythmic agent flecainide, which has been shown to reduce the dwell time of RyR open states (47).

Figure 11, C and D, shows \(Ca^{2+}\) spark frequency and mean spark duration as a function of the RyR kinetic constants \(k_{co}\) and \(k_{oc}\) when these spark statistics are well defined (score \(\geq 0.2\)). The contours dividing the plane into the areas where sparks are present (gray) and absent (white) follow constant \(K = k_{oc}/k_{co}\), indicating that sparks occur provided the single channel RyR open probability (Eq. 25) is neither too low or too high. If RyR parameters were changed from the standard values (asterisk), the resulting change in SR \([Ca^{2+}]\) also depend only on RyR open probability (Fig. 11A). SR \([Ca^{2+}]\) was too high for sparks in the top left white region and too low for sparks in the bottom right (cf. Fig. 3A). In contrast, spark frequency and mean spark duration strongly depended on whether a pharmacological perturbation of RyR kinetics is assumed to affect \(k_{co}\), \(k_{oc}\), or both (diamonds). A reduction in \(k_{co}\) (increase in \(\tau_C\)), analogous to effect of tetracaine, led to fewer sparks but an increase in spark durations. Conversely, an increase in \(k_{oc}\) (decrease in \(\tau_O\)), analogous to the effect of flecainide, caused more sparks with decreased mean duration. Examples of Monte-Carlo simulations under these different conditions are shown in Fig. 12. Similarly, we can assume that low-dose caffeine leads to an increase \(k_{co}\) accompanied by a smaller decrease in \(k_{oc}\) (+ symbol) (29). The model predicted that this would cause a decrease in SR \([Ca^{2+}]\), an increase in spark frequency, and little change in spark duration, roughly consistent with the results observed by Lukyanenko et al. (35).

These results illustrate a fundamental point about the interplay between local and global \(Ca^{2+}\) signals during pharmacological interventions designed to manipulate spontaneous cel-
The three mechanisms of RyR inhibition have different consequences on mean spark duration and spark frequency

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<th>Corresponding figure</th>
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<th>Tetracaine ($\tau_c \uparrow$)</th>
<th>Flecainide ($\tau_o \downarrow$)</th>
<th>Dual Mechanism ($\tau_c \uparrow$ and $\tau_o \downarrow$)</th>
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Shown are results for the whole cell model with standard parameters and modified Ca$^{2+}$ activation rate constant ($k_{oc}$), which represents the application of tetracaine and led to an increase in ryanodine receptor (RyR) open time (cf. solid and open circles in Fig. 3, respectively). When the same level of RyR inhibition was modeled as a decrease in open dwell time ($\tau_c \downarrow$), as may occur upon the application of flecainide, or a dual mechanism ($\tau_c \uparrow$ and $\tau_o \downarrow$), the mean spark duration changed. *Results identical to the tetracaine case.

lular responses. Local (microscopic) aspects of cell response, such as spark frequency and duration, are highly dependent on the molecular details, that is, precisely how the kinetics of RyR stochastic gating kinetics have been modified. Global (macroscopic) aspects of the cell response, such as steady-state bulk SR [Ca$^{2+}$], are less dependent on the kinetic details of the perturbation but remain determined by equilibrium quantities such as the dissociation constant for Ca$^{2+}$ binding to the RyR.

DISCUSSION

This report presents a minimal whole cell model of local and global Ca$^{2+}$ signals in quiescent ventricular myocytes. The modeling formalism accounts for the effect of random spontaneous Ca$^{2+}$ sparks, changes in bulk myoplasmic and SR [Ca$^{2+}$] mediated by the balance of stochastic release and reuptake by the SR, and feedback of myoplasmic and SR [Ca$^{2+}$] on spark frequency. The functional organization of the model (Fig. 1) is similar to previously published Monte Carlo models of local control (17). The assumptions made here regarding the rapid equilibration of domain Ca$^{2+}$ are similar to assumptions made in previously published local control models that represent the stochastic dynamics of a large number of Ca$^{2+}$-release sites (16, 25), but these previous studies have not made a distinction between domain and bulk SR Ca$^{2+}$ as done in our minimal whole cell model.

To our knowledge, this is the first theoretical study of the relationship between RyR kinetics, spontaneous and stochastic Ca$^{2+}$ release, and the resulting balance of bulk myoplasmic and SR [Ca$^{2+}$] in quiescent ventricular myocytes. Because of the computational challenge of large-scale simulations, a traditional Monte Carlo approach is not well suited to investigate these phenomena. The whole cell modeling approach introduced here bypasses Monte Carlo simulation by assuming a large number of Ca$^{2+}$-release sites and rapid Ca$^{2+}$ domain dynamics, resulting in a minimal formulation that facilitates parameter studies.

The minimal model of local and global Ca$^{2+}$ responses in quiescent ventricular myocytes presented here is able to recapitulate recent experiments by Zima et al. (52) showing that tetracaine, an inhibitor of RyRs, causes a transient suppression of Ca$^{2+}$ sparks that is followed by an increase in bulk SR [Ca$^{2+}$], partial recovery of spark frequency, and an increase in Ca$^{2+}$ spark duration (Fig. 11). Conversely, if flecainide is assumed to decrease RyR mean open time while not affecting Ca$^{2+}$-release sites (16, 25), but these previous studies have not made a distinction between domain and bulk SR Ca$^{2+}$ as done in our minimal whole cell model.

FIG. 11. Effect of rate constants $k_{oc}$ and $k_{co}$ in the single channel RyR model (Eq. 1) on bulk SR [Ca$^{2+}$] (A), fraction of open channels (B), spark frequency (C), and mean spark duration (D). *Standard parameters. Open diamonds indicate various changes in the open and closed dwell times of the RyR (cf. tetracaine, flecainide, and dual mechanism in Table 1 and Fig. 12). Open squares indicate that an increase in $k_{co}$ was accompanied by a smaller decrease in $k_{oc}$ corresponding to a low dose of caffeine. The steady-state SR load ($c_{sr}$) and fraction of open channels ($\theta_O$) are functions of the RyR Ca$^{2+}$-binding constant $K = k_{oc}/k_{co}$, which was constant along the lines parallel to the diagonal band for which spark frequency and duration are well defined (dashed lines, score > 0.2).
in this first study of the relationship between Ca\(^{2+}\) sparks and homeostasis allows us to focus on aspects of the cellular response to perturbations that are likely to be fundamental and general. A more complicated RyR model would call into question the specific details of how we (arbitrarily) suppose pharmacological perturbations influence the kinetic constants that determine RyR stochastic gating, assumptions that could easily influence the whole cell response and our conclusions. We find it intriguing that this model of local and global Ca\(^{2+}\) responses in quiescent ventricular myocytes is able to reproduce changes in spark frequency and duration caused by the application of both tetracaine and flecainide, despite the fact that the RyR model used does not include regulatory processes such as Ca\(^{2+}\)-dependent inactivation and/or sensitization by SR [Ca\(^{2+}\)]. The effect of these well-established aspects of RyR Ca\(^{2+}\) regulation is beyond the scope of this work.

The Ca\(^{2+}\) activation process in the RyR model is mediated by myoplasmic domain Ca\(^{2+}\) (\(c_{\text{myo}}\), Eq. 9), and it would be possible to augment the model to include the sensitization of RyRs by SR domain Ca\(^{2+}\) (\(c_{\text{sr}}\), Eq. 9). However, the assumption of instantaneous coupling of RyRs (i.e., rapid equilibration of myoplasmic and SR domain Ca\(^{2+}\)) may not work well when luminal Ca\(^{2+}\) plays a role in spark termination (27, 42). A computational study of the contribution of luminal RyR regulation to the bidirectional coupling of spontaneous Ca\(^{2+}\) release and Ca\(^{2+}\) homeostasis will likely require more subtle mathematical formulations, similar to the probability density and moment closure techniques that accelerate “local control” simulations of high-gain graded Ca\(^{2+}\) release in voltage-clamped cardiac myocytes (49, 50). These representations of heterogeneous domain Ca\(^{2+}\) concentrations associated with a large number of Ca\(^{2+}\)-release sites remain valid even when the dynamics of SR domain Ca\(^{2+}\) are not fast compared with channel gating. Indeed, the model presented here can be viewed as a reduction of the moment closure formulation (50) that is valid when SR Ca\(^{2+}\) domains rapidly equilibrate with myoplasmic domain and bulk SR Ca\(^{2+}\), in which case there is a negligible variance of SR domain [Ca\(^{2+}\)] for any given Ca\(^{2+}\)-release site state.

In preliminary work, we studied whole cell responses using RyR models that included both Ca\(^{2+}\) activation and inactivation by myoplasmic domain Ca\(^{2+}\), for example, the following three-state model:

\[
\begin{align*}
    k_{\text{co}} (c_{\text{myo}}) & C \\
    k_{\text{oc}} & O \\
    k_{\text{ro}} & R
\end{align*}
\]

which includes a long-lived closed state (R). As expected, release sites composed of such channels exhibit Ca\(^{2+}\) sparks and homeostasis similar to that observed for the two-state RyR when the dissociation constant \(K_{\text{inact}} = k_{\text{ro}}/k_{\text{or}}\) is large (see supplemental Fig. S3). While fast Ca\(^{2+}\) inactivation (large \(k_{\text{ro}}\) and \(k_{\text{or}}\) with fixed \(K_{\text{inact}}\)) is associated with decreased spark duration and increased spark frequency, extremely fast Ca\(^{2+}\) inactivation can preclude sparks (supplemental Fig. S3, C and D). Provided Ca\(^{2+}\) inactivation is sufficiently fast, decreasing \(K_{\text{inact}}\) leads to increased SR Ca\(^{2+}\) load, decreased RyR activity, increased spark frequency, and decreased spark duration (supplemental Fig. S3, A–D). Interestingly, decreasing the Ca\(^{2+}\) activation rate constant \((k_{\text{co}})\) in the three-state model with Ca\(^{2+}\) inactivation (Eq. 29) to simulate the application of tetracaine
may lead to longer or shorter duration sparks depending on whether the rate of Ca\(^{2+}\) inactivation is slow or fast, respectively, despite the fact that neither RyR activity nor SR load are strongly affected by the rate of Ca\(^{2+}\) inactivation in either condition (see supplemental Fig. S4). This sensitivity of the stochastic dynamics of Ca\(^{2+}\) release to the rate of Ca\(^{2+}\) inactivation is consistent with results obtained using Ca\(^{2+}\)-release site models that do not account for Ca\(^{2+}\) homeostasis (18). However, the significance of these observations is unclear given recent experimental results showing that, at even 50 µM myoplasmic [Ca\(^{2+}\)], inactivation is unable to suppress SR Ca\(^{2+}\) release in permeabilized myocytes (45).

While the simulated spark duration histograms shown in Fig. 5A are unimodal, the experimentally observed spark duration histogram shows two peaks in the presence of tetracaine, suggesting two distinct populations of sparks (52). Zima and coworkers (52) suggested that the prolonged sparks occurred at release sites with highly interconnected junctional SR and high refilling rates. Our simulations shown in Fig. 8 address this idea by investigating the effects of a larger domain refilling rate (\(v_r\)). Consistent with the hypothesis of Zima et al. (52), an increase in \(v_r\) from 10 to 50 s\(^{-1}\) caused a slight increase in baseline Ca\(^{2+}\) spark duration as well as a greater percent increase upon the application of tetracaine (57% vs. 47%, not shown). This suggests that the long sparks observed upon the application of tetracaine (52) may indeed be associated with fast rather than slow SR refilling.

Finally, it is intriguing that robust Ca\(^{2+}\) sparks were observed in the whole cell model even when the domain rate constants were ranged over several orders of magnitude (Fig. 8). Because the range of domain rate constants leading to Ca\(^{2+}\) sparks is considerably smaller when bulk myoplasmic and SR [Ca\(^{2+}\)] are fixed at baseline values (compare Fig. 9, A and B), we conclude that this robust Ca\(^{2+}\) spark behavior is a consequence of homeostatic changes in bulk SR [Ca\(^{2+}\)] accounted for in our model formulation. The fact that the feedback of spontaneous SR leak on spark frequency extends the regime where spontaneous sparks occur is potentially physiologically relevant. Speaking teleologically, the homeostatic mechanisms appear to encourage SR leak mediated by Ca\(^{2+}\) sparks and discourage the alternatives: SR leak mediated by quarks or tonically active release sites. These observations underscore the importance of accounting for global Ca\(^{2+}\) balance in models of localized Ca\(^{2+}\)-release events.

ACKNOWLEDGMENTS

Some of the results of this study have previously appeared in abstract form (22).

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


