Role of coupled gating between cardiac ryanodine receptors in the genesis of triggered arrhythmias

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Chen W, Wasserstrom JA, Shiferaw Y. Role of coupled gating between cardiac ryanodine receptors in the genesis of triggered arrhythmias. Am J Physiol Heart Circ Physiol 297: H171–H180, 2009. First published May 8, 2009; doi:10.1152/ajpheart.00098.2009.—Mutations in the ryanodine receptor (RyR) have been linked to exercise-induced sudden cardiac death. However, the precise sequence of events linking RyR channel mutations to a whole heart arrhythmia is not completely understood. In this paper, we apply a detailed, mathematical model of subcellular calcium (Ca) release, coupled to membrane voltage, to study how defective RyR channels can induce arrhythmogenic-triggered activity. In particular, we show that subcellular Ca activity, such as spontaneous Ca sparks and Ca waves, is highly sensitive to coupled gating between RyR channels in clusters. We show that small changes in coupled gating can induce aberrant Ca release activity, which, under Ca overload conditions, can induce delayed afterdepolarization (DAD). We systematically investigate the properties of subcellular Ca during DAD induction and show that the voltage time course during a DAD is dependent on the timing and number of spontaneous Ca sparks that transition to Ca waves. These results provide a detailed mechanism for the role of coupled gating in the genesis of triggered arrhythmias.

Keywords: calcium; modeling; arrhythmia

VENTRICULAR FIBRILLATION (VF) occurs when electrical waves on the heart fractionate to form reentrant activity. The likelihood of wave fractionation is enhanced by the presence of triggered excitations in the heart, which may lead to abnormal wave propagation (3, 16, 23). Triggered activity is caused by voltage depolarization during repolarization (early afterdepolarizations) or after full repolarization [delayed afterdepolarizations (DADs)]. Many experiments have shown that animal hearts prone to VF are more likely to exhibit a higher frequency of triggered activity (2, 15). Therefore, to understand VF, it is also necessary to explore the mechanisms that lead to triggered activity.

Recent studies of genetic mutations of animal hearts have revealed important insights on the subcellular processes that initiate a cardiac arrhythmia (11, 12, 20, 21). In these studies, the ryanodine receptor (RyR) has emerged as an important player because of its role as the main calcium (Ca) release channel required for excitation-contraction (EC) coupling. In several important studies, Wehrens et al. (20, 21) have shown that mice with a mutation on the 12.6-kDa FK506 binding protein (FKBP12.6) binding site of the RyR are more prone to exercise-induced VF. Single-cell studies of these myocytes revealed abnormal Ca cycling properties, such as increased leak from the sarcoplasmic reticulum (SR), Ca waves, and DADs (8). Further studies (10, 11) have also identified an RyR mutation in patients with inherited catecholaminergic polymorphic ventricular tachycardia. Again, myocytes from these hearts exhibited a greater propensity for DADs (4, 5). Subsequent studies showed that this mutation led to abnormal Ca cycling via an increased susceptibility to spontaneous Ca release from the SR. These studies show that various cardiac arrhythmias can be caused by defects in the RyR macromolecular complex.

Despite extensive work, the underlying mechanisms relating an RyR mutation and a whole heart arrhythmia are not completely understood. Weiherens et al. (21, 22) have hypothesized that dissociation of FKBP12.6 from RyR channels leads to enhanced Ca leak from the SR, which can lead to abnormalities in intracellular Ca release, which can trigger VF. On the other hand, Jiang et al. (4) have argued that RyR mutations responsible for catecholaminergic polymorphic ventricular tachycardias lower the SR Ca threshold for spontaneous release, thus making the cell more prone for spontaneous release under Ca overload conditions. In both cases, the detailed sequence of events leading from the RyR abnormality to a triggered event at the whole cell level is not completely understood.

In this paper, we apply mathematical modeling to explore the detailed mechanisms by which an RyR channel defect can lead to a triggered arrhythmia. To accomplish this, we have developed a mathematical cell model that couples voltage and Ca and that also accounts for the spatial distribution of Ca release sites within a single cell and groups of cells. Large ensembles of ion channels are simulated in a probabilistic fashion to correctly bridge the scale between single-ion channel behavior and whole cell electrophysiology. Our aim is to elucidate the role of coupled gating (14) between RyR channels within clusters and the emergence of arrhythmogenic Ca release activity at the multicell level. We apply our mathematical model to investigate the relationship between RyR properties at the single-channel level, the formation of spontaneous Ca sparks and waves, and the induction of DADs via Ca-sensitive membrane currents. In this way, we elucidate the detailed mechanisms by which a defect in the RyR can lead to arrhythmogenic triggered activity.

METHODS

A spatially distributed Ca cycling model. To model the spatiotemporal distribution of Ca in cardiac cells, we have implemented a recently developed mathematical model due to Restrepo et al. (17). In this model, the cardiac cell is represented as a collection of spatially distributed subcellular compartments, which contain key elements of the Ca cycling machinery (Fig. 1A). Each subcellular unit consists of the
following: 1) the proximal or dyadic junction, where a few L-type Ca channels (LCC, 1–5 in number) on the cell membrane are in close proximity to a cluster of 50–150 RyR channels attached to the junctional SR; 2) the submembrane space, which represents a volume in the vicinity of the sarcolemma; it is necessary to include this space, since Ca-sensitive membrane currents are regulated by Ca near the cell membrane, which varies more rapidly than the average bulk Ca concentration; 3) the bulk myoplasm, which represents the volume of space into which Ca diffuses; 4) the local junctional SR, which is the portion of the SR that is close to the cell membrane; and 5) the network SR, which represents the bulk SR network. The Ca concentrations in these compartments are denoted as \( c_{\text{ps}} \), \( c_{\text{s}} \), and \( c_{\text{network}} \), respectively, where the superscript labels the \( k \)th subcellular unit.

The spatiotemporal distribution of Ca is modeled by allowing Ca to diffuse between adjacent subcellular units. In this study, a single cardiac cell is modeled as a linear array of 200 subcellular units, which are diffusively coupled. This represents a minimal model of longitudinal diffusion along the cell. In this study, we consider five cells, each with 200 subcellular units, coupled end to end, as illustrated in Fig. 1A. Under normal conditions, Ca diffusion between neighboring cells is small, and, therefore, we set Ca diffusion to be zero across the cell boundaries. Since Ca diffusion is fast, we follow Restrepo et al. (17) and assume that the Ca concentration within the dyadic space is spatially uniform. This approximation is valid, owing to the fast diffusion time of Ca, across the \( \sim 100 \)-nm diameter of the dyadic junction. Model parameters used are the same as in Restrepo et al. (17), with minor modifications which are described in the Online Supplement. (The online version of this article contains supplemental data.)

**Markov schemes for the LCC and RyR channels.** Each ion channel within the dyadic junction is modeled according to experimentally based Markov schemes. The LCC is modeled using the five-state Markov scheme shown in Fig. 1B following Mahajan et al. (13), which was based on whole cell LCC measurements in rabbit myocytes. In this model, LCC inactivation occurs via a Ca- and voltage-dependent rates, while recovery from inactivation is only voltage dependent (18). The four-state RyR Markov model is based on a model proposed by Stern et al. (19) and modified by Restrepo et al. (17). The transition rates have the form \( k_{21} = K_{\text{ac}} c_{\text{ps}}^2, k_{23} = 1/\tau_s, k_{32} = k_{41} = 1/\tau_e, k_{43} = K_{\text{cr}} c_{\text{ps}}^2, \) and \( k_{34} = k_{43} k_{32} / k_{43} \). Here \( K_c \) is a constant parameter that determines the opening rate of the RyR channel, whereas \( \tau_e \) is the RyR closing time scale. Also, \( \tau_s \) and \( \tau_e \) denote the time scale of inactivation and recovery of the RyR channel. Time evolution of the channels is modeled so that state transitions occur at each time step, with the probabilities dictated by the transition rates.

**Mathematical model of coupled gating between RyR channels.** The RyR cluster is modeled as a square array of 10 \( \times \) 10 channels. To model coupled gating, we follow Stern et al. (19) and introduce an allosteric interaction between nearest neighbor RyRs. Allosteric interactions require that the transition rates obey

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Fig. 1. Illustration of subcellular calcium (Ca) cycling architecture and ion channel distribution. A: spatial distribution of ion channels and compartments involved in intracellular Ca cycling. Each subcellular unit is denoted by a superscript \( k \) and is diffusively coupled with its nearest neighbor. B: Markov model of L-type Ca channels (LCC) and ryanodine receptor (RyR) channel. Within a single dyadic junction, we have 1–5 LCCs and a 10 \( \times \) 10 array of RyR channels in a cluster. Each LCC channel is modeled using five states: two closed states (C1, C2), an open state (O), and two inactivation states (I1, I2). JSR, junctional sarcoplasmic reticulum; NSR, network sarcoplasmic reticulum; \( I_{\text{NaK}} \), sodium current; \( I_{\text{NaCl}} \), sodium/potassium exchanger current; \( I_{\text{Ca}} \), Ca current; \( I_{\text{Kr}} \), rapid-component transient outward \( K^+ \) current; \( I_{\text{Ks}} \), slow-component \( K^+ \) current; \( I_{\text{Kd}} \), fast-component transient outward \( K^+ \) current; \( c_{\text{ps}} \), Ca concentration in the dyadic junction; \( c_{\text{s}} \), Ca concentration in the submembrane space; \( c_{\text{b}} \), Ca concentration in the bulk myoplasm.
where the subscript $ij$ represents any two connected states of the RyR Markov scheme, $k_{ij}$ is the transition rate for an isolated RyR channel, $\Delta E_{ij}$ represents the energy penalty for an $i \rightarrow j$ transition due to allosteric interactions between two neighboring RyR channels, and $T$ is temperature. If a neighboring RyR channel is in state $i$, then $\Delta E_{ij} = E_{ii} - E_{ij}$, where $E_{ii}$ and $E_{ij}$ are the energies of the $ii$ and $ij$ conformations, respectively. Summing over the four nearest neighbors gives

$$\Delta E_{ij} = \sum_{t=1}^{4} E_{i(t)} - E_{j(t)}$$

where $l_k$ represents the state of the $k$th nearest neighbor. In this study, we consider a minimal model with allosteric interactions only between neighboring RyRs, which are either in the open (O), or closed (C) state. Therefore, $E_{OC} = E_{CO} = E$, and $E_{ij} = 0$, for all other combinations of states $i$ and $j$. For convenience, we will introduce the dimensionless parameter $\Delta = E/kT$, which represents the strength of coupled gating between RyR channels. Under physiological conditions, we will fix $\Delta = 1$, since it is known that RyR channels that are physically connected exhibit coupled gating (14). To model the effect of dissociation of the FKBP12.6 regulatory subunit, we simply decrease $\Delta$, to simulate a reduction in the degree of coupled gating.

**Coupling to membrane voltage.** The voltage across the cell membrane is modeled using a detailed physiological model based on experimental measurements in the rabbit ventricular myocyte (13). The ionic currents implemented in the model are shown in Fig. 1. Given that the diffusion time of membrane voltage across the span of five cells is small (~0.5 ms), we assume that voltage is the same across all cells, i.e., spatial differences of voltage are quickly averaged out via electrotonic currents. Therefore, we only need to keep track of the average voltage $V$, which is dictated by the sum of local currents spatially distributed over the five cells. The dynamics of $V$ is then given by

$$\frac{dV}{dt} = -\frac{1}{C_m} \left( I_{Na} + I_K + I_{stim} + \sum_{k=1}^{N} I_{NaCa} + I_{Ca} \right)$$

where $C_m$ is the cell membrane capacitance, $I_{stim}$ denotes the stimulus current, $I_{Na}$ is total Na current, $I_K$ is total K current, and $N$ is the total number of subcellular units within the collection of five coupled cells. For the five cells, we use $N = 1,000$. Here, the local LCC current within the $k$th subcellular unit is denoted by $I_{Ca}$, which is the sum of the total current due to the 1–5 LCC channels within that subcellular unit. Similarly, $I_{NaCa}$ represents the Ca-sensitive Na/Ca exchange current within sarcomere $k$.

To confirm that known features of EC coupling and subcellular Ca release are reproduced by the model, in Fig. 2, we plot voltage and Ca at a cycle length (CL) of 500 ms. We study the steady-state properties of the system by pacing for 100 beats, after which there are no systematic changes in the magnitude of the Ca transient and SR Ca load, i.e., all beat-to-beat variations arise only due to the stochasticity of Ca signaling. In Fig. 2A, we show the voltage time course for the last three beats, and below (Fig. 2B) we show the corresponding space-time plot of the spatial distribution of Ca within the five cells. In this case, most Ca sparks (shown in white) occur during the AP, and there are few spontaneous Ca release events. This is consistent with previous studies (6). In Fig. 2C, we plot the dyadic junction Ca concentration ($c_{p}$) within a junction indicated by the dashed white line in Fig. 2B. In this model, the $c_{p}$ can rise as high as ~500 $\mu$M during a spark, which is consistent with previous studies (6). In Fig. 2D, we plot the number of RyR channels open within the same dyadic junction, showing that, during a spark, the number of open RyR channels increases rapidly from 0 to close to the 100 channels in that cluster. In Fig. 2E, we plot the total LCC current due to the 5,000 LCC channels distributed across the five cells. In Fig. 2, F and G, we plot the average Ca concentration in the myoplasm ($c_i$) and the SR ($c_{Ca}$) defined as

![Fig. 2. Voltage and subcellular Ca under normal conditions. Voltage (V) and space-time plot (B) of Ca when five coupled cells are paced for three beats are shown. White regions denote high Ca concentration due to Ca release from sparks. Cell boundaries are shown as solid white lines. C: $c_p$ located at the 20th subcellular unit (dashed white line in B). D: number of RyR channels open in the same dyadic junction. E: LCC $I_{Ca}$ summed over all five cells showing stochastic features of the whole cell L-type $I_{Ca}$. F: $c_i$ averaged over the five cells. G: sarcomplasmic reticulum (SR) Ca concentration ($c_{Ca}$) averaged over the five cells.](image-url)
The fundamental unit of Ca release in the cardiac myocyte is the Ca spark (1). A Ca spark occurs when the cp is raised to a level that can induce, via Ca-induced Ca release, all or most RyR channels in the cluster to open. Under normal conditions, a Ca spark is triggered by an opening of one or a few LCC channels in the vicinity of an RyR cluster. To understand the basic properties of local Ca signaling, we first study the probabilistic nature of the response of an RyR cluster to an LCC channel opening. For simplicity, we will first consider LCC openings with a fixed duration of 5 ms, which is roughly the same as the mean open time of the LCC channel during the plateau of an action potential (0–20 mV). In Fig. 3A, we plot the local Ca concentration cp after a single LCC channel is opened for 5 ms in the vicinity of an RyR cluster. The four current traces correspond to four independent simulations under identical initial conditions. Note that, since the RyR cluster is stochastic, each simulation is different, even though all parameters are identical. The top two traces are cases when the Ca current entry due to the LCC opening induces a Ca spark, which appears as a substantial increase in cp to ~200 μM, which decays to the resting concentration in roughly 15 ms. The bottom two traces are examples where the LCC opening did not induce a Ca spark. To quantify the trigger-response relationship, we compute the probability, denoted by PS, that a spark is induced due to a local LCC opening. We compute PS by stimulating a single dyadic junction 5,000 times and computing the fraction of times in which a spark occurs. A triggered spark is counted when the number of open RyR channels in the local cluster exceeds 90% of the total number of channels, within a 20-ms time interval after the LCC channel opening. In Fig. 3B, we plot the spark probability PS for a range of Δ, as a function of the amplitude of the Ca current due to the local LCC channel opening. Here, we denote the amplitude of the local Ca current to be I_lcc. The figure indicates that PS ≈ 0 for small I_lcc and then rapidly increases to PS ≈ 1. The rapid increase of PS occurs at a current amplitude, denoted by I_lcc, where PS(I_lcc) = 1/2, and where I_lcc can be interpreted as the threshold current for Ca sparks to be activated via an LCC opening. As shown in Fig. 3B, the current amplitude I_lcc decreases substantially as Δ is decreased. This result shows that the probabilistic response of an RyR cluster, to changes in the local Ca concentration, is strongly dependent on the degree of coupled gating between RyR channels.

**RESULTS**

**Dependence of Ca sparks on RyR allosteric interactions.** The probabilistic response of an RyR cluster, to changes in the local Ca concentration, is strongly dependent on the degree of coupled gating between RyR channels.
cluster. In this simulation, the membrane voltage is clamped at \(-85 \text{ mV}\), so that all LCCs channels are in their closed state and there is no trigger current. Despite the absence of a trigger current, several spontaneous Ca sparks occur within the 2-s interval shown. The time interval between spontaneous events, denoted by \(T_{ss}\) (Fig. 3C), is then measured. To quantify the frequency of spontaneous Ca sparks, we compute \(T_{ss}\), which is the average time between spontaneous release events, where the average is computed using 1,000 independent simulations. In Fig. 3D, we plot \(T_{ss}\) vs. \(\Delta\), for three initial SR Ca loads. The plot of \(T_{ss}\) vs. \(\Delta\) is well fit by an exponential function. Note that, for normal or low SR loads, the time interval between spontaneous sparks is larger than 2 s. In this case, spontaneous release is unlikely to occur within a physiological CL, which is typically \(<1 \text{ s}\).

**Dependence of Ca wave characteristics on RyR allosteric interactions.** Ca waves occur when the Ca released at one dyadic junction can diffuse and stimulate neighboring junctions to spark. Important characteristics of a Ca wave are the speed of wave propagation and the distance traveled by the wave before it extinguishes. Here, we investigate how Ca wave characteristics depend on the degree of coupled gating between RyR channels. Ca waves can be induced in our model by elevating SR Ca load. We then initiate a Ca spark at one end of the cell and then measure both the distance the wave travels and the propagation speed. In Fig. 4A, we plot the average wave propagation speed \(V_{Ca}\) as function of \(\Delta\) for a fixed SR Ca load of 1.8 mM. This result shows that wave speed increases substantially as the degree of coupled gating is decreased. In Fig. 4B, we plot the average distance \(L_{Ca}\) that a Ca wave travels as a function of \(\Delta\). In this case, we find again that \(L_{Ca}\) increases as the degree of coupled gating is reduced. Here, we only plot \(L_{Ca}\) for \(\Delta\) in the range 0.6 to 1. For \(\Delta\ < 0.6\), \(L_{Ca}\) cannot be reliably estimated, since propagating waves collide with Ca released spontaneously far from the stimulation site. At fixed \(\Delta\), we also find that Ca wave characteristics are highly sensitive to SR Ca content. In Fig. 4, C and D, we plot both wave speed and propagation distance as a function of SR Ca load, with \(\Delta\) fixed at 0.7. In Fig. 4C, we see that \(V_{Ca}\) increases abruptly from zero as a function of the SR Ca load. This result indicates that there is a sharp threshold from nonpropagating to propagating Ca sparks. In Fig. 4E, we plot the threshold SR Ca

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content vs. Δ, showing that the SR Ca content at which sparks transition to waves increases with increasing Δ.

Spontaneous sparks, Ca waves, and DADs. The voltage across the cell membrane is sensitive to subcellular Ca release due to the presence of Ca-dependent inward currents. Thus spontaneous Ca sparks and Ca waves can lead to membrane depolarization, which can potentially induce DADs. In experiments, triggered arrhythmias are induced in rats via β-adrenergic stimulation, along with rapid pacing (8). To model this effect, we increase Ca current entry by increasing the LCC conductance by 10%, which is a known effect of β-adrenergic stimulation. These modifications have the effect of increasing SR Ca load, since total Ca entry is increased. Following the experimental protocol, the cell is paced rapidly for 20 beats at a fixed CL = 500 ms. The initial SR load is chosen to be the diastolic c_{sr} at the 500-ms steady state. In Fig. 5, we plot the last three action potentials, along with the spatial distribution of Ca for a range of Δ. In Fig. 5A, we show the case Δ = 1, which shows that Ca release occurs mostly via isolated sparks following the AP upstroke, with a few spontaneous release events after pacing ceases. In Fig. 5, B and C, we decrease the allosteric coupling to Δ = 0.7 and Δ = 0.5. We find that, for lower Δ, Ca release following the AP upstroke occurs due to triggered Ca sparks, which transition to Ca waves. Thus Ca release can be more synchronized than the case when Δ = 1, where release occurs only due to isolated Ca sparks. Thus a reduction in RyR cooperativity tends to increase the degree of synchrony of triggered Ca release. Furthermore, after pacing is ceased, we observe a substantial increase in the number of spontaneous Ca release events that transition to Ca waves. This Ca release activity leads to DADs in the corresponding voltage trace. In Fig. 6, we show the corresponding average c_i and c_{sr}. In Fig. 6A, we see that the peak of the Ca transient is larger due to the increased Ca current. However, as the RyR coupling is decreased (Fig. 6, B and C), the diastolic Ca concentration rises while the peak of the Ca transient decreases. Also, we find that the SR Ca content immediately before the AP upstroke is substantially decreased from roughly 1.8 to 1.4 mM. Thus SR refilling is slowed dramatically due to spontaneous Ca release events. At more rapid rates, we observe similar results. In Fig. 7, we plot voltage and Ca after pacing the cell for 20 beats at 350 ms. In Fig. 8, we show the corresponding Ca transient and SR Ca load. Interestingly, we found that the Ca transient exhibits alternans for the case Δ = 1, but that these alternans disappear for small values of Δ. Again, we find that SR Ca content also decreases as Δ is decreased.

DADs and subcellular Ca. To explore the relationship between subcellular Ca and DADs, we observe the spatiotemporal dynamics of Ca during a DAD. In Fig. 9A, we show a typical DAD, along with the simultaneous subcellular Ca activity in which several spontaneous Ca sparks transition to Ca waves. To quantify the relationship between voltage and Ca, we measure δV, which is the voltage difference between the first minimum and maximum voltage after the last AP, and the time \( t_d \), which is the corresponding time interval (Fig. 9A). To quantify the Ca activity that induces that DAD, we count the number of distinct spontaneous Ca wave sources during the time interval \( t_d \). We then compute \( n_s \), defined as the number of Ca wave sources per cell. Finally, we compute a new quantity \( r = n_s/t_d \), which is the number of waves sources per cell per unit time, during the rising phase of the voltage deflection. This quantity gives a direct measure of the rate at which Ca waves sources are generated during the formation of a DAD. We measured δV vs. r for 100 independent simulation runs, where the initial SR Ca load is fixed at the indicated values. In Fig. 9B, we plot the average DAD amplitude δV vs. r for all simulation runs. The results demonstrate that the amplitude of a DAD increases linearly with the number of Ca waves sources.

Fig. 5. Plot of voltage V and subcellular Ca after steady-state pacing followed by a pause. To simulate Ca overload conditions, we increase LCC conductance by 10% from the value used in Fig. 2. The five cells are then paced for 20 beats at 500 ms, before cessation of pacing. Initial conditions are set at the steady-state values found from the simulation in Fig. 2. All parameters are kept the same, except for the degree of RyR allosteric interaction with \( \Delta = 1.0 (A), \Delta = 0.7 (B), \) and \( \Delta = 0.5 (C) \).
per unit time. This linear relationship holds for the three initial SR Ca loads, with $\delta V$ increasing with SR Ca load.

**DISCUSSION**

In this study, we applied numerical simulations to explore the relationship between RyR channel properties and the distribution of subcellular Ca. We demonstrated that the $P_S$ that an LCC opening triggers a Ca spark exhibits a sigmoid dependence on the stimulus amplitude, with a threshold that is sensitive to the degree of coupled gating. Thus a small decrease in $\Delta$ led to substantial leftward shift in the $P_S$ curve (Fig. 3B). To explain this shift, it is first necessary to understand RyR channel dynamics during the activation of a Ca spark. If the Ca concentration within a dyad is low, then a cluster of RyR channels will be “closed” in the sense that most of the ~100 channels will be in their closed conformational state. Now a small increase in the local Ca concentration, due to an LCC opening, will promote channel openings via Ca-induced Ca release, which may then cascade into a Ca spark, where almost all of the RyR channels transition to the open state, i.e., the cluster can “open”. In this way, an RyR cluster as a whole behaves as a switch between a “closed” and “open” state. Here, we argue that the kinetics of switching between closed and open state is highly sensitive to the allosteric coupling between

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**Fig. 6.** Average $c_i$ and $c_{sr}$ during the beats shown in Fig. 5, with $\Delta = 1.0$ (A), $\Delta = 0.7$ (B), and $\Delta = 0.5$ (C). CL, cycle length.

**Fig. 7.** Plot of $V$ and subcellular Ca after steady-state pacing at 350 ms. All parameters are the same as those in Fig. 5, with $\Delta = 1.0$ (A), $\Delta = 0.7$ (B), and $\Delta = 0.5$ (C).
RyR channels. If we pick an arbitrary closed RyR channel in a “closed” cluster, then it will be surrounded by four neighboring channels, which will, with high probability, also be in the closed state. Now, if $\tilde{k}_{CO}$ is the transition rate from a closed to an open state in the absence of allosteric interactions, then allosteric interactions will decrease this rate to $k_{CO} = \tilde{k}_{CO} \exp\left(-4\Delta\right)$, since transitions to the open state will be less favorable, owing to the energy cost for the four neighboring channels to be in different conformational states, i.e., each neighboring channel will decrease the transition rate by a factor $\exp\left(-\Delta/4\right)$. In this sense, allosteric interactions stabilize a “closed” cluster by decreasing the probability that RyR channels transition to the open state. Thus, as $\Delta$ is decreased, the threshold for the $P_S$ shifts to lower Ca concentration levels, thus making the cluster more sensitive to activation by Ca influx.

The dependence of RyR cluster sensitivity on allosteric interactions has important implications. First, a decrease in allosteric coupling $\Delta$ leads to a substantial increase in the frequency of spontaneous Ca sparks. To understand this sensitive dependence, we first note that a spontaneous Ca spark occurs as a result of the inherent stochastic behavior of RyR channels. Even when the $c_p$ is low, there is still a small but finite probability that an RyR channel will transition from the closed to the open state. When such a transition occurs, the Ca flux from the SR into the dyadic space can raise the Ca concentration further to induce the entire cluster to open. In this way, Ca sparks can occur spontaneously, independently of LCC openings. As a result, the frequency of spontaneous Ca sparks is sensitive to the probability that a given RyR channel transitions to the open state. Thus, if allosteric interactions are decreased, then the frequency of spontaneous Ca sparks

Fig. 8. Average $c_i$ and $c_{sr}$ during the beats shown in Fig. 7, with $\Delta = 1.0$ (A), $\Delta = 0.7$ (B), and $\Delta = 0.5$ (C).

Fig. 9. Relationship between subcellular Ca and delayed afterdepolarizations (DADs). A: $V$ and subcellular Ca during a DAD for $\Delta = 0.7$. The DAD is characterized by the time ($t_d$) and voltage change ($\delta V$) between the minimum and maximum voltage following the previous AP. White arrows indicate spontaneous Ca sparks, which transition to Ca waves. B: plot of DAD amplitude $\delta V$ vs. $r$, the rate at which Ca wave sources are formed during the time interval $t_d$. All data points represent 100 independent simulations. The lines represent best line fits at the indicated SR Ca loads.

AJP-Heart Circ Physiol • VOL 297 • JULY 2009 • www.ajpheart.org
rally increases, since the probability of RyR channel openings is increased. Another important finding in our simulations is that the spontaneous spark frequency is exponentially sensitive to RyR allosteric interactions (Fig. 3D). Therefore, even small changes in the degree of RyR coupling can substantially change the frequency of spontaneous Ca sparks. This exponential sensitivity highlights the importance of coupled gating in the regulation of subcellular Ca release.

Allosteric RyR interactions also influence the behavior of Ca waves. To quantify this relationship, we measured the propagation speed and distance of a Ca wave as function of the strength of allosteric interactions ($\Delta$). Our results demonstrate that both speed and propagation distance increase rapidly with decreasing $\Delta$. To understand this effect, we first note that a Ca wave occurs when the Ca released at a dyadic junction diffuses and triggers a Ca spark in a neighboring dyad. A chain reaction of these events leads to a Ca wave. A key determinant of wave speed will be the sensitivity of a typical RyR cluster to changes in the Ca concentration in the dyad. Thus a decrease in $\Delta$, which causes a higher sensitivity to Ca, owing to the leftward shift of the $P_S$ curve, will naturally promote faster and further wave propagation. Furthermore, we found that the $c_{ \text{Ca}}$ threshold for Ca waves to form is also highly sensitive to $\Delta$. This shows that both SR Ca load and RyR-coupled gating are key factors that determine efficacy and timing of whole cell spontaneous Ca release.

The pacing rate dependence of Ca cycling is also sensitive to the degree of RyR-coupled gating. In our simulations, we find that, as $\Delta$ is decreased, the diastolic Ca concentration in the cytoplasm increases, while the SR Ca load is decreased substantially (Figs. 6 and 8). This is because spontaneous Ca release events drain the SR and, therefore, prevent the SR from refilling to normal levels. In effect, the SR Ca leak is substantially increased so that more Ca is sequestered in the cytoplasm rather than the SR. Consequently, the amount of Ca released from the SR, in response to LCC openings, is also reduced. This is due to the reduction in SR Ca load, which dictates how much Ca is released in response to an LCC opening. Therefore, the magnitude of the Ca transient, which is measured as the difference between peak Ca and diastolic Ca, decreases as RyR-coupled gating is decreased. These results suggest that the pacing rate dependence of Ca cycling is dependent on the degree of RyR-coupled gating.

In our simulations, we find that DADs occur under Ca overload conditions and when the strength of RyR allosteric interactions is decreased. A close examination of Ca release during a DAD shows that subcellular Ca waves originate at spontaneous release sites distributed across the five cells considered. Our main finding is that the amplitude of a DAD ($\delta V$) is strongly dependent on the number of spontaneous release events that transition to waves within the rise time ($t_{\text{rise}}$) of the DAD (see Fig. 9A). This result is not surprising, since a DAD is due to the increase in inward current via the sodium/Ca exchanger ($I_{\text{NaCa}}$), which is directly proportional to the magnitude of Ca release into the cytoplasm. Thus, as more Ca is released per unit cell, per unit time, the amplitude of DADs also increases. Furthermore, we also find that DAD amplitude is sensitive to SR Ca load, which is not surprising, since higher SR load leads to larger Ca release, which should, in turn, lead to larger $I_{\text{NaCa}}$.

Lehnart et al. (7, 9) have shown that FKBP12.6-deficient mice are prone to exercise-induced triggered arrhythmias. According to these authors, the mechanism for the arrhythmia is likely due to the “leakiness” of the SR caused by the dissociation of FKBP12.6 from the RyR complex, which has been shown to mediate RyR-coupled gating (14). Our simulation results are consistent with this finding and present a detailed picture of the underlying subcellular mechanisms. In our simulations, “leakiness” of the SR is a complex process that involves an increased frequency of spontaneous Ca sparks, in conjunction with faster and further propagating Ca waves. These subcellular events are caused by the increased Ca sensitivity of RyR clusters due to the reduction of RyR allosteric interactions. Furthermore, we find that the formation and amplitude of DADs is dependent on the rate of formation of spontaneous Ca sparks, which subsequently transition to Ca waves. These results show that a reduction in RyR-coupled gating, which may be caused by a molecular defect in RyR, can promote triggered arrhythmias via Ca-mediated DADs. Perhaps most importantly, however, our studies reveal that the frequency of spontaneous Ca sparks is an important factor in DAD initiation because of their exponential sensitivity to the degree of RyR-coupled gating. This exponential sensitivity could explain why specific molecular defects in RyR function that modulate coupled gating are particularly prone to spontaneous Ca release, a result that is consistent with experimental findings showing that triggered arrhythmias can be caused by dissociation of the FKBP12.6 binding protein (7–9).

GRANTS

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REFERENCES