Modulation of local Ca^{2+} release and depletion signals through calsequestrin-mediated RyR luminal regulation

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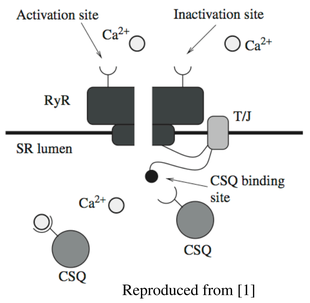
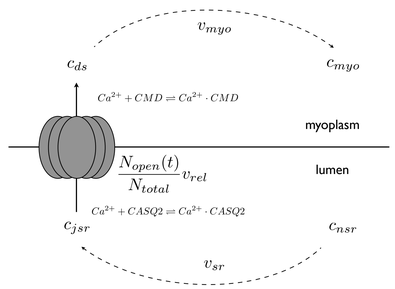
— Abstract —

Luminal regulation of ryanodine receptors (RyRs) is likely an important factor in Ca^{2+} release in cardiac myocytes. The Ca^{2+}-free form of cardiac calsequestrin (CSQ or CASQ2 below), a luminal Ca^{2+} buffer, binds to the RyR/triadin/junctin complex and modulates spark activity. When jSR [Ca^{2+}] is low, CSQ binds to the channel and lowers the RyR open probability. We present hybrid Markov chain/ODE simulations of a release site consisting of 100 eight-state Lee-Keener RyRs and associated Ca^{2+} domain dynamics [1]. Below, we simulate different possible CSQ-RyR interactions. The resulting emergent spark properties are compared to experimental observations of spark activity in control cells and several arrhythmogenic CSQ mutants [2].



Reproduced from [1]

— Bidomain Model Description —



The evolution in time of the [Ca^{2+}] in the diadic subspace (c_{ds}) and jSR (c_{jsr}) is given by

$$\frac{dc_{ds}}{dt} = \frac{\beta_{ds}}{\lambda_{ds}}(J_{ryr} - J_{efflux}) \qquad \frac{dc_{jsr}}{dt} = \frac{\beta_{jsr}}{\lambda_{jsr}}(J_{refill} - J_{ryr})$$

where

$$J_{efflux} = v_{myo}(c_{ds} - c_{myo}) \qquad J_{refill} = v_{sr}(c_{nsr} - c_{jsr})$$

$$J_{ryr} = \frac{N_{open}(t)}{N_{total}}(c_{jsr} - c_{ds}),$$

λ_{ds} and λ_{jsr} are volume fractions, and β_{jsr} and β_{ds} are Ca^{2+}- dependent buffering factors. $N_{open}(t)$ is the number of open channels in the model Ca^{2+} release site composed of 100 stochastically gating Lee-Keener RyRs [1]. The channels are identical and coupled under the assumption that each experiences the same ds and jSR [Ca^{2+}]. We assume rapid equilibration of the free and bound forms of CSQ.

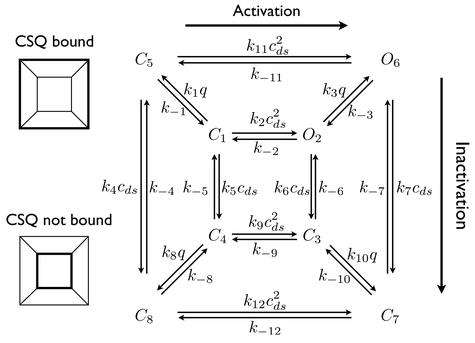


Figure 1: The eight-state Lee-Keener RyR model [1]. q represents the concentration of the Ca^{2+}-free form of CSQ. We assume rapid equilibration between CSQ and the RyR.

—Experimental results —

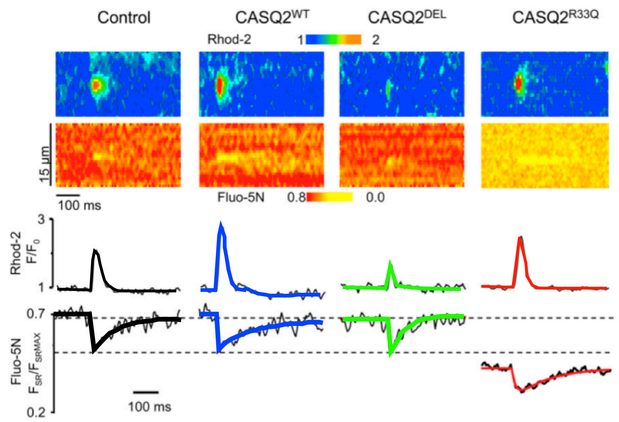


Figure 2: Simultaneous measurement of Ca^{2+} changes in myoplasmic and jSR compartments during spontaneous sparks. $CASQ2^{WT}$, cells with overexpressed CSQ. $CASQ2^{DEL}$, cells expressing mutant CSQ with reduced affinity for Ca^{2+}. $CASQ2^{R33Q}$, cells expressing mutant CSQ with reduced affinity for RyRs. Reproduced from [2].

— Spark Simulation, Alignment, Averaging —

Simulated sparks were aligned and then averaged. The resulting emergent spark properties were compared to experimental observations of spark activity in control and CASQ2 mutant cells. Parameter studies were performed to test various aspects of the model against experimental evidence. Aspects of experiment replicated (√) or not replicated (×) are shown to the right. Color in averaged simulated sparks indicates corresponding experimental results.

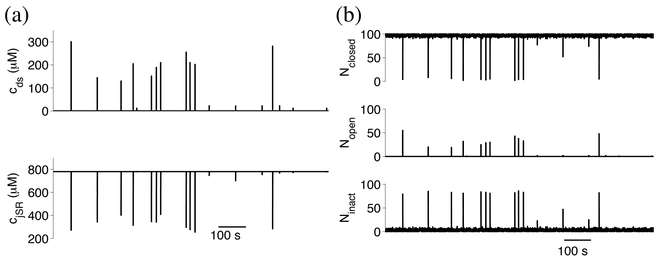


Figure 3: **(a)** Representative times and amplitudes of spontaneous SR Ca^{2+} release and associated depletion signals exhibited by a Ca^{2+} release site model composed of 100 Lee-Keener RyRs. **(b)** Associated changes in channel state.

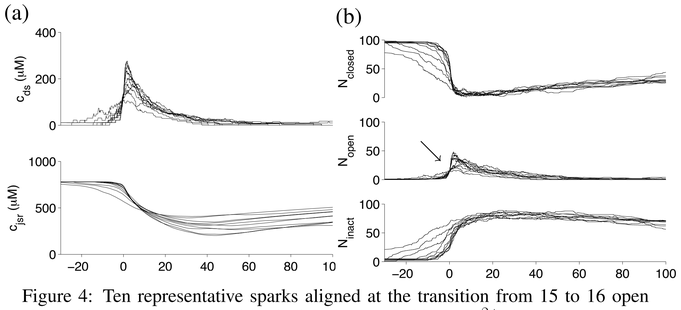


Figure 4: Ten representative sparks aligned at the transition from 15 to 16 open channels (shown by arrow). **(a)** Diadic subspace and jSR [Ca^{2+}] as well as changes in **(b)** channel activity are shown.

— $[CSQ]_T$ modulates spark activity —

- √ • $[CSQ]_T \uparrow \Rightarrow$ spark amplitude ↑
- √ • $[CSQ]_T \uparrow \Rightarrow$ jSR recovery time ↑
- × • $[CSQ]_T \uparrow \Rightarrow$ resting nSR [Ca^{2+}] and blink nadir ↑
- • $[CSQ]_T \uparrow \Rightarrow$ channel activity is invariant

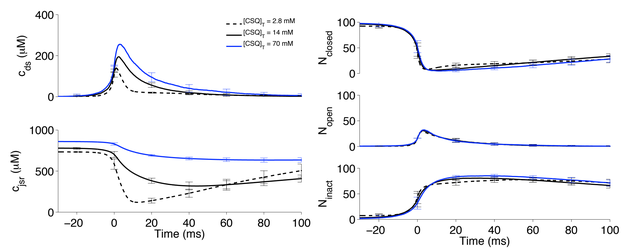


Figure 5: Average [Ca^{2+}] and channel activity for simulated sparks.

— Reducing CSQ-Ca^{2+} affinity — modulates spark activity

- √ • K_{CSQ-Ca} varies $\Rightarrow$ spark amplitude ↓
- √ • $K_{CSQ-Ca} \uparrow \Rightarrow$ jSR recovery time ↓
- × • $K_{CSQ-Ca} \uparrow \Rightarrow$ resting nSR [Ca^{2+}] ↑
- • $K_{CSQ-Ca} \uparrow \Rightarrow$ $N_{inact} \downarrow$ $N_{C1} \uparrow$

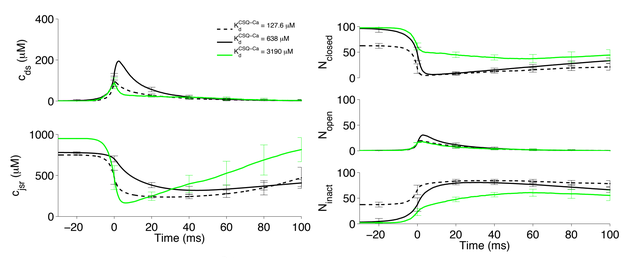


Figure 6: Average [Ca^{2+}] and channel activity for simulated sparks.

— Reducing CSQ-RyR affinity — modulates spark activity

- √ • $K_{CSQ-RyR} \uparrow \Rightarrow$ amplitude & average channel state invariant
- √ • $K_{CSQ-RyR} \uparrow \Rightarrow$ resting nSR [Ca^{2+}] and blink nadir ↓

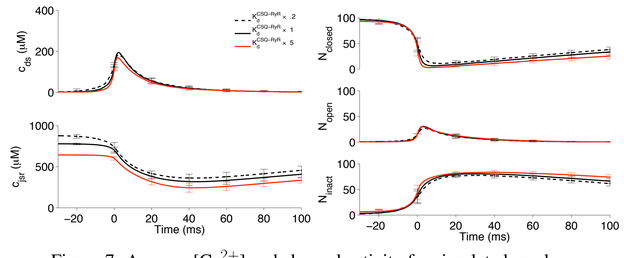


Figure 7: Average [Ca^{2+}] and channel activity for simulated sparks.

— Effects of CSQ buffering on — spark activity

- • $K_{CSQ-RyR}$ & $[CSQ]_T \uparrow \Rightarrow$ amplitude ↑
- • $K_{CSQ-RyR}$ & $[CSQ]_T \uparrow \Rightarrow$ $N_{inact} \uparrow$ $N_{C1} \downarrow$

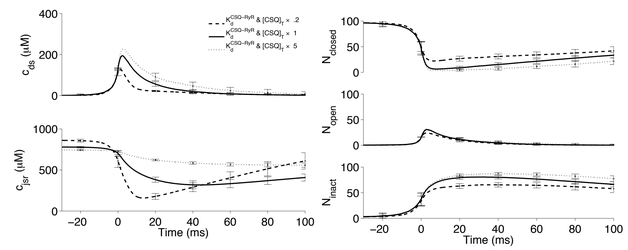


Figure 8: Average [Ca^{2+}] and channel activity for simulated sparks. Both $K_{CSQ-RyR}$ and $[CSQ]_T$ are modified so that the proportion of CSQ bound to the channel would be invariant over different parameters. This allows a focus on differences in spark statistics based purely on the buffering properties of CSQ.

— RyR inactivation studies —

- The Lee-Keener RyR model includes CSQ-mediated facilitation of cytosolic Ca^{2+} inactivation as well as CSQ-mediated suppression of cytosolic Ca^{2+} activation. The direct inactivation of the RyR by CSQ is a possible mechanism that is not included in the Lee-Keener model. Further studies were performed to ascertain how these mechanisms may determine CSQ's role in regulating spontaneous Ca^{2+} release.
- Direct inactivation of channels by CSQ results in fewer open channels and an associated rise in the number of closed channels. This results in lower spark amplitudes with few changes in associated blinks, as well as the inability of sparks to initiate (not shown).
- Down-regulation of inactivation via cytosolic Ca^{2+} resulted in the inability of CSQ to regulate the channel effectively. Sparks were often terminated as a result of luminal depletion (not shown).

— Conclusions —

- The effect of CSQ on modulating RyR activity was studied in a Ca^{2+} release site model composed of 100 Lee-Keener RyRs coupled with a bidomain model of spontaneous Ca^{2+} release.
- Many aspects of experimental evidence of CSQ mutations are replicated by the model. However, certain key features, such as blink nadir and resting nSR [Ca^{2+}] in specific mutants are not observed. Thus, a constant blink nadir regardless of CSQ buffering strength might not be associated with a luminal Ca^{2+}-dependent spark termination mechanism.
- The buffering properties of CSQ have an effect on the change in resting nSR [Ca^{2+}], by modulating the spark amplitude. However, the binding of CSQ to the RyR causes spark frequency to fall, which overshadows the effects of spark amplitude on resting nSR [Ca^{2+}].

— References —

[1] Y.-S. Lee and J. P. Keener. A calcium-induced calcium release mechanism mediated by calsequestrin *J Theor Biol*, 253:668 - 679, Jan 2008.

[2] D. Terentyev, et al. Modulation of sr ca release by luminal ca and calsequestrin in cardiac myocytes: Effects of casq2 mutations linked to sudden cardiac death *Biophys J*, 95:2037 - 2048, Jan 2008.

This material is based upon work supported by the National Science Foundation under Grant No. 0443843.

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