Letter to the Editor

Ryanodine Receptor Permeation and Gating: Glowing Cinders that Underlie the Ca^{2+} Spark

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Schneider (1999) recently addressed the question of whether Ca^{2+} sparks arise from the opening of a single ryanodine receptor (RyR) channel or the simultaneous opening of several channels. The discussion highlighted the importance of single RyR channel permeation and gating in the interpretation of Ca^{2+} spark data. The Schneider (1999) perspective inspired us to extend this theoretical discussion by using a published kinetic model of modal RyR gating to actually simulate RyR channel gating and permeation that may underlie a Ca^{2+} spark in cardiac muscle.

The Single Channel Ca^{2+} Spark Interpretation

Cheng et al. (1993) was the first to propose that the spontaneous Ca^{2+} spark is the elementary intracellular Ca^{2+} release unit that underlies excitation–contraction coupling in cardiac muscle. They estimated that the local Ca^{2+} flux underlying the Ca^{2+} spark would need to be $\sim 2 \times 10^{-17}$ mol/s, assuming a volume of $\sim$10 fl (i.e., an $\sim$2-μm cube), duration of 10 ms (time to peak), and a final $[Ca^{2+}]$ of $\sim$300 nM (resting $[Ca^{2+}]$ = 100 nM). This type of calculation predicts that the underlying unitary RyR channel Ca^{2+} current would need to be 1–4 pA to generate the observed Ca^{2+} spark (Cheng et al., 1993; Pratusevich and Balke, 1996; Blatter et al., 1997; Jiang et al., 1998; Schnieder, 1999). An early estimate of the unitary Ca^{2+} current through the cardiac RyR channel was 2.5 pA (at 0 mV with 50 mM charge carrier; Rousseau and Meissner, 1989). This lead Cheng et al. (1993) to propose that the Ca^{2+} spark may arise from the opening of a single RyR Ca^{2+} release channel. The RyR channel, however, is a poorly selective Ca^{2+} channel, and thus other ions (e.g., K^+ and Mg^{2+}) are likely to compete with Ca^{2+} for occupancy of the pore. Consequently, the unitary Ca^{2+} current must be smaller under more physiological conditions (1 mM lumenal Ca^{2+}, 150 mM K^+, and 1 mM Mg^{2+}). Tinker et al. (1993) used a RyR permeation model to estimate that the unitary Ca^{2+} current was 1.4 pA (at 0 mV, 1.2 mM lumenal Ca^{2+} charge carrier in symmetrical 120 mM K^+ and 0.5 mM Mg^{2+}). This updated estimate lead Blatter et al. (1997) to propose that simultaneous opening of two RyR channels may generate the Ca^{2+} spark. If Ca^{2+} sparks arise from the opening of one or two RyR channels, then certain pharmacological manipulations that alter single channel properties should be reflected at the Ca^{2+} spark level. Cheng et al. (1993) reported that lower amplitude, long duration Ca^{2+} sparks occur in the presence of ryanodine. This resembles the ryanodine-induced long-lasting subconductance states observed at the single channel level. Shtifman et al. (1999) reported that prolonged small-amplitude Ca^{2+} sparks occurred after application of Imperatoxin A ($IpTx_A$). This resembles the prolonged subconductance of $IpTx_A$-modified RyR channels in bilayers (Tripathy et al., 1998).

In summary, the single channel Ca^{2+} spark interpretation is largely based on two lines of evidence: first, the relatively large estimates of unitary RyR channel Ca^{2+} current and, second, the parallel pharmacological actions at the spark and single channel levels.

The Multichannel Ca^{2+} Spark Interpretation

The hypothesis that multiple RyR channels open simultaneously to generate the Ca^{2+} spark is consistent with the clustered arrangement of RyR channels in heart (Sun et al., 1995; Franzini-Armstrong and Protasi, 1997). It is also consistent with the stereotypic amplitude of the Ca^{2+} spark. If Ca^{2+} sparks were generated by spontaneous openings of a single channel, then the distribution of Ca^{2+} spark amplitudes should be exponential in nature because single channel open times are distributed exponentially. Observed Ca^{2+} spark amplitudes, however, are normally distributed. There is also a curious lack of small Ca^{2+} spark events that is not easy to reconcile with the single channel spark hypothesis. Recently, Mejía-Alvarez et al. (1999) have directly measured the amplitude of unitary Ca^{2+} current

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through a single cardiac RyR channel under quasi-physiological ionic conditions. The unitary Ca^{2+} current was considerably smaller than previously predicted (0.35 vs. 1.4 pA; Tinker et al., 1993). This suggests that Ca^{2+} sparks may arise from 3 to 10 RyR channels opening simultaneously.

In summary, the multichannel Ca^{2+} spark interpretation is based on three lines of evidence: first, the stereotypic nature of the Ca^{2+} spark; second, new smaller estimates of unitary RyR channel Ca^{2+} current; and, third, tantalizing correlations with the clear physical clustering of RyR in heart.

Simulating the RyR Channel Gating that Underlies the Ca^{2+} Spark

A published kinetic Markovian scheme of RyR channel gating was used to generate simulated single RyR channel records. The simulated gating reflects single RyR channel measurements made in planar lipid bilayer studies (e.g., Sitsapesan and Williams, 1995). The unitary Ca^{2+} current was fixed at 0.35 pA (Mejía-Alvarez et al., 1999). To predict free [Ca^{2+}] fluctuations, a multicompartment unidimensional diffusion model was evaluated (Cannell and Allen, 1984; Pizarro et al., 1991). The diffusion model includes Ca^{2+} binding/unbinding to known buffers and SR Ca^{2+} reuptake. The only entity allowed to diffuse is the Ca^{2+} ion. The predicted fluorescence (Fluo-3) signals due to the local Ca^{2+} fluxes produced by the simulated single RyR channel activity were calculated and are presented in Fig. 1.

At a steady state Ca^{2+} concentration of pCa 7, the applied RyR gating scheme predicts that spontaneous single channel events occur at low open probability (P_o). (The gating scheme does not consider other regulatory factors [e.g., Mg^{2+}] that may impact the stationary P_o of the channel.) Most single channel open events are brief and bursts of open events are rare (Fig. 1 A). Every RyR channel opening elevates the local Ca^{2+} concentration. However, nearly all local Ca^{2+} elevations

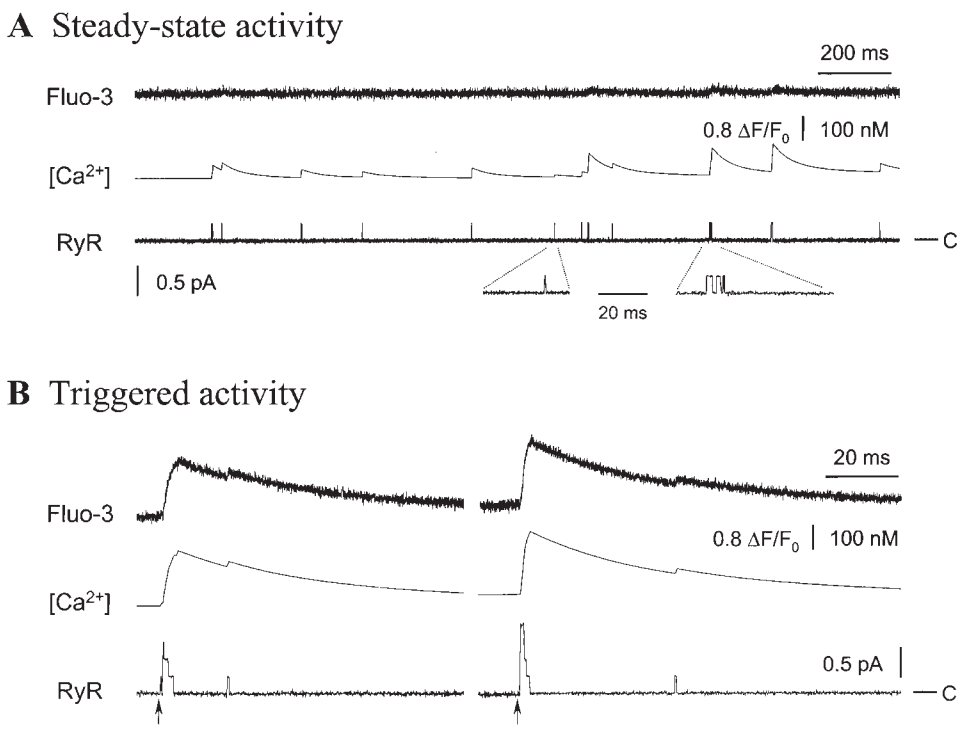

Figure 1. Single RyR channel gating and Ca^{2+} spark simulations. (A) Steady state single channel simulation of RyR gating, predicted local Ca^{2+} flux, and resulting fluorescence signal. Individual single channel openings fail to elevate local Ca^{2+} sufficiently to be detected as a fluorescence signal. Local Ca^{2+} elevations induced by a burst of single channel openings generates a small but detectable fluorescence signal. (B) Triggered nonstationary gating simulation of five RyR channels. The trigger stimulus was applied at the arrow. The predicted local Ca^{2+} flux and resulting fluorescence signals are shown. Brief simultaneous opening of multiple RyR channels generate fluorescence signals reminiscent of experimentally observed Ca^{2+} sparks. The kinetic RyR channel gating scheme is from Villalba-Galea et al. (1998) and was based on the model proposed by Zahradníková and Zahradník (1996). The RyR channel mean open time was 0.5 ms and the unitary current amplitude was 0.3 pA, which corresponds to $\sim 9.4 \times 10^5$ ions/s. Simulations were run under steady state conditions (pCa 7) assuming the presence of a single RyR channel. Simulations of triggered RyR channel activity were run assuming the presence of five RyR channels. The applied trigger Ca^{2+} pulse (10 μM for 500 μs) was from pCa 7. This trigger Ca^{2+} stimulus (arrows) mimics that which may be generated by an opening of a single DHPR Ca^{2+} channel. For simplicity, the simulation assumed that the trigger Ca^{2+} pulse and RyR-mediated Ca^{2+} release occur in different pools. A multicompartment diffusion model was used to evaluate how the simulated single channel behavior (i.e., the underlying driving Ca^{2+} waveform) impacts local Fluo-3 (100 μM) fluorescence. Model parameters include: Fluo-3 $k_{on} = 0.238\ \mu M^{-1}\ ms^{-1}$, Fluo-3 $k_d = 740$ nM, 1 mM endogenous Ca^{2+} buffers with $k_{on} = 0.002\ \mu M^{-1}\ ms^{-1}$ and $k_d = 400$ nM in a volume of 10^{-15} liters. The Ca^{2+} flux through a single RyR channel was 1.55×10^{-15} μM/ms. If five RyR channels open simultaneously for 500 μs in a volume of 10^{-15} liters, then the $\Delta[Ca^{2+}]$ was 775 nM. This value is considerably higher than that needed to generate a Ca^{2+} spark. However, the inclusion of parameters like binding and diffusion result in a $\Delta[Ca^{2+}]$ of 232 nM, which corresponds to a $\Delta F/F_0$ of $\sim$1.53.

would not be detected as Fluo-3 fluorescence signals. The largest local Ca^{2+} elevations induced by bursts of RyR openings are just barely detectable at the fluorescence level. The same RyR gating scheme was also used to predict the response of five RyR channels to a trigger Ca^{2+} pulse (10 μM for 500 μs). The trigger Ca^{2+} pulse was applied to synchronize the opening of the RyR channels. Simultaneous opening of multiple RyR channels elevates the local Ca^{2+} concentration to levels consistent with that predicted to underlie the Ca^{2+} spark (Fig. 1 B). These local Ca^{2+} concentrations generate Fluo-3 fluorescence signals reminiscent of the experimentally observed Ca^{2+} spark.

Conclusions

Our simulations suggest that individual openings of a single RyR channel under steady state conditions at a resting Ca^{2+} level are unlikely to generate detectable local Ca^{2+} release events. Barely detectable Ca^{2+} release events occasionally occur when bursts of open events (lasting many milliseconds) occur. This implies that an abnormally long opening of a single RyR channel would generate a prolonged detectable local Ca^{2+} release. Simultaneous opening of multiple RyR channels generated fluorescence signals that were consistent with the observed Ca^{2+} spark waveform. We propose that the stereotypical Ca^{2+} sparks are generated by the simultaneous opening of multiple RyR channels. This proposition is consistent with our recent estimates of unitary Ca^{2+} current, the stereotypical nature of the spark, and the clustering of RyR channels in the diadic space. We also propose that pharmacological manipulations that generate small-prolonged local Ca^{2+} fluxes could arise from the opening of single RyR channels.

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