Modelling and imaging cardiac function during excitation-contraction coupling

C. Soeller and M.B. Cannell, Department of Physiology, School of Medical Science, University of Auckland, Private Bag 92019, Auckland, New Zealand.

Cardiac excitation-contraction (E-C) coupling takes place in the narrow diadic cleft between the transverse-tubular membrane and the closely apposed terminal cisternae of the sarcoplasmic reticulum (SR). Within the cleft (which is only ~15nm high and some 100nm wide) large clusters of SR Ca^{2+} release channels or ryanodine receptors (RyRs) are located. It is now generally accepted that the opening of these clusters of RyRs underlies the elementary events of muscle activation called “calcium sparks”, brief microscopic increases in intracellular Ca^{2+}, which can be observed in heart cells loaded with fluorescent Ca^{2+} indicators such as fluo-3.

As a result of Ca^{2+} binding reactions and indicator diffusion Ca^{2+} spark records do not provide a direct measure of the time course of Ca^{2+} release. To robustly reconstruct the underlying Ca^{2+} release time course we developed novel algorithms in which a parametric Ca^{2+} spark model is fit to experimental records. Using this approach we calculated that the peak flux amplitude is ~7-12pA suggesting that at least 15 RyRs contribute to a Ca^{2+} spark. To obtain further insight into the gating of RyRs underlying Ca^{2+} sparks we constructed a detailed Monte Carlo model of RyR gating and associated Ca^{2+} movements within the diad. In this model the movement of individual Ca^{2+} ions was traced and diffusion was implemented as a random walk. RyR gating was described by a phenomenological 4-state scheme (Stern *et al.*, 1999) that included explicit inactivation. Our calculations suggest that the geometry of the diad and the RyR cluster can significantly affect the time course of release. In our spatially explicit model we observe waves of RyR openings originating at the initial site of activation. In elongated clusters of RyRs the time course of release therefore depends on the site of wave initiation while the total amount of Ca^{2+} that is released stays nearly constant.

We also explored the effect of allosteric coupling between RyRs on the gating of large RyR clusters in the model. Allosteric coupling was implemented as a nearest neighbour interaction where transition rates of receptors in the cluster were modified based on the state of adjacent RyRs. With moderate coupling our model generated a mean Ca^{2+} release time course that was similar to that reconstructed from experimental sparks. On the other hand, strong coupling resulted in increased variability and duration of the Ca^{2+} release time course.

It has been suggested that the protein FKBP12.6 may be the molecular basis of allosteric coupling between RyRs. To test this idea we recorded sparks in the presence of FK506, a drug which removes FKBP12.6 from RyRs. Analysis of our data suggests that, although the amplitude of Ca^{2+} sparks is reduced in FK506 (as compared to control sparks), the decay time and variability of Ca^{2+} sparks is only weakly changed by 50 μM FK506 which argues against a significant role of FKBP12.6 in coupling RyR gating.

We are currently extending the model to investigate other RyR gating schemes and the effect of local SR depletion on cluster gating. Our work suggests that the combination of mathematical modelling with high resolution Ca^{2+} imaging will provide valuable insight into cardiac E-C coupling.

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