

Commentary

Exploring Local Calcium Feedback: Trying to Fool Mother Nature

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The job of nature is to design robust organisms. To this end, it often employs a classical engineering strategy: provide excess functional capacity and enclose it in regulatory, preferably redundant, feedback loops that function not only at the sub-cellular level, but also at the molecular, or nanoscale, levels. The job of the physiologist is to discover the mechanisms that lie within the feedback loops. Because the properties of a well designed feedback loop are determined by its function rather than its mechanism, physiologists can expect to meet with nature's active resistance to their efforts. Both stealth and cunning are called for: stealth to get within the feedback loops and disable, or at least weaken, them, and cunning because it seldom will be possible to entirely disable a feedback loop, so the results of any weakening of the loop must be compared with quantitative models. To make matters worse, molecular-scale mechanisms are, perforce, both stochastic and nonlinear.

Nowhere are these complexities more clear than in the case of the ryanodine receptor calcium release channel (RyR). The RyR is of physiological interest, not only because it is widely distributed in many cell types throughout the animal kingdom, but also because it is capable of mediating both a positive and a negative feedback process, Ca^{2+} -induced calcium release (CICR) and calcium-dependent inactivation, respectively. Teasing apart the manifestations of these processes in muscle excitation-contraction (EC) coupling requires a close interplay between biophysical experiments and mathematical modeling. This issue of *The Journal* contains two excellent examples of the genre. Pape et al. (1998) employ and compare two different methods for weakening local calcium feedback loops: exogenous calcium buffers and controlled depletion of sarcoplasmic reticulum (SR) calcium. Using these interventions, together with their EGTA/phenol red method for measuring SR calcium release, reveals evidence of not one but three different negative feedback processes tending to stabilize the rate of calcium release from the SR. By comparing the effects of buffers with those of depletion, they infer that the distance over which calcium acts as a negative modulator of its own release is <22 nm. On a slightly larger spatial scale, Baylor and

Hollingworth (1998) revisit a "classical" problem, the modeling of calcium transport within the sarcomere, first explored by Cannell and Allen (1984). Armed with these new and better experimental results, they discover a crucial factor omitted from previous models: the role of ATP as a buffer and transporter of calcium.

Baylor and Hollingworth (1998) illustrate the importance of detail in biophysics. The conventional way to use and calibrate calcium-sensing dyes is to compare whole cell optical data with single-compartment kinetic models. Both data and the models ignore the spatial inhomogeneity of calcium concentrations and, because of the nonlinearity of reaction kinetics, the errors do not compensate. By modeling spatially resolved calcium transport in a half sarcomere, and comparing with release-flux estimates obtained with comparatively low affinity, modern dyes, the authors arrive at a glaring inconsistency about the magnitude and time course of the Ca^{2+} transient. The inconsistency is resolved by the inclusion of ATP as a calcium ligand. Usually ignored because of its low Ca^{2+} affinity, ATP is a significant calcium buffer because of its high concentration in the myoplasm. It turns out to be even more important in transporting calcium, by virtue of its diffusibility. The model (Baylor and Hollingsworth, 1998) shows that more calcium is transported as CaATP than as free Ca^{2+} . Strictly speaking, the model shows only that 5–8 mM of a diffusible buffer with certain kinetic parameters could resolve the paradox. The required characteristics are exactly those of ATP, and it is hard to doubt that ATP fulfills the role of the diffusible buffer, with obvious implications for spatially inhomogeneous calcium signaling in all cell types. There are also immediate payoffs. The additional calcium transport by ATP equalizes and synchronizes the binding of calcium to troponin C—exactly what the designer would want in order to optimize contraction. And, by a closely reasoned sequence of forward and backward model computations, using single-compartment and spatially resolved models, the authors are able to quantify the probable errors associated with a variety of calcium dyes, and to show that the intracellular kinetic parameters of, for example, fluo-3, probably should be revised—by as much as a factor of 2. It is a tribute to the

quality of our present understanding of events on the sarcomere scale that it is possible to analyze such significant, but moderate, quantitative discrepancies.

Pape et al. (1998) are fishing in murkier waters. It is clear that all types of ryanodine receptors can mediate CICR, which is the primary means of excitation-contraction coupling in cardiac and crustacean skeletal muscle. It also is clear that, in vertebrate skeletal muscle, calcium is released by a direct coupling, probably allosteric in nature, between voltage sensors and ryanodine receptors. Beyond this, the possible role of CICR in skeletal EC coupling is controversial. Under voltage clamp conditions, transient and steady components of calcium release are observed. According to one interpretation, the transient component represents a partial, calcium-dependent inactivation of a primarily voltage-controlled release. Another interpretation holds that the steady release is a primary, voltage-controlled process, while the transient component is secondary, mediated by CICR, and inactivates either fatefully or in response to the released calcium. This idea was made precise in a proposal by Ríos and Pizarro (1988), based on ultrastructural observations from Block (1988). In this model, release channels, which form a regular, rectangular, 2-dimensional lattice, alternate between voltage-controlled ("V") channels, which are allosterically coupled to voltage sensors, and calcium-controlled ("C") channels. A noninactivating gating scheme for the V channels was proposed (Ríos et al., 1993) on the basis of studies in conditioned and partially calcium-depleted muscles. This formed the basis for the "couplon" model (Stern, 1997), in which C channels located contiguously on one side of a triad junction are triggered initially by calcium released from the intercalated V channels, and then interact collectively and regeneratively by local CICR. In the original version of this model, which I will refer to as the "standard" couplon model, the C channels were represented by a phenomenological "cartoon" gating scheme, consisting of an activation gate that is opened by the simultaneous, cooperative binding of two Ca^{2+} ions to an activating site, and an inactivation gate that is closed by the binding of a single Ca^{2+} to an inactivating site. In simulations, this complex, stochastic system displays a variety of emergent macroscopic effects, often counterintuitive, that are remarkably effective in explaining diverse observed properties of skeletal EC coupling.

It would seem to be a simple matter to distinguish between competing models by interrupting the local calcium coupling. If CICR is the dominant interaction, then release, particularly the transient component, should be markedly reduced. If, on the other hand, calcium inactivates a primarily voltage-dependent release, then release, particularly the steady component, should

be enhanced. Unfortunately, studies in which calcium buffers were used to interrupt the coupling have given different results in different laboratories. Some find that the steady component is enhanced ("less calcium-dependent inactivation"), while others find that the transient component is depressed ("less CICR"). The problem is compounded by the fact that calcium buffers are incapable, for kinetic reasons, of suppressing the calcium microdomain in close proximity to the mouth of a channel (Neher, 1986; Stern, 1992; Pape et al., 1995), and that high concentrations of most calcium buffers produce often irreversible side-effects. When examined over a limited range of parameters, the standard couplon model predicts that increasing concentrations of fast, diffusible calcium buffers should suppress first the transient, and then the steady component of release—to a degree that depends on voltage, even though the V channels are not, themselves, directly regulated by calcium. This prediction is in agreement with observations in some laboratories (Ríos and Pizarro, unpublished results).

In this issue, Pape et al. (1998) use an alternative strategy to diminish calcium coupling: graded depletion of SR calcium, which is expected to produce a roughly proportional diminution of the unitary current of release channels (in addition, 20 mM EGTA was always present, as required by the measurement method). Calcium release was triggered either by action potentials or by 10-ms depolarizations to -20 mV, while SR calcium was depleted into the range of 140–1,200 μM (referred to myoplasmic water). They made the following observations: (a) fractional calcium release by action potentials is markedly increased by SR depletion. This is due to increased duration of release. (b) The prolongation of release is due partly to prolongation of the action potential, probably caused by reduction of calcium-activated K current, but prolonged release is also seen under voltage clamp conditions. (c) The peak fractional rate of release shows only a modest, biphasic variation across a 10-fold range of SR content. (d) The "off" component of gating charge movement is slowed by SR depletion. (e) Fast calcium buffer (fura-2) in concentrations of 0.5–1 mM enhances fractional release, whereas larger concentrations have little further effect, or decrease it. Interrupting calcium signalling by means of buffers did not reproduce the marked prolongation of release seen with SR depletion. Based on an extensive theoretical analysis, the authors make a strong case that fast buffers act by interrupting the component of calcium-dependent inactivation that is due to calcium from distant sources (i.e., other release channels); moreover, fast buffers in concentrations up to 6 mM are incapable of interrupting the inactivation due to calcium permeating the same release channel. From this they infer that the inactivation site must lie

within 22 nm of the pore, a distance comfortably accommodated on the foot process of a single ryanodine receptor.

On the face of it, these results favor the hypothesis of calcium inactivation of voltage-dependent release over that of CICR, and the authors end their discussion with a very good question: how could the couplon model account for the relative constancy of fractional release flux over an order of magnitude range of SR calcium content, while disallowing any regenerative spread of activation beyond the boundaries of the couplon, even at the highest calcium loads?

In point of fact, the standard couplon model, with the set of parameters given by Stern et al. (1997), does not reproduce the marked prolongation of release observed by Pape et al. (1998) at low SR calcium. This is primarily because closing of the *C* channel depends as much on deactivation as on inactivation. But the behavior of the couplon model is controlled by a dynamic interplay between the positive and negative feedback processes of the RyR. By altering the parameters to favor inactivation, both release prolongation and the biphasic variation of fractional release flux as a function of SR calcium content can be reproduced by the couplon model, as shown in Fig. 1, which should be compared with Fig. 7, A and B, of Pape et al. (1998).

Little effort was made to optimize the parameters, and only qualitative agreement should be expected when using the naive cartoon gating scheme for the *C* channels. Still, it is worth noting some of the quantitative discrepancies. First, the apparent agreement between the predicted absolute magnitude of the fractional rate of release and the experimental results is somewhat misleading. The fractional release rates in the simulation were obtained by the same method used in the experiments: the simulation was run with 20 mM EGTA in the global calcium removal model, and “fractional release rate” was determined as the rate of change of CaEGTA divided by the SR content, also calculated from the change in CaEGTA. This method severely underestimates the initial peak rate of calcium release, by a factor of about 2, and also distorts its time course, because faster, endogenous buffers (troponin, parvalbumin, pump sites) initially can compete with EGTA for the released calcium, even though 98% of it eventually ends up on EGTA. Pape et al. (1998) have very carefully studied the EGTA/phenol red method (Pape et al., 1995) and find very little evidence for such calcium recycling from endogenous buffers to EGTA. The reason for this is unclear, although a similar “excessive accuracy” of the EGTA method is observed in cardiac myocytes (Song et al., 1998). In any case, the qualitative behavior shown in the figure is not an artifact of the method, since the same pattern is seen if true fractional release rate is plotted (not shown).

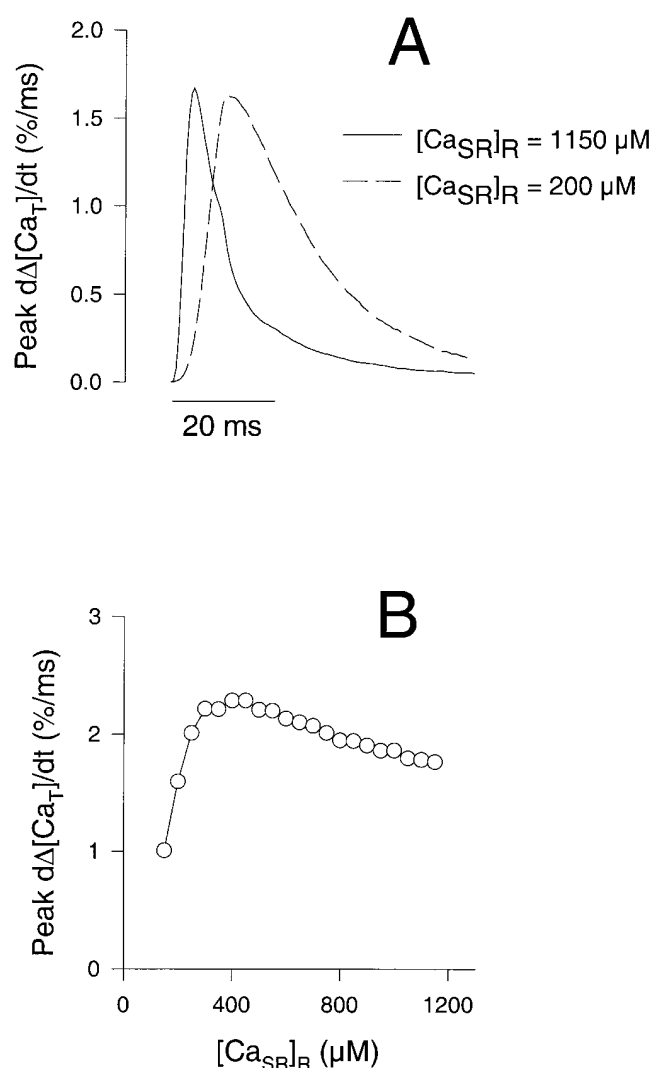


FIGURE 1.

The second discrepancy is that the initial rate of rise of fractional release flux depends on the SR load in the simulation, whereas it was observed experimentally to be constant. This discrepancy could likely be remedied by a more sophisticated gating scheme for the *C* channel, considering that the fractional release rate is a measure of the open probability of the RyR and its rate of rise depends on the speed with which RyR activation spreads through the couplon, as a function of the local $[\text{Ca}^{2+}]$.

There is one important experimental observation of Pape et al. (1998) that certainly cannot be reproduced by the standard couplon model: the dependence of gating charge movement on SR calcium content. The standard couplon model assumes, naively, that *V* channels and their voltage sensors are not directly modulated by calcium; this is unlikely to be true of any ryanodine receptor. It is not clear whether the observed slowing of the off gating charge movement produced by SR depletion is a primary cause of the prolongation of release,

or is secondary to relief of a putative calcium-inactivation of the allosterically coupled V channels. In any case, the gating charge data will force some revision of the couplon model. It is also worth bearing in mind that this and other effects of SR depletion might be mediated by a direct effect of SR luminal calcium, either free or bound to calsequestrin, on RyR gating. This is an issue that has not been addressed in either model.

At this point, it remains to be determined whether the observations of Pape et al. (1998) can be accommodated by the couplon model without sacrificing its other predictive powers. This will require a careful examination of the effects of parameter choices and refinements to the V and C channel gating schemes, a substantial amount of computation. Stay tuned.

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