Numerical Analysis of Ryanodine Receptor Activation by L-Type Channel Activity in the Cardiac Muscle Diad

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ABSTRACT Computer simulations were used to examine the response of ryanodine receptors (RyRs) to the sarcolemmal calcium influx via L-type calcium channels (DHPRs). The effects of ryanodine receptor organization, diad geometry, DHPR single-channel current, and DHPR gating were examined. In agreement with experimental findings, the simulations showed that RyRs can respond rapidly (~0.4 ms) to calcium influx via DHPRs. The responsiveness of the RyR depends on the geometrical arrangement between the RyRs and the DHPR in the diad, with wider diads being generally less responsive. When the DHPR single-channel current is small (~25 fA), the organization of RyRs into small clusters results in an improved responsiveness. With experimentally observed DHPR mean open and closed times (0.17 ms and 4 ms, respectively) it is the first opening of the DHPR that is most likely to activate the RyR. A measure of the efficiency (Q) by which DHPR gating evokes sarcoplasmic reticulum release is defined. Q is at maximum for $\tau \approx 0.3$ ms, and we interpret this finding in terms of the "tuning" of DHPR gating to RyR response. If certain cardiac myopathies are associated with a mismatch in the "tuning," then modification of DHPR gating with drugs to "retune" calcium-induced calcium release should be possible.

INTRODUCTION

Recently, cardiac excitation contraction coupling has been shown to consist of the temporal and spatial summation of elementary release events called "calcium sparks" (Cheng et al., 1993; Cannell et al., 1994, 1995). Calcium sparks are due to the activation of sarcoplasmic reticulum (SR) calcium release channels, which are organized into "functional release units" in the junctional space (Cannell et al., 1994; Isenberg and Han, 1994). The SR release channels, which have been purified and sequenced, are ryanodine receptors (RyRs) (Inui et al., 1987; Anderson et al., 1989; Otsu et al., 1990; Nakai et al., 1990). The organization of sarcolemmal L-type calcium channels (DHPRs) and RyRs forms the molecular basis of the well-known "calcium-induced calcium release" (CICR) mechanism described by Fabiato (1983). Although calcium sparks were first observed in quiescent preparations (as a result of the RyR open probability being nonzero at rest) (Cheng et al. 1993), they can also be triggered by calcium influx via sarcolemmal calcium channels (Cannell et al., 1994, 1995; Cheng et al., 1995; López-López et al., 1994, 1995; Santana et al., 1996) and it has been shown that a single DHPR opening can activate a spark (Cannell et al., 1995; Santana et al., 1996), which is central to the model presented here.

When calcium sparks are activated by trigger calcium influx, there is no measurable delay between the appearance of trigger calcium in the myoplasm and the release of calcium by the SR, showing that the CICR mechanism responds in $\ll$2 ms (Cheng et al., 1994). In reconstitution experiments, Györke and Fill (1993) showed that the activation of RyRs in response to flash photolysis of caged calcium occurs exponentially with a time constant of 1.2 ms. Although this kinetic response might seem adequate to explain the observations of Cheng et al. (1994), it is important to note that DHPRs have a mean open time of ~0.2 ms (Rose et al., 1992), which raises the question of how RyRs respond to such a brief calcium influx.

It is also unclear how many RyRs are activated by a single calcium channel, although Cannell et al. (1994) have suggested that the basic unit of E-C coupling may consist of four RyRs in a square packing array opposite a single DHPR. In any case, the small size of the calcium spark suggests that the number of RyRs in a functional release unit must be small, given the high conductance of the RyR (Cheng et al., 1993). The physical size of the RyR (~25 nm across) suggests that individual RyRs within the cluster will be exposed to different $[Ca^{2+}]$ levels during the opening of a sarcolemmal calcium channel, given previous computations of the distribution of $[Ca^{2+}]$ after a DHPR channel opening (Chad and Eckert, 1984; Simon and Linas, 1985; Stern, 1992; Langer and Peskoff, 1996; Soeller and Cannell, 1997). Therefore, a realistic model of E-C coupling must also account for the likely temporal/spatial distribution of trigger $[Ca^{2+}]$ levels within the junctional space.

In the previous paper we calculated the $[Ca^{2+}]$ levels in the junctional space that should develop after a DHPR opens. In the present study, we investigate how the open probabilities of RyRs will respond to calcium in the junctional space after the opening of an DHPR channel. In this paper we address several important questions: 1) Given the brief open time of the DHPR (~0.2 ms; Rose et al., 1992) and a simplified model for RyR gating, could a RyR or cluster of RyRs respond quickly enough (to the local increase in $[Ca^{2+}]$) to explain the activation of SR calcium

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release? 2) What is the relationship between the probability of RyR activation and the local [Ca^{2+}] when DHPR single-channel current is varied (as would occur at different membrane potentials)? 3) How does the probability of RyR cluster activation depend on the number of RyRs in a cluster? 4) How does the organization of RyRs and the DHPR in the junctional space affect the probability of release unit activation? The calculations presented here are directed toward examining the activation of RyR and do not consider what happens after RyR activation, which requires more detailed knowlege of RyR gating at high [Ca^{2+}] levels.

Our calculations show that the geometry between the DHPR and the RyR in the diad is critical in determining the RyR activation time course. However, the close apposition of the sarcolemmal (SL) and SR membranes results in the development of very high local [Ca^{2+}] when a DHPR opens, which ensures rapid activation of relatively low-affinity RyRs.

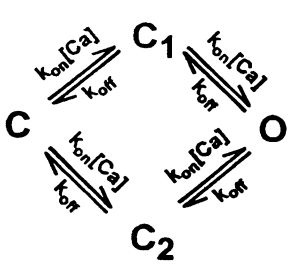
METHODS

The temporal and spatial patterns of [Ca^{2+}] changes in the diadic space after DHPR activation were calculated by using the model described by Soeller and Cannell (1997). The distance between the SR and t-tubule membranes was 15 nm, and this narrow region is referred to as the "diadic space." The differential equations describing RyR activation (see below) were added to these equations and solved simultaneously with the equations describing the calcium movements in the "full" model (which included all classes of calcium-binding sites and electric field).

RyR arrangement and gating

Information about the geometric arrangement of RyRs in the diad is required for the simulation of RyR cluster behavior (see below). In our simulations, RyRs were located on a regular square grid with a unit cell size of 31 × 31 nm, as reported in electron microscopic studies of the "foot" structures (Radermacher et al., 1994; Saito et al., 1988).

The primary goal of these computations was to examine the response of ryanodine receptors to the likely changes in [Ca^{2+}] across the diadic space. From published values for the sensitivity of RyR gating to calcium (see below), we suggest that the sensing site lies outside the Debye layer(s). Tests showed that placing the RyR calcium sensing site between 3 and 12 nm above the SR membrane had only a minor effect when compared to other parameters (e.g., radial position; see Fig. 2 *A*). In view of this insensitivity, we assume that the sensing sites were 4 nm above the SR membrane (with the exception of Fig. 2 *A*). The RyR on rate was assumed to be diffusion-limited at $1.25 \times 10^8\ M^{-1}\ s^{-1}$, and the off rate constant was chosen to obtain half-maximum activation at 0.6 μM with a Hill coefficient of 2 (i.e., $k_{off} = 75\ s^{-1}$) in the following model:



(1)

where C represents RyRs with both calcium binding sites unoccupied, C1 and C2 are closed states with a single bound calcium ion, and O is the open-channel state with two calcium ions bound. We modeled the two sites C1 and C2 as completely equivalent with regard to their kinetics and to the [Ca^{2+}] levels experienced by those sites, so that these two states can be represented by a single species C* with concentration [C*] = [C1] + [C2]. Consideration of the differential equations describing the kinetics of the above model (Eq. 1) leads to a simpler equivalent model with changed rate constants:

$$C \underset{k_{off}}{\overset{2k_{on}[Ca]}{\rightleftharpoons}} C^* \underset{2k_{off}}{\overset{k_{on}[Ca]}{\rightleftharpoons}} O \qquad (2)$$

With this model at 60 nM [Ca^{2+}], we would expect a mean open time of 6 ms and a mean closed time of 800 ms. These values are similar to those recorded in some bilayer reconstitution experiments (e.g., Rousseau and Meissner, 1989). Furthermore, the rise time for a sudden increase in [Ca^{2+}] from 0.1 to 1 μM would be 3.6 ms, which, although slightly slower than the value measured in flash photolysis experiments (Györke and Fill, 1993), is not unreasonable, given the problem of short-term overshoots in calcium occurring during flash photolysis. It has recently been reported that RyR gating may be modal, with calcium-dependent changes in open probability being due to the calcium dependence of the mode occurrence (Zahradníková and Zahradnik, 1995). Zahradníková and Zahradnik (1996) suggest that calcium-dependent activation may be associated with a rapid transition to "H" mode, and inspection of the gating parameters of that mode suggests the most likely closed times of 0.38 and 3.27 ms (observed 34% and 48% of the time, respectively), giving a mean closed time of 1.7 ms at 10 μM [Ca^{2+}] (we ignore the less frequent closed time of 59.1 ms measured by Zahradnikova and Zahradnik (1995, table 4), as it may represent a brief sojourn in the "I" mode). Our model has a mean closed time of 0.83 ms at 10 μM [Ca^{2+}], which therefore represents a compromise between the times expected from the two bilayer studies described above. Given the widely varying estimates of open and closed times for any given [Ca^{2+}] within the literature, a more complicated model for RyR gating was felt to be unwarranted, and the kinetic parameters used here to be a reasonable first-order approximation to RyR gating behavior. It should be noted that, for this study, only the response of the receptors to fast changes in [Ca^{2+}] (on the millisecond time scale) is of interest. Phenomena such as adaptation develop with a time constant on the order of 0.1–1 s (Györke and Fill, 1993; Valdivia et al., 1995) and so can be ignored for the first few milliseconds after the L-type channel opening. Furthermore, the relatively long close time of the L-type channel (~4 ms; Rose et al., 1992) implies that the local [Ca^{2+}] levels decay between successive openings to near-resting levels (see Soeller and Cannell, 1997), so it was felt unnecessary for the purpose of this study to include "desensitization" phenomena in the model of the RyR receptor activation behavior.

It is well known that receptor activation is a stochastic process that must be described by probabilistic methods. The open probability for any time after the start of release can be calculated using the known rate constants in Eq. 2. Because the on rates are, in this case, time dependent, there is no closed solution to the open probability (unlike that for constant transition rates; Colquhoun and Hawkes, 1977). Instead, the differential equations for the open probability $P_{o,T}$ of a RyR (at a given location in the diad) resulting from an DHPR opening of duration T must be solved numerically using the known time course of the effective on rates (Cox and Miller, 1965, p. 181). This leads to

$$\frac{dC}{dt} = -2k_{on}[Ca]_T C + k_{off} C^* \qquad (3)$$

$$\frac{dC^*}{dt} = 2k_{on}[Ca]_T C - k_{off} C^* - k_{on}[Ca]_T C^* + 2k_{off} P_{o,T} \qquad (4)$$

$$\frac{dP_{o,T}}{dt} = k_{on}[Ca]_T C^* - 2k_{off} P_{o,T} \qquad (5)$$

where $[Ca]_T(t)$ is the calculated time course of $[Ca^{2+}]$ at the RyR-sensing site resulting from a DHPR opening of duration T (see Methods, Soeller and Cannell, 1997). Initially, all species were at their equilibrium values at the resting $[Ca^{2+}]$ level (0.1 μM) and subject to the normalization condition

$$C + C^* + P_{o,T} = 1 \tag{6}$$

Whereas the open probability is a measure of the total fraction of open SR release channels found on average at any time, the activation of the RyRs due to sarcolemmal influx can be conveniently characterized by calculating what we call the activation probability $P_{a,T}$ in response to a DHPR calcium influx of duration T. To this end we consider the "absorbing" system derived from Eq. 2 in which the open state is made a trapping state by setting its off rate to zero (in direct analogy to the calculation of closed state lifetimes for fixed transition rates; Colquhoun and Hawkes, 1977; Cox and Miller, 1965, p. 196). The system of differential equations for this probability $P_{a,T}$ is the same as Eqs. 3–5, except that the off rate in the second step of Eq. 2 is zero, to yield

$$\frac{dC^*}{dt} = 2k_{on}[Ca]_T C - k_{off}C^* - k_{on}[Ca]_T C^* \tag{4a}$$

$$\frac{dP_{a,T}}{dt} = k_{on}[Ca]_T C^* \tag{5a}$$

Here the initial conditions are $P_{a,T}(0) = 0$ and C, C^* have relative equilibrium occupancy at resting $[Ca^{2+}]$. Therefore, $P_{a,T}(t)$ can be interpreted as the probability that a RyR that was in a closed state at the start of DHPR calcium influx (which will be of duration T) will have switched to the open state at a time $\leq t$. Furthermore, as $P_{a,T}$ is a cumulative probability that approaches 1 as time increases, we can calculate the corresponding probability distribution function (pdf) $f_{a,T}$, which we call the RyR activation time distribution, as

$$f_{a,T}(t) = \frac{dP_{a,T}}{dt} \tag{7}$$

which gives the probability for activation in the period between t and $t + dt$ as $f_{a,T}(t)dt$. When the duration (T) of DHPR calcium influx is clear from the context (or unimportant), the suffix T will be omitted for clarity, i.e., we write P_a and f_a. The system of Eqs. 3, 4a, and 5a was integrated numerically during solution of the diffusion/reaction scheme analyzed in the previous manuscript (Soeller and Cannell, 1997). The behavior of these probabilities as a function of duration and magnitude of DHPR influx and diad geometry was taken to be a suitable measure of the efficiency with which calcium entry into the diadic space activates release from the SR.

Two limiting cases (in terms of fixed DHPR open time) of special interest are $P_{a,0}(t)$ (i.e., no DHPR calcium influx at all), which represents the "reference case" without L-type channel activity, and $P_{a,\infty}(t)$ (i.e., continuous calcium influx through the DHPR). It is clear from causality and inspection of Eqs. 3, 4a, and 5a that the following relations hold for any given $P_{a,T}(t)$, $f_{a,T}(t)$:

$$P_{a,0}(t) \leq P_{a,T}(t) \leq P_{a,\infty}(t)$$

$$\left.\begin{aligned} P_{a,T}(t) &= P_{a,\infty}(t) \\ f_{a,T}(t) &= f_{a,\infty}(t) \end{aligned}\right\} t \leq T \tag{8}$$

which we note for later use.

Monte Carlo simulations

Monte Carlo simulations of the activation properties of RyR channels were performed, assuming monoexponential open and closed time distributions for DHPR gating.

During integration, DHPR transition times were calculated according to

$$t_{n+1} = t_n - \frac{\ln R}{k} \tag{9}$$

where R is a random number between 0 and 1, k is the rate constant for leaving the current state, t_n is the time of the last transition, and t_{n+1} is the time of the next transition. When the next transition time was reached, the DHPR calcium flux was switched off (or on) and the integrator restarted. The computational time penalty associated with this procedure was extremely large, typically increasing the integration time in almost direct proportion to the number of DHPR state transitions.

RESULTS

Time course of the RyR response

Fig. 1 shows how the RyR activation probability (P_a) and the RyR activation time distribution (f_a) change as a function of time in response to a 0.2-pA calcium influx lasting for periods (T) of 0.1 and 0.3 ms and for the duration of the plot (10 ms). The calcium-sensing sites on the RyR were assumed to be 4 nm above the SR membrane, and the RyR was placed at $r = 0$ (directly opposite the DHPR). After calcium influx started, P_a increased to significant values after ~0.1 ms (Fig. 1 *A*). As soon as the influx was stopped, the rate of rise of P_a declined in ~0.4 ms to the small value observed at resting $[Ca^{2+}]$ levels. This important result is shown more clearly in Fig. 1 *B*, where f_a is plotted for the same DHPR opening times. It is notable that the maximum RyR opening rate (for any L-type channel opening $\geq$ 0.13 ms) occurs at ~0.13 ms, which is close to the measured mean open time of the DHPR of 0.17 ms at −10 mV (Rose et al., 1992). After that time, the activation rate decays as the cumulative P_a approaches unity at ~1 ms. In this case (a single receptor immediately opposite the DHPR mouth), P_a is close to 1 after 1 ms, so even longer DHPR openings would simply increase net calcium influx into the cell without significantly altering the RyR response.

Fig. 1 *C* shows the effect of a twofold increase in the rate of calcium binding and unbinding by the RyR on P_a (with the same K_d). As might be expected, the increase in the kinetics of calcium binding resulted in a higher P_a (0.98 compared to 0.8) by the time of DHPR closure at 0.3 ms. Fig. 1 *D* shows that f_a peaks earlier (0.07 ms compared to 0.13 ms). The peak f_a is less than twice as large, although the on rate was doubled. This indicates that RyR calcium binding is not the only rate-limiting process present, but is also influenced by the time course of rise of $[Ca^{2+}]$. f_a initially declines more rapidly after the peak, an effect due to the higher levels of P_a being reached and the development of significant saturation effects in RyR calcium binding. However, after DHPR closure, the increased rate of calcium binding causes f_a to be larger as the RyR responds to the "tail" of elevated $[Ca^{2+}]$ that persists in the diad (Fig. 1 *C*, *dotted curve*).

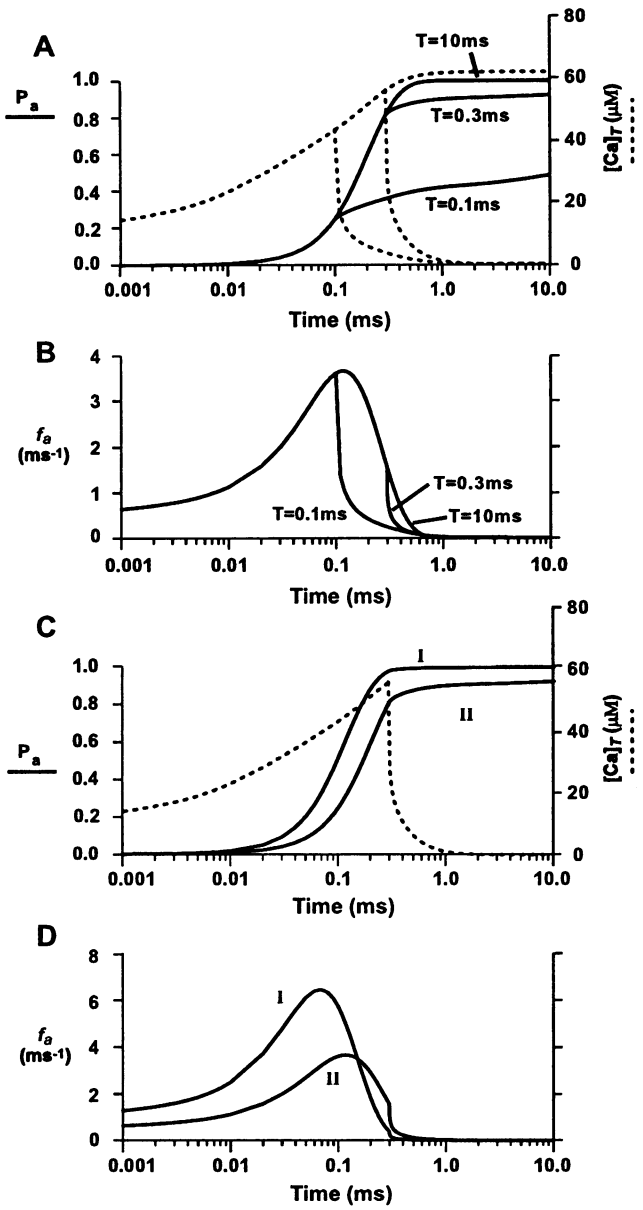


FIGURE 1 RyR activation probability (P_a) and activation time distribution (f_a) in response to a 0.2-pA DHPR calcium influx of fixed duration (T). (*A*) P_a (——) for a single RyR that was calculated from the time course of $[Ca^{2+}]$ at the center of the diad and 4 nm from the SR membrane (– – –). Curves show the time course of P_a resulting from DHPR openings of 0.1 ms, 0.3 ms, and 10 ms in a diad cleft 100 nm across and 15 nm high. (*B*) Activation time distribution f_a corresponding to the probabilities shown in *A*. Note that the opening rate is at maximum at ~0.13 ms after the DHPR opened. (*C*) The effects of a twofold increase in the on and off rates of RyR calcium binding (for the same K_d) on P_a are shown. Curve I corresponds to $k_{on} = 1.25 \times 10^8$ M^{-1} s^{-1}, $k_{off} = 75$ s^{-1}. For curve II, $k_{on} = 2.5 \times 10^8$ M^{-1} s^{-1}, $k_{off} = 150$ s^{-1}. Both sets of curves were calculated for a DHPR calcium influx of 0.2 pA and 0.3 ms duration. The dotted curve shows the changes in $[Ca^{2+}]$ at the RyR calcium-sensing site. (*D*) Corresponding activation time distribution f_a for the two sets of rate constants.

Effect of geometry in the diad

Fig. 2 examines P_a as a function of time for RyRs at various positions across the diadic space. Fig. 2 *A* shows peak P_a for DHPR open times of 0.1, 0.3, and 1 ms after DHPR opening for the RyR-sensing sites at varying positions across the diad. Increasing the height of the RyR calcium sensing site (z) had little effect on P_a, except when the sensing site was within 10 nm of the DHPR mouth. In contrast, the radial distance from the DHPR profoundly affected P_a; for example, after 0.3 ms P_a decreased from 0.9 to 0.2 from the center to the edge of a 100-nm diad. The lack of sensitivity of P_a to height of the RyR-sensing site was due to the relatively small gradients of calcium levels in that direction (see Soeller and Cannell, 1997). For simplicity, all subsequent simulations will be carried out for the RyR-sensing sites 4 nm above the SR membrane.

Fig. 2 *B* shows that the time course of P_a is progressively delayed with increasing distance from the DHPR. This result is as expected from the variation in the amplitude and time course of the $[Ca^{2+}]$ transient with position across the diad (as shown in the previous paper; Soeller and Cannell, manuscript submitted for publication). Nevertheless, when the DHPR opening is longer than ~3 ms, P_a approaches 1, even when the RyR is located at the edge of the diad. Fig. 2 *C* shows the time course of P_a when the duration of DHPR calcium influx was limited to 0.3 ms. With such a shorter period of influx the peak P_a was also determined by radial position. P_a is close to 1 only for RyR calcium-sensing sites very close to the DHPR mouth ($r = 2.5$ nm). In comparison, P_a reaches a peak value of only 0.3 at the edge of the diad ($r = 45$ nm), even after 10 ms (9.7 ms after DHPR closure).

We have shown previously (Soeller and Cannell, manuscript submitted for publication) that the time course of $[Ca^{2+}]$ is influenced by the diameter of the diad, with $[Ca^{2+}]$ declining more slowly in a wider diad after DHPR closure. Fig. 3 shows the time course of P_a for RyRs at varying distances from the centers of 100-nm and 400-nm diads for a DHPR open time of 0.3 ms. For a RyR at the center of the diad, increasing the diameter of the diad slightly decreases P_a at all times until DHPR closure, but subsequently results in a higher P_a. This effect can be seen most clearly for RyRs placed some distance from the center of the diad. For example, at $r = 45$ nm in the 100-nm diad, P_a after 2 ms is ~0.25, whereas in the 400-nm diad, P_a is 0.32 and continues to increase at almost the same rate for a further 5 ms. It is clear that the longer lasting elevation in $[Ca^{2+}]$ associated with the wider diad results in a marked increase in P_a long after DHPR closure. However, the wider diad does not significantly increase the P_a of a RyR in the center of the cleft. These effects can be summarized by considering that the main effect of increasing diad diameter is to insulate RyR gating from DHPR closure (see below).

Effect of RyR clustering

From electron micrographs it is apparent that there are several RyRs in each diad (e.g., Sommer and Waugh, 1976; Forbes and Sperelakis, 1982), and from the previous figures it is clear that the RyRs will have different responses at

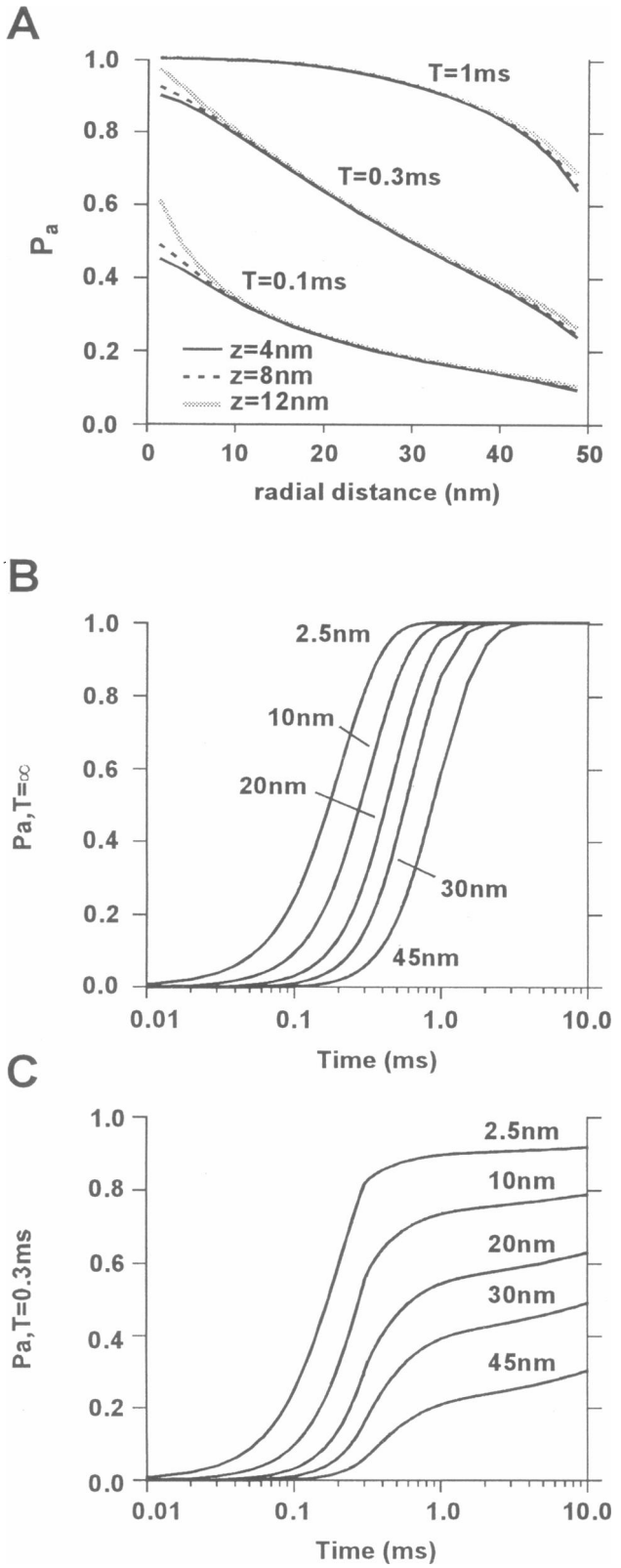


FIGURE 2 P_a of RyRs as a function of time and position in a diad 100 nm across. (*A*) Values of P_a 1 ms after calcium influx started as a function of position within the diad. The three groups of curves show the responses to calcium influx lasting 0.1 ms, 0.3 ms, and 1.0 ms. Within each group, the RyR calcium-sensing site was placed at 4, 8, and 12 nm above the SR membrane. Note that P_a is much more sensitive to radial position than height (except when the sensing site is within 10 nm of the DHPR). (*B*) Time course of P_a for RyRs at 2.5, 10, 20, 30, and 45 nm from the DHPR when the calcium influx was continuous. The calcium influx producing these changes was equivalent to a DHPR current of 0.2 pA. (*C*) Effect of turning off the influx after 0.3 ms. The activation probabilities are shown for RyRs at the same locations and calcium influx as in *B*.

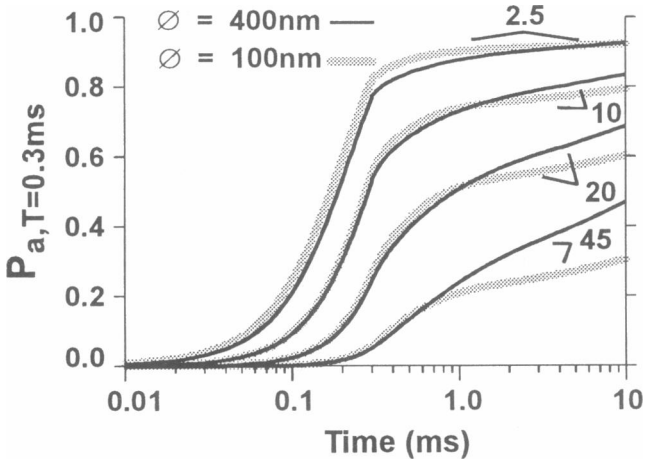


FIGURE 3 Effect of diad diameter on the time course of P_a. The gray curves correspond to the data shown in Fig. 2 *B* and were computed for a diad 100 nm across. The black curves correspond to a diad 400 nm across. Note that the wider diad results in slightly reduced P_a at 0.3 ms (when the 0.2-pA DHPR influx was terminated) in the center of the diad, but P_a continued to increase long after the influx was terminated (especially at the edge of the diad).

different positions within the diad. For a DHPR opening of duration T, the cluster activation probability ($P_{C,T}$) for n RyRs was calculated from the equation

$$P_{C,T}(t) = 1 - (1 - P_{a,T}(r_1, t)) \cdot (1 - P_{a,T}(r_2, t)) \cdot \ldots \cdot (1 - P_{a,T}(r_n, t)) \quad (10)$$

where $P_{a,T}(r_i, t)$ is $P_{a,T}$ of the RyR number i at a distance r_i from the DHPR. $P_{C,T}$ is the probability of activating at least one RyR within the cluster and therefore represents the probability of evoking SR calcium release. Fig. 4 *A* shows how the open probability of a cluster depends on the number of RyRs making up the cluster. $P_{C,T}$ is shown for clusters of different sizes and a DHPR open time of 0.3 ms in a 100-nm diad, in which the individual RyRs were placed in a close-packed square array (the arrangements of the RyRs forming each cluster are sketched in the figure). Fig. 4 *B* shows the activation time distribution ($f_{C,T}$) for these clusters. For clusters of RyRs with two, four, and eight members, in which there is no RyR immediately opposite the DHPR, P_C and f_C start to rise to significant levels ~0.05 ms later than in clusters where a RyR is directly opposite the DHPR (one and nine members). It is notable that a cluster of two RyRs has a lower P_C than a single RyR, again emphasizing the importance of the geometric arrangement of the RyR cluster. The four- and eight-member clusters increase P_C from

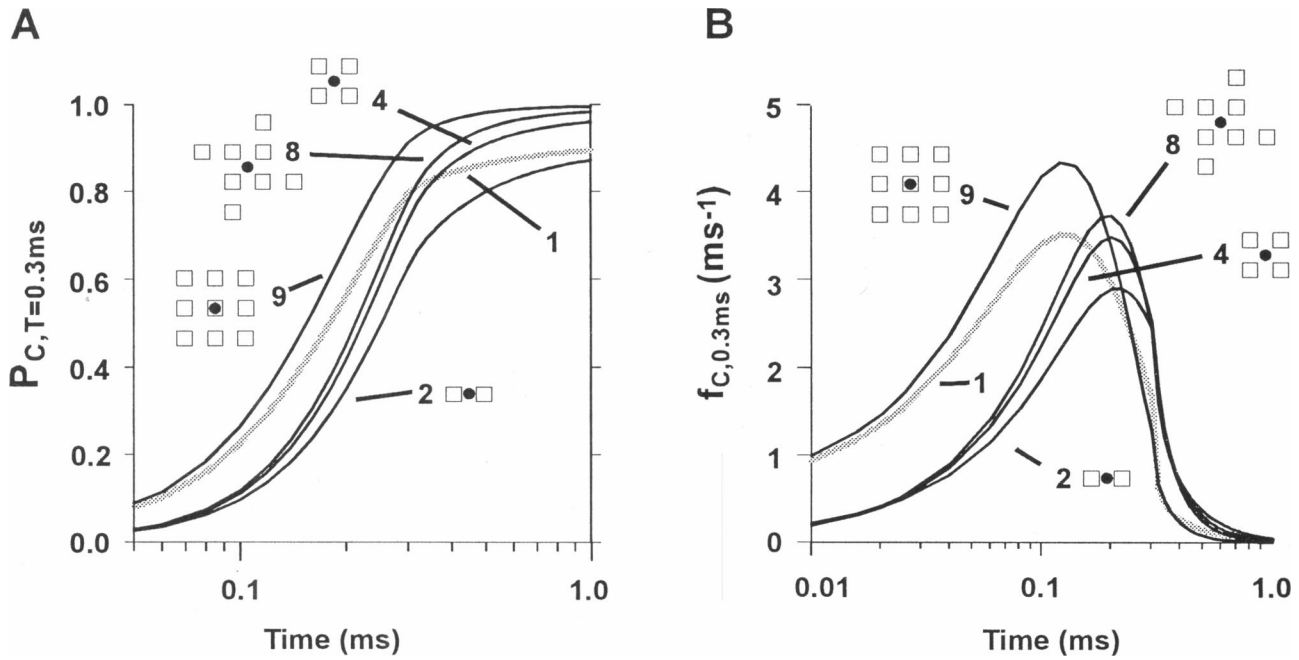


FIGURE 4 Cluster activation probability (P_C) and activation time distribution (f_C) for RyR clusters with various numbers of RyRs. (*A*) P_C is shown for clusters consisting of two, four, eight, and nine RyRs in response to a DHPR calcium influx (0.2 pA) of 0.3 ms duration into a diad of 100 nm diameter. The radial arrangement of RyRs (□) around the central DHPR (●) is indicated for each curve. Compare the responses to the gray curve showing P_a for a single RyR in the center of the diad. (*B*) Corresponding time course of f_C for those clusters in comparison to f_a for a single RyR (*gray curve*).

0.89 (single RyR) to 0.95 and 0.97 after 1 ms, respectively. The nine-member cluster reaches a substantial P_C within 0.1 ms and a $P_C > 0.98$ after 1 ms. From Fig. 4 *B* it is clear that this arrangement has the highest activation rate among those clusters shown, i.e., the steepest increase in probability with time. For a diad 100 nm across, it was not possible to increase the number of RyRs in a cluster beyond nine, because the outermost RyRs were already at the edge of the diad.

Dependence on DHPR current

We have shown that $[Ca^{2+}]$ levels in the diad have a nearly linear dependence on the amplitude of the calcium influx via the DHPR (Soeller and Cannell, manuscript submitted for publication). It is therefore not surprising that P_a also depends on the amplitude of the calcium influx, with larger currents producing higher open probabilities for clusters and single RyRs (Fig. 5 *A*). However, it should be noted that the shape of the time dependence of P_a changes with DHPR current amplitude. As the current decreases, the effect of the resting $[Ca^{2+}]$ level on the time dependence of P_a becomes more significant as the local $[Ca^{2+}]$ levels associated with DHPR opening become smaller (an effect that is more pronounced for clusters; Fig. 5 *A*, *dotted lines*). Reducing the DHPR calcium influx also slowed the time course of rise of P_a, but this effect was much smaller than the effect on the level of P_a reached at a given time. Therefore, reducing the DHPR calcium influx had a much smaller effect on the latency for calcium release (after the DHPR opens) than on the probability of release. In connection with this point, it has been shown that the latency for calcium release appears to closely follow the latency to the first opening of the DHPR at different potentials (Isenberg and Han, 1994).

An unexpected result of changing the amplitude of the DHPR calcium influx is shown in Fig. 5 *B*. In this figure we examine the relative behavior of a cluster of four RyRs compared to a single RyR as the DHPR current is varied from 25 fA to 200 fA. At short times after DHPR opening (0–0.4 ms), P_C/P_a is almost constant with current amplitude and less than 1.0. The probability of cluster activation is lower than for a single RyR because of the geometric effects described earlier (Fig. 4). However, at later times (>0.4 ms; see Fig. 5 *A*), P_C/P_a becomes greater than 1.0 and larger for smaller DHPR currents. So, for smaller DHPR currents, placing the RyRs in clusters carries a benefit in terms of increasing the probability of SR activation after ~0.3 ms of calcium influx.

Ensemble behavior

Although we have examined the response of RyRs to constant DHPR open times, in the intact cell DHPRs have exponentially distributed open times. The statistical properties of the activation process can be estimated from the convolution of the activation probabilities ($P_{a,T}$ or $P_{C,T}$) and the pdf of the DHPR open times. Assuming that the DHPR open times have a monoexponential pdf with mean open time τ, the ensemble activation probability $P_\tau(t)$ of the

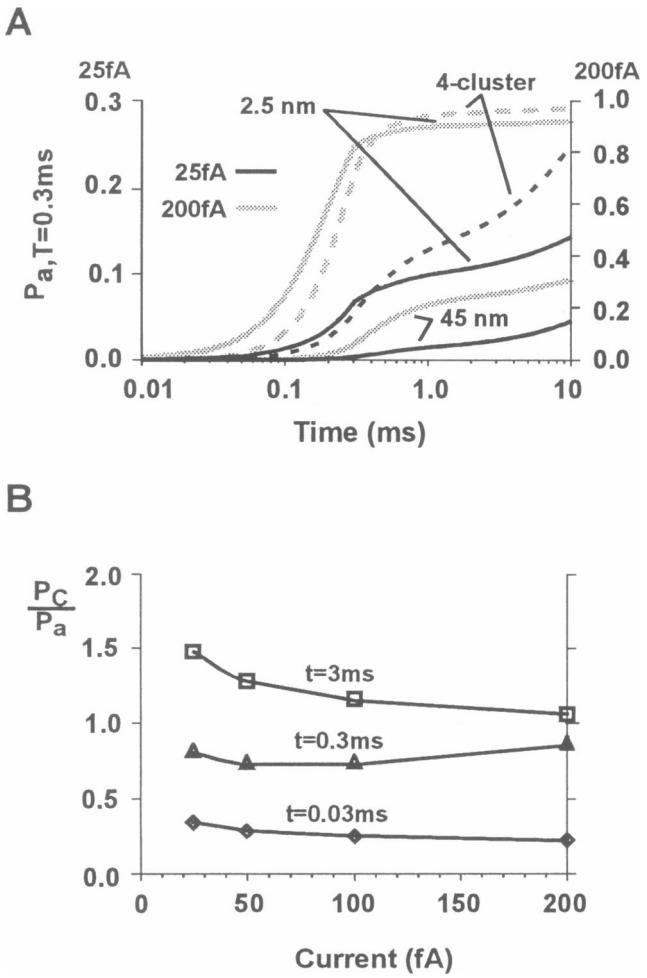


FIGURE 5 Effect of DHPR current amplitude on P_a for a single RyR and P_C for cluster of 4 RyRs. (*A*) Time course of P_a for single RyRs at the center (at 2.5 nm) and edge (at 45 nm) of a 100-nm diad. The DHPR calcium influx lasted for 0.3 ms. P_a for an influx of 200 fA is shown in gray and calibrated on the right axis (and is shown in Fig. 2 *B*), and the data for 25-fA influx is in black and calibrated on the left axis. The P_C of a cluster of four RyRs in response to the same influxes is also shown (*dashed curves*). Note the different scales for the two values of influx. (*B*) Relative behavior of a cluster of four RyRs to a single RyR. This graph shows the ratio P_C/P_a as a function of DHPR influx amplitude at different times after the start of DHPR calcium influx. It is notable that the relative benefit of placing RyRs in a cluster increases with decreasing influx amplitude and at later times (≥0.4 ms) after the DHPR opens.

DHPR-RyR system for activation times $\leq t$ is

$$P_\tau(t) = \int_0^\infty \frac{1}{\tau} e^{-s/\tau} P_{\text{a or C,s}}(t)\, ds \tag{11}$$

The integration interval can be split using the relations given in Eq. 8 to give

$$P_\tau(t) = \int_0^t \frac{1}{\tau} e^{-s/\tau} P_{\text{a or C,s}}(t)\, ds + e^{-t/\tau} P_{\text{a or C},\infty(t)} \tag{12}$$

and similarly, the ensemble pdf is

$$f_\tau(t) = \int_0^t \frac{1}{\tau} e^{-s/\tau} f_{\text{a or C,s}}(t)\, ds + e^{-t/\tau} f_{\text{a or C},\infty}(t) \tag{13}$$

In this approximation, the latency to first DHPR opening and the possibility of subsequent openings have been neglected (see below). Nevertheless, it seems reasonable that the above should estimate P_τ and f_τ for $t \leq \tau$ if $\tau \lll \tau_c$ (the DHPR mean closed time) and for $\tau \gg \tau_c$, because $P_\tau \to 1$. This simple method has the benefit that it gives an estimate of the ensemble behavior of RyRs (for any given τ and arrangement of RyRs) without having to resort to computationally expensive Monte Carlo methods.

Fig. 6 *A* shows the time courses of $P_\tau(t)$ derived from Eq. 12 for a cluster of four RyRs. For comparison, the time course of P_C for constant DHPR open times is shown in Fig. 6 *B*. From these figures, we see that $P_\tau(t) < P_{C,T=\tau}$, although for short τ (≤0.03 ms) the difference is not large. This effect arises from saturation of RyR calcium binding, so that long DHPR openings do not increase the average P_C in proportion to their duration. The curves also show that for mean open times > 0.1 ms, the exponential distribution of open times (Fig. 6 *A*) leads to a greater sensitivity to the mean open time. Although increasing the DHPR open time generally increases P_τ, the sigmoidal nature of the relationship arises from the finite rate of rise of $[Ca^{2+}]$ to steady state in the diadic space. This leads to a lower P_τ for short open times, whereas at long DHPR open times, the relationship flattens as $P_\tau \to 1$.

Although it would be desirable to define a single measure for the efficiency with which the DHPR calcium influx triggers RyR opening, this is not straightforward when E-C coupling results from two stochastic processes (DHPR and RyR gating) that are not linked linearly (Cannell et al., 1994). Here we introduce a "quality" measure (Q) for the coupling between the DHPR opening and RyR response as a function of the DHPR mean open time (τ). As a convenient measure of Q we propose that the ratio of P_τ (after one mean open time) to the average amount of calcium that entered through the DHPR (Ca_τ) be used. As an equation:

$$Q(\tau) = \frac{P_\tau(\tau)}{Ca_\tau} \tag{14}$$

This measure will decrease for excessive calcium influx as $P_\tau \leq 1$. Noting that the average amount of Ca_τ entering the cell is

$$Ca_\tau = \int_0^\infty \frac{1}{\tau} e^{-s/\tau} s \cdot J_{Ca}\, ds = \tau J_{Ca} \tag{15}$$

we obtain

$$Q(\tau) = \frac{1}{J_{Ca}} \frac{P_\tau(\tau)}{\tau} \tag{16}$$

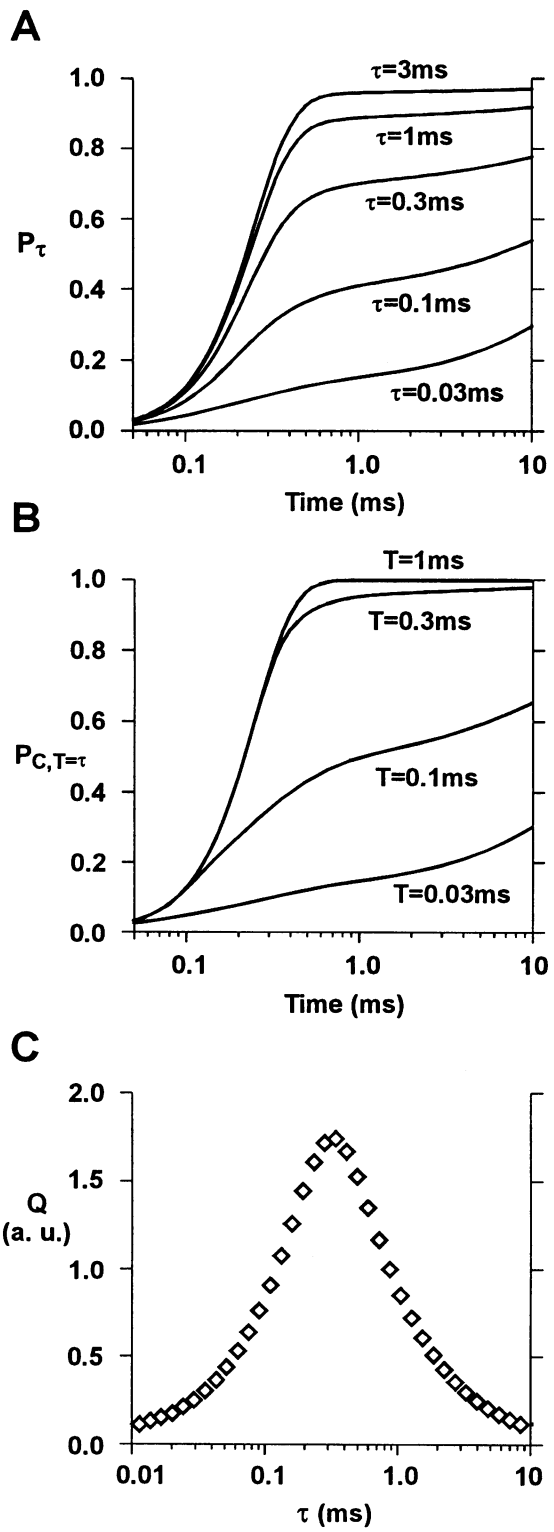


FIGURE 6 Dependence of ensemble activation probability P_τ of the DHPR-RyR system on DHPR mean open time (τ). (*A*) Time course of P_τ for a cluster of four RyRs with τ between 30 μs and 3 ms (diad diameter 100 nm, DHPR calcium current 0.2 pA). For comparison, *B* shows the corresponding time courses of P_C for fixed DHPR open times (rather than exponentially distributed open times, as shown in *A*). (*C*) The Q factor (as defined by Eq. 16) as a function of τ. It is notable that this measure of the "quality" of DHPR-RyR coupling has a maximum at ~0.3 ms, which is similar to experimentally observed mean open times (see text).

where J_{Ca} denotes the calcium flux through the open DHPR (which will, of course, be determined by the experimental conditions). This Q factor can be thought of as the "efficiency" with which a given calcium influx activates release, given a fixed (or known) pdf for calcium influx.

Fig. 6 *C* shows the variation in Q for DHPR mean open times between 0.01 and 10 ms for a cluster of four RyRs. It is notable that Q shows a strong dependence on DHPR open time, increasing by 50% for DHPR mean open times between 0.1 and 1 ms. Because Q has a peaked dependence on mean open time, it follows that there is an optimal mean DHPR open time for activating the RyR cluster. The model suggests that Q is at maximum for DHPR mean open times of ~0.3 ms, which is close to experimentally measured DHPR mean open times.

Although the simplified analysis presented in Fig. 6 can be used to give new insight into the matching of the DHPR gating to the responsiveness of the RyR (see Discussion), the ensemble behavior of the diad will actually depend on the full stochastic behavior of DHPR gating. Put another way, the closed times will also influence P_τ, and there is no direct relationship between any particular DHPR mean open time (τ_o) and closed time (τ_c) (although on average $P_{o,\,DHPR} = \tau_o/\tau_o + \tau_c$). To examine this point, we carried out Monte Carlo simulations of DHPR gating for a mean open time of 0.17 ms and a mean closed time of 4 ms (Rose et al., 1992) while calculating the resulting behavior of a cluster of four RyRs. The calculations provide an estimate of the expected latency to first opening of an RyR in the cluster after the first opening of the DHPR. Fig. 7 *A* shows sample records of the gating of the DHPR, $[Ca^{2+}]$ at the calcium-sensing sites, and P_C of the RyR cluster. Inspection of the sample data in Fig. 7 *A* shows that for $\tau_c = 4$ ms, P_C for the cluster is determined primarily by the τ_o of the DHPR. In fact, P_C appears to reflect the integral of the DHPR current, with significant changes in P_C only occurring during the opening of the DHPR. This observation is supported by Fig. 7 *B*, where the time course of activation probability $P_{\tau,MC}$ computed from 300 simulation runs is shown and compared with the computed P_τ (calculated from Eq. 12 with $\tau = 0.17$ ms) that neglects the contribution of multiple openings. The good agreement for $t < 0.6$ ms demonstrates that the first opening dominates the stochastic behavior of the activation process (as suggested by Cannell et al., 1994, and Isenberg and Han, 1994). However, at times longer than ~0.6 ms, $P_{\tau,MC}$ calculated from the Monte Carlo simulation rises more steeply than P_τ (e.g., $P_{\tau,MC} = 0.9$ compared with $P_\tau = 0.66$ after 10 ms), showing that multiple openings make a significant contribution at later times. Finally, the Monte Carlo simulations predicted that 50% of clusters would be activated within ~0.4 ms, which is in general agreement with the idea that it is the latency to first opening of the DHPR that determines activation latency rather than the latency of the RyR response per se (Cheng et al., 1994; Cannell et al., 1994; Isenberg and Han, 1994).

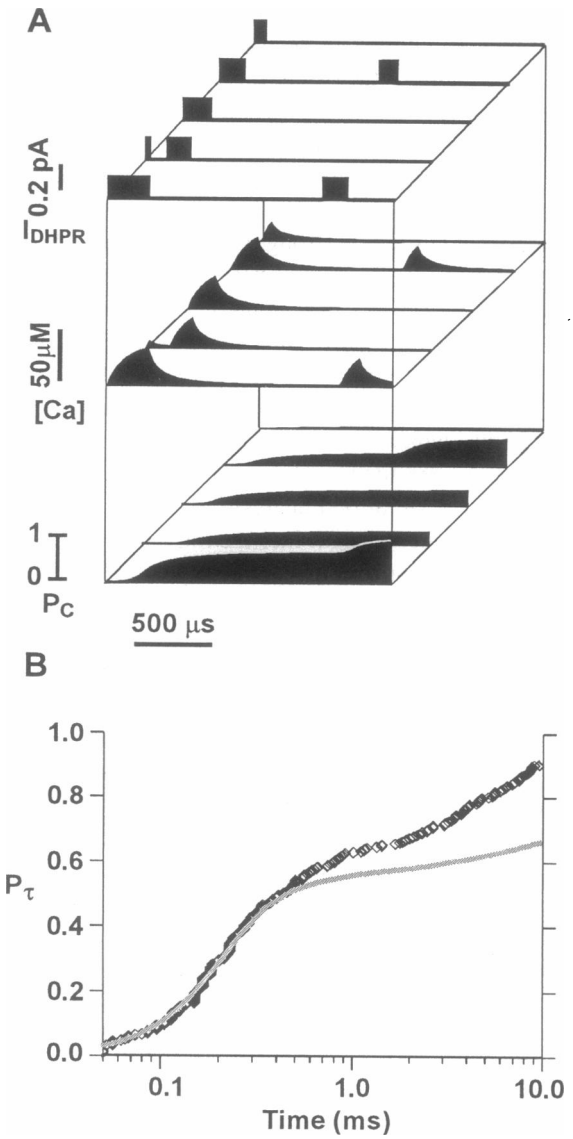


FIGURE 7 Monte Carlo simulation of the interaction between the DHPR and a cluster of four RyRs. (*A*) Model records of DHPR gating (*upper panel*, calcium current 0.2 pA, τ_{open} = 0.17 ms, τ_{closed} = 4 ms), the resulting [Ca^{2+}] changes at the RyR calcium-sensing sites (*middle panel*), and P_C of the cluster (*lower panel*). The [Ca^{2+}] time course was calculated for a diad 100 nm across. (*B*) Ensemble time course of activation probability $P_{\tau,MC}$ ($\diamond$) computed from 300 simulation runs. The solid line shows P_τ calculated from Eq. 12 with τ = 0.17 ms. Note the reasonable agreement between both curves for times greater than or equal to 0.5 ms.

DISCUSSION

Although the modeling presented here is certainly a simplification of the true nature of cardiac CICR, it has given useful insight into the interaction between DHPRs and RyRs. These calculations indicate that the geometry of the diadic space has a profound effect on the ability of the calcium flux through a DHPR to activate SR calcium release. The narrow diadic space ensures that high [Ca^{2+}] levels develop near the SR membrane within a few microseconds. Such high [Ca^{2+}] levels lead to a rapid rate of binding of calcium to the RyR calcium-sensing sites, thereby reducing the latency between DHPR opening and SR calcium release. This allows CICR to respond rapidly to the DHPR activation and may help explain why such a structure has evolved.

The modeling presented here can be viewed as a test of our current understanding about how cardiac CICR is achieved. It is therefore encouraging that the model predicts significant increases in the P_a of RyR on the time scale over which E-C coupling is observed. However, more detailed knowledge about RyR gating will be needed before a more complete description of E-C coupling can be achieved. Nevertheless, the model has shown that there is an optimal DHPR gating pattern for the activation of CICR, and this concept should be retained in future models.

Efficiency of the coupling between the DHPR and RyR

As shown in Fig. 6, increasing the mean open time of the DHPR increases P_τ of the RyR cluster. However, it is also clear that for mean open times longer than ~0.3 ms, the most likely opening that will activate a RyR is less than the mean open time. This implies that longer mean open times are not more efficient at activating SR calcium release, as they are associated with excess calcium influx. On the other hand, mean open times less than ~0.3 ms result in the majority of DHPR openings being unable to activate SR calcium release, which also results in an excess of calcium influx. This view was quantified by our estimate of the "efficiency" of RyR activation (Fig. 6 *C*), for which we introduced the "*Q*" factor.

As pointed out earlier, the pdf resulting from the convolution of the DHPR open-time pdf and time dependence of the RyR cluster P_C (Fig. 5 *A*) can be considered to be a "tuning curve" that describes the quality of the match between the DHPR gating and the response of the RyR. For example, any intervention that increases the P_c of the RyR will shift the tuning curve to the left, thereby making shorter DHPR openings more efficient at activating SR calcium release. Similarly, a decrease in RyR calcium sensitivity will shift P_C to the right and cause longer DHPR openings to be more efficient. Nevertheless, it is remarkable that the optimum tuning curve for the model parameters considered here occurs with a DHPR mean open time of ~0.3 ms, which is comparable to experimentally observed mean open times (e.g., Hess et al., 1984). Thus the model suggests that cardiac E-C coupling may be well matched to the gating of the DHPR and operate with a near-optimal calcium influx.

It has been shown recently that probability of SR calcium release occurring (as measured by calcium spark probability) is sensitive to the amplitude of the single-channel current (López-López et al., 1995; Santana et al., 1996). These results are in general agreement with the model predictions, because if E-C coupling were operating in a saturated condition (with mean open times much longer than required),

one would not expect the probability of SR release to be so sensitive to the DHPR channel current. With regard to the relationship between DHPR current amplitude and RyR behavior, an unexpected finding was that clustering RyRs lead to a relative increase in the probability of SR release activation (compared to the activation of single RyRs) as DHPR current decreased (Fig. 5 *B*). Put another way, very short DHPR openings are effectively penalized by clustering RyRs, because such openings do not efficiently activate the RyRs at the edge of the diad at early times. This effect arises from the finite time taken to establish the steady-state concentration gradient within the diad (see Soeller and Cannell, 1997). However, the magnitude of the relative increase in P_C/P_a is also current amplitude dependent and becomes smaller as current amplitude increases. This effect is due primarily to the nonlinear relationship between P_a and local $[Ca^{2+}]$ concentration, because as the current amplitude increases, both P_C and $P_a \rightarrow 1.0$. Nevertheless, it is interesting to consider that the DHPR current would normally be less than 200 fA at the peak of the action potential, so that clusters would be more advantageous for activating SR release than single RyRs.

The model presented here shows that the narrow diadic space allows a rapid response of the RyRs to DHPR opening. In fact, the model suggests latencies for RyR activation on the order of 0.3 ms, which is almost negligible compared to the latency to first opening of the DHPR of ~2.1 ms (Isenberg and Han, 1994). This result also explains why there was no detectable delay between the appearance of trigger calcium in the cytosol and SR release (Cheng et al., 1994). Our simulations also suggest that caution should be applied to the interpretation of experiments that attempt to simulate the trigger calcium influx, or produce calcium influx via systems other than the DHPR. Unless the simulated trigger calcium levels are ~50 μM and are very localized, the trigger will not reproduce the time course of RyR response. For example, a lower trigger calcium level over the entire volume of the cell may eventually lead to RyR activation, but the latency and calcium dependence of such a response will not reflect the normal behavior of the system. A similar argument has been put forward to explain why flash photolysis of caged calcium was unable to evoke calcium sparks (Lipp and Niggli, 1996). In connection with this point, we note that the classic experiments that first demonstrated cardiac CICR (Fabiato, 1983) are associated with very long latencies.

Effect of diad geometry

As pointed out earlier, the narrow diad cleft ensures a rapid response of the RyR to DHPR opening. Larger clefts allow larger numbers of RyRs to be incorporated, which will allows a greater peak P_C. However, increasing the cleft diameter also brings a penalty in terms of a decrease in responsiveness. It is clear from Fig. 4 that the greatest fractional increases in P_C are associated with adding small numbers (<8) of RyRs to the cluster. In the model presented here, there is no benefit to increasing the cluster size beyond nine, as the peak $P_C \approx 1.0$ at 0.3 ms. However, it is possible that nine RyRs per DHPR may not be the most suitable stoichiometry, because a cluster of nine RyRs may exhibit uncontrolled regenerative behavior (Stern, 1992). Although this model could evaluate this possibility more directly, we have not examined behavior beyond the point of RyR activation, because there is no consensus as to the mean open and closed times (or their calcium dependence) of RyRs in the literature.

Small diads allow the activator calcium to diffuse away rapidly (Soeller and Cannell, 1997), which helps ensure that the cluster activation is tightly coupled to the DHPR opening. Much larger diads (which are needed to accommodate even larger number of RyRs) do not allow such tight control of the cluster activation by the DHPR opening and, in any case, do not increase the overall P_C. It therefore follows that there is an optimal geometry for the diad in terms of cleft diameter and number of RyRs in a cluster. Although we have considered only circular diads, the same arguments should be applicable to the minimum dimension of diads with different shapes.

It has been suggested that the fundamental unit of CICR may be composed of one DHPR opposite a cluster of four RyRs (Cannell et al., 1994), and this arrangement seems reasonable in view of the results presented here (and previous discussions), as it would allow the cluster to have a high P_C while being quite responsive to the duration of DHPR opening. It is also possible that there may be more than one DHPR in a fundamental release unit, but such an arrangement would only be beneficial if the probability of a DHPR opening during the time course of E-C coupling is low. If the probability of both channels opening over the time scale of E-C coupling is significant, the additional calcium influx after release is initiated would serve little purpose in regulating CICR, because SR calcium release should then dominate local calcium levels, as pointed out previously (Cannell et al., 1987).

CICR tuning

The novel "tuning" concept presented here can be extended to any DHPR/RyR organization, and it is likely that there will always be an optimal gating behavior for any given junctional architecture. Unfortunately, further examination of these ideas requires more detailed quantification of DHPR and RyR gating, as well as quantification of diad microarchitecture. Nevertheless, it is likely that evolution would have optimized the DHPR gating and conductance to the geometry of the diadic space (or vice versa) to achieve efficient E-C coupling. In connection with this point, we suggest that the previously unexplained large size of the RyR protein may 1) serve to place the RyR calcium-sensing site(s) at an optimal distance from the sarcolemmal membrane and 2) ensure that the distance between sarcolemmal

and SR membranes is fixed. The latter may represent an optimal geometry for meeting the conflicting needs of large changes in P_C as well as achieving speed of response (see Soeller and Cannell, 1997). In connection with this point, it is possible that some types of cardiac myopathy may reflect a mismatch in the "tuning" of the RyR to the DHPR, and this could come about by 1) changes in the calcium-dependent gating of the RyR, 2) changes in DHPR gating, or 3) changes in the spatial relationship between the channels. If this suggestion proves correct, it may then be possible to "retune" CICR by altering the mean open and closed times of the DHPR with drugs. Such an approach would minimize calcium influx into the cell (which might otherwise lead to the undesirable sequelae of calcium overload) while maximizing the strength of contraction.

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