A Model of Propagating Calcium-induced Calcium Release Mediated by Calcium Diffusion

PETER H. BACKX, PIETER P. DE TOMBE, JURJEN H. K. VAN DEEN, BARBARA J. M. MULDER, and HENK E. D. J. TER KEURS

From the Department of Medicine and Medical Physiology, University of Calgary, Health Sciences Centre, Calgary, Alberta T2N 4N1, Canada

ABSTRACT The effect of sudden local fluctuations of the free sarcoplasmic $[Ca^{++}]_i$ in cardiac cells on calcium release and calcium uptake by the sarcoplasmic reticulum (SR) was calculated with the aid of a simplified model of SR calcium handling. The model was used to evaluate whether propagation of calcium transients and the range of propagation velocities observed experimentally (0.05–15 mm s^{-1}) could be predicted. Calcium fluctuations propagate by virtue of focal calcium release from the SR, diffusion through the cytosol (which is modulated by binding to troponin and calmodulin and sequestration by the SR), and subsequently induce calcium release from adjacent release sites of the SR. The minimal and maximal velocities derived from the simulation were 0.09 and 15 mm s^{-1} respectively. The method of solution involved writing the diffusion equation as a difference equation in the spatial coordinates. Thus, coupled ordinary differential equations in time with banded coefficients were generated. The coupled equations were solved using Gear's sixth order predictor-corrector algorithm for stiff equations with reflective boundaries. The most important determinants of the velocity of propagation of the calcium waves were the diastolic $[Ca^{++}]_i$, the rate of rise of the release, and the amount of calcium released from the SR. The results are consistent with the assumptions that calcium loading causes an increase in intracellular calcium and calcium in the SR, and an increase in the amount and rate of calcium released. These two effects combine to increase the propagation velocity at higher levels of calcium loading.

INTRODUCTION

Spontaneous contractions are known to propagate in cardiac cells (Fabiato and Fabiato, 1972; ter Keurs and Mulder, 1984; Capogrossi and Lakatta, 1985; Allen et al., 1985; Bahinski et al., 1986; Golovina et al., 1986), skeletal fibers (Coleman et al., 1972; Natori et al., 1976), and in cardiac trabeculae (ter Keurs and Mulder, 1984).

Direct microscopic examination in cardiac cells (Fabiato and Fabiato, 1972; ter Keurs and Mulder, 1984; Capogrossi and Lakatta, 1985; Bahinski et al., 1986) and direct microscopic examination and laser diffraction measurements in cardiac trabeculae (ter Keurs and Mulder, 1984) demonstrate unequivocally that local contractile activity propagates as contractile waves. The velocity of propagation is known to vary with conditions that alter diastolic calcium concentrations in the cardiac cytosol (ter Keurs and Mulder, 1984). The range of observed velocities was 0.05–3.0 mm s^{-1} in cardiac cells (Golovina et al., 1986) and up to 15 mm s^{-1} in cardiac trabeculae (ter Keurs and Mulder, 1984). These contractions have been shown to result from propagating waves of calcium in the myofibrillar space (Wier et al., 1987) and they are independent of an intact sarcolemma (Fabiato and Fabiato, 1972). The peak calcium concentration, as measured by means of aequorin, that is reached in the cell during these oscillations has been reported to be between 4 and 40 μM (Wier et al., 1983; Orchard et al., 1985). The transient rise of the cytosolic calcium level may give rise to a transient depolarization, which can lead to triggered arrhythmias (Kass and Tsien, 1982; Matsuda et al., 1982; ter Keurs and Mulder, 1984; Capogrossi and Lakatta, 1985; Bahinski et al., 1986).

Under conditions where the diastolic calcium concentration in skinned myocytes is less than most estimates of the resting diastolic calcium concentration in intact muscle (i.e., pCa = 7.1), propagated spontaneous contractions were observed to occur in a cyclical fashion (Fabiato, 1983). Fabiato has pointed out the cyclic release of calcium, which initiates the propagating contractile waves in cardiac cell fragments, is not related to calcium-induced calcium release, but rather corresponds to release as a consequence of calcium overload (Fabiato, 1985a). The occurrence of Ca^{++} overloading and subsequent spontaneous Ca^{++} release is not expected to occur as readily in the undamaged intact myocardium even during conditions where the diastolic $[Ca^{+}]$ is well above a pCa of 7.1 (i.e., after rapid stimulation). The reason is that the diastolic $[Ca^{++}]$ is dynamic and an elevation would be expected to be short-lived due to extrusion mechanisms. Therefore, the elevated $[Ca^{++}]$ would not be sustained as in skinned preparations.

Due to the cyclic nature of local spontaneous contractions, multicellular preparations like cardiac papillary muscles and trabeculae can show scattered light intensity fluctuations, spontaneous tension oscillations, and diastolic oscillations of intracellular Ca^{++} as measured by Ca^{++} indicators (Glitsch and Potter, 1975; Lakatta and Lappe, 1981; Wier et al., 1983; Allen et al., 1984, 1985; Kort and Lakatta, 1984; Orchard et al., 1985).

Spontaneous contractions of this type have a profound effect on force generation for a given level of activator calcium (ter Keurs and Mulder, 1984; Orchard et al., 1985; Capogrossi et al., 1986a, b). Provided that the frequency of spontaneous contractions is lower than the rate of stimulation of the preparation, the force generated by the muscle will be unaffected. Alternatively, the force may be reduced when a synchronous release of calcium from intracellular stores occurs before stimulation (Orchard et al., 1985; Capogrossi et al., 1986a, b). The frequency of oscillations and the velocity of propagation are influenced by conditions and chemical agents known to alter intracellular calcium concentration and calcium handling by the sarcoplasmic reticulum (SR) e.g., $[Sr^{++}]_o$, caffeine, ryanodine, verapamil, procaine, the fre-

quency of stimulation, temperature, pH, Na^+/K^+ pump inhibitors (Fabiato and Fabiato, 1975; Natori et al., 1976; Lakatta and Lappe, 1981; Kass and Tsien, 1982; Allen et al., 1984; Kimura et al., 1984; Kort and Lakatta, 1984).

Once calcium overloading has occurred at a focal region in the cell it seems plausible that the released calcium could diffuse into the adjacent regions that are not calcium overloaded, and cause calcium release from the SR by calcium-induced calcium release. That such a mechanism may be responsible for a propagating contractile wave can be best appreciated in rat cardiac trabeculae (ter Keurs and Mulder, 1984). Propagating spontaneous contractions arise from the damaged end region of the preparation where calcium overloading has occurred, resulting in a regional spontaneous contraction (Mulder et al., 1989); these contractions propagate in a synchronized fashion at constant velocity along the remainder of the muscle that otherwise shows no signs of calcium overload (i.e., no local spontaneous contractions; ter Keurs and Mulder, 1984).

In this paper we examine via a computer simulation whether the phenomenon of propagated contractions can be explained by a simplified model based on calcium-induced calcium release and calcium diffusion. In particular our model is based on focal release of calcium from the SR, which is followed by diffusion of calcium into the adjacent cytosol, thereby inducing calcium release. Quantitative simulation predicts the range of velocities that have been observed experimentally. Previously a model very similar to ours was proposed but the quantitative consequences were not fully examined (Regirer et al., 1986).

METHODS

The experimental results of the previous article can be understood in terms of the model to be described below. In essence the model is a one-dimensional representation of cardiac cells and trabeculae in which focal release of Ca^{++} from the SR diffuses into adjacent regions and thereupon induces Ca^{++} release from the adjacent SR by virtue of Ca^{++}-induced Ca^{++} release. The release of Ca^{++} from the SR occurs at release sites (10 per sarcomere) when the $[Ca^{++}]$ reaches a threshold for release (usually 1.2 μM). The release function was described as an exponential rise and fall of the Ca^{++} flux from the SR. This represents two first order processes: one for activation and the other for inactivation of the SR Ca^{++} channels. The model incorporates the effect of binding of the released free Ca^{++} to Ca^{++} binding sites in the sarcoplasm. Ca^{++} is sequestered in the SR by a Ca^{++}-ATPase.

From the above considerations the model can be represented in mathematical terms as a set of seven coupled first order differential equations in time for each of the spatial grid points (see below). In obtaining these equations the diffusion equation has been converted from a partial differential equation to an ordinary differential equation using the finite difference method (Cannel and Allen, 1984; Crank, 1975). As a result, in the following equations the subscripts will refer to spatial grid points that are equally spaced. The actual number of coupled ordinary differential equations is $7 * N$ where N is the total number of grid points. Specifically, we simulated 20–500 sarcomeres with 10 release sites and 100 grid points per sarcomere. The differential equations for a single grid point are:

$$dC_1(x_i)/dt = D[C_1(x_{i+1}) - 2C_1(x_i) + C_1(x_{i-1})] + F(x_i) \tag{1}$$

$$dC_2(x_i)/dt = -k_{on}^C C_1(x_i)C_2(x_i) + k_{off}^C[cal^T - C_2(x_i)] \tag{2}$$

$$dC_3(x_i)/dt = -k_{on}^T C_1(x_i)C_3(x_i) + k_{off}^T[trop^T - C_3(x_i)] \tag{3}$$

$$dC_4(x_i)/dt = -k_{on}^{TC}C_1(x_i)C_4(x_i) + k_{off}^{TC}C_5(x_i) - k_{on}^{TM}C_4(x_i)C_7(x_i) + k_{off}^{TM}C_6(x_i) \qquad (4)$$

$$dC_5(x_i)/dt = k_{on}^{TC}C_1(x_i)C_4(x_i) - k_{off}^{TC}C_5(x_i) \qquad (5)$$

$$dC_6(x_i)/dt = k_{on}^{TM}C_4(x_i)C_7(x_i) - k_{off}^{TM}C_6(x_i) \qquad (6)$$

$$dC_7(x_i)/dt = -k_{on}^{TM}C_4(x_i)C_7(x_i) + k_{off}^{TM}C_6(x_i) \qquad (7)$$

where $C_i(x_i)$ refers to a concentration at a location along the specimen:

$C_1(x_i)$ calcium

$C_2(x_i)$ unbound calmodulin

$C_3(x_i)$ unbound calcium sites on troponin

$C_4(x_i)$ unbound calcium-magnesium sites on troponin

$C_5(x_i)$ calcium-magnesium sites on troponin bound to calcium

$C_6(x_i)$ calcium-magnesium sites on troponin bound to magnesium

$C_7(x_i)$ magnesium;

$F(x_i)$ = the calcium flux, which equals

$$dC_2(x_i)/dt + dC_3(x_i)/dt - dC_5(x_i)/dt - F_P(x_i) + F_R(x_i); \qquad (8)$$

$F_P(x_i)$ = flux due to the SR calcium pump (see below); and $F_R(x_i)$ = the influx of calcium from the release site (see below). Notice that only Ca^{++} diffuses; the other six molecular species defined above change in concentration by virtue of binding and dissociation only. The grid points labeled i, i + 1, and i − 1 are 0.02 μm apart.

The boundary conditions used in the simulation were $dC_1(x_i)/(dt)_{x=0,L}$ = 0, which correspond to reflective boundary conditions where O and L refer to either end of the fiber. The solution to the above set of equations was obtained by using a sixth order predictor-corrector method with a variable step size (Gear, 1971). A vectorized version of this program was used on the Cyber 205 at the University of Calgary.

The various constants and parameters used in Eqs. 1–7 are tabulated in Table I. The rate constants for Ca^{++} binding to troponin and calmodulin were taken from the work of Robertson et al. (1981) and Potter et al. (1981). The concentrations of troponin and calmodulin used correspond to those used recently by Wier and Yue (1986).

Calcium was assumed to diffuse along the preparation with a diffusion constant of 400 μm² s⁻¹. The diffusion constant for pure water is 700 μm² s⁻¹ (Wang, 1953), which was used in previous simulations (Cannel and Allen, 1984; Wier and Yue, 1986). The combination of the fact that the intracellular milieu has an ionic strength of 0.17 and the presence of large protein moieties, causes the diffusion constant in the cell to be lower than in pure water (Phillies, 1974; Kruus, 1977). The "apparent" diffuse constant is of course much lower since the binding sites included in the simulation reduces the amount of free calcium available for diffusion (Kushmerick and Podolsky, 1969; Crank, 1975). The diffusion constant was assumed to be homogeneous throughout the length of the preparation and thus no variations were assumed to exist at cell boundaries.

The initial $[Mg^{++}]$ was assumed to be 3.15 mM (Fabiato, 1983) while the initial $[Ca^{++}]$ was varied from 50 nM to 1 μM. From the initial conditions and the total concentrations of the various Ca^{++} binding sites used in the simulation, the initial concentrations of bound and free binding sites could be determined (Gillis et al., 1982).

The SR Ca^{++} pump was assumed to obey first order enzyme kinetics (Cannel and Allen,

1984; Haynes and Mandueno, 1987):

$$F_p = d[Ca^{++}]/dt = V_{max}[Ca^{++}]/([Ca^{++}] + K_m)$$ (9)

where F_p is the pump flux rate, $V_{max} = 1.0$ mM s^{-1}, and $K_m = 1.0$ μM (Ogawa et al., 1981; Cannel and Allen, 1984). The use of first order Michaelis-Menten equation to describe calcium reuptake by the SR appears to be justified since kinetic schemes devised to describe the Ca^{++}-ATPase activity of the SR have demonstrated that a rate-limiting step occurs in this process (Haynes and Mandueno, 1987).

The release of calcium from the SR was assumed to occur from the release sites provided that the [Ca^{++}] at the release site reached a value above a particular threshold. This threshold was usually assumed to be 1.2 μM. For some of the results discussed below the threshold was varied in an attempt to examine the predictions of the model when different thresholds were used. Both experimental work (Fabiato, 1985b) and previous modeling work (Wong et al., 1987) on cardiac skinned fibers indicate that the release of calcium occurs only if the [Ca^{++}]

TABLE I

Parameter	Value	Source
Diffusion constant (D)	400 μm^{2}s^{-1}	Assumed
Troponin Ca^{2+} specific		
Binding sites		
Concentration (trop^T)	70 μM	Wier and Yue, 1986
Ca^{2+} on-rate (K_{on}^T)	39 μM^{-1}s^{-1}	Robertson et al., 1981
Ca^{2+} off-rate (K_{off}^T)	19.6 s^{-1}	Robertson et al., 1981
Troponin Ca^{2+} – Mg^{2+}		
Binding sites		
Concentration (trop^T)	70 μM	Wier and Yue, 1986
Ca^{2+} on-rate (K_{on}^{TC})	100 μM^{-1}s^{-1}	Robertson et al., 1981
Ca^{2+} off-rate (K_{off}^{TC})	0.33 s^{-1}	Robertson et al., 1981
Mg^{2+} on-rate (K_{on}^{TM})	0.7 μM^{-1}s^{-1}	Robertson et al., 1981
Mg^{2+} off-rate (K_{off}^{TM})	33.3 s^{-1}	Robertson et al., 1981
Calmodulin binding sites		
Concentration (cal^T)	50 μM	Wier and Yue, 1986
Ca^{2+} on-rate (K_{on}^C)	100 μM^{-1}s^{-1}	Robertson et al., 1981
Ca^{2+} off-rate (K_{off}^C)	238 s^{-1}	Robertson et al., 1981
Sarcomere length	2 μm	Assumed
Magnesium concentration	3.15 mM	Fabiato, 1983
Diastolic calcium concentration	0.05–1 μM	Assumed

is above a particular value. In skinned cardiac cells (Fabiato, 1985b), for example, no release takes place if the triggering [Ca^{++}] does not exceed a pCa of 6.5. Thereafter, the release is graded with the size and rate of increase of [Ca^{++}] applied to the outside of the sarcoplasm. Model parameters used to describe the experimental results in skinned fibers (Wong et al., 1987) reproduce this property but do not predict propagation of calcium transients. Probable reasons for this reside in the slow kinetics of the calcium release process that have been chosen in Wong's model to predict the kinetic behavior of calcium transients in skinned cells (Fabiato, 1985b), which respond clearly more slowly than intact cardiac tissue (Allen and Kurihara, 1980, 1982; Wier and Yue, 1986). When we used the kinetic scheme presented in the work of Wong et al. (1987) (with the difference that we decreased their time constants by a factor of >100) the predicted calcium transients could be made to match those that have been reported for intact cardiac muscle. Secondly, Wong's model assumed a shallow depen-

dence of the steady state values of the activation and inactivation parameters on the $[Ca^{++}]$. This assumption again led to good correlation with the observations in skinned cardiac cells (Fabiato, 1985b), but did not help to predict contractile wave propagation even when the time constants for release were decreased as already mentioned above. This suggested to us that the sensitivity of the release mechanism in the SR in intact cells is more sensitive to calcium than was described by Wong's model. Moreover, with acceleration of the kinetics of SR-calcium release in Wong's model, the amount of calcium that was released for any trigger $[Ca^{++}]$ appeared to increase. The increase of the amount of released calcium depended on the relative increases of the rates of activation and inactivation. Hence, because no accurate information about the release kinetics were available to us we decided to simplify the release process by assuming variable release kinetics in combination with a fixed threshold in this model. This is equivalent to assuming a steep calcium dependence of the steady state value of the activation and inactivation parameters or the existence of a regenerative calcium release. The use of a threshold should be viewed as a first order approximation to a more realistic description of the release process. The release process in our model is qualitatively similar to those used by previous authors (Cannel and Allen, 1984; Yamaguchi et al., 1985).

If the $[Ca^{++}]$ was above the threshold at a release site, then the release rate of calcium from the SR was assumed for simplicity to be a time-dependent process described by an exponential rise and fall. In particular:

$$F_R = \begin{cases} A(1 - e^{-kt}), & \text{with} \quad 0 < t < t_a \\ A'e^{-k(t-t_a)}, & \text{with} \quad t_a < t < \infty \end{cases} \tag{10}$$

where k is the rate constant for activation and deactivation of the SR calcium channel (k ranged from 40–0.1 ms^{-1}), $t_a = 2/k$, $A' = A(1 - e^{-kt_a})$, and A is adjusted to obtain systolic calcium concentrations between 6–15 μM.

The actual range of rate constants for the release of calcium from the SR used in Eq. 10 was 0.1–40 ms^{-1}. The variation of the rate of Ca^{++} release from the SR used in our model (i.e., an increasing rate of Ca^{++} release with increasing Ca^{++} loading of the SR) is consistent with the data on birefringence changes in myocardium (Weiss and Morad, 1983). The second component of the birefringence change shortly after stimulation reflects the Ca^{++} release process from the SR (Weiss and Morad, 1983). By increasing the extracellular $[Ca^{++}]$ from 0.18 to 2.4 mM, the rate of rise of the change in the birefringence signal varied by a factor of about 20 in rat myocardium (Weiss and Morad, 1983). Other interventions such as the application of epinephrine also increased the rate of rise of this signal. The time period of release as indicated by the birefringence signal was decreased as the rate of rise of the signal was decreased (Weiss and Morad, 1983). A larger range of rates of rise of the birefringence signal would be expected than that reported by Weiss and Morad (1983) since these authors did not examine a large range of different levels of loading of the SR. A large variation in the rate of rise of the aequorin signal for different levels of SR loading, and therefore the Ca^{++} release rate from the SR, is not expected since the aequorin signal is expected to act as a filter with a time constant of 5 ms at 30°C (Wier and Yue, 1986) and 20 ms at 22°C (Fabiato, 1985a).

The rate constants used in Eq. 10 for the rise and fall of the calcium release from the SR were assumed to be the same for simplicity. This assumption was not necessary and it was found that the major determinant of the propagation velocity was the rate of rise of the calcium release from the SR.

Wong's model (Wong et al., 1987) predicts a more rapid rate of release for larger amounts of Ca^{++} loading in the SR release compartment and for higher resting diastolic $[Ca^{++}]$. Thus in the model it was assumed that with high levels of Ca^{++} loading of the SR and/or with high diastolic $[Ca^{++}]$ the release process took place more rapidly. That is, the rate constants for release were higher. Lower diastolic $[Ca^{++}]$ and lower levels of SR loading result experimen-

tally in a reduced rate of Ca^{++} release, an increased period of release, and a lower peak $[Ca^{++}]$ for both cyclical spontaneous release and Ca^{++}-induced calcium release. These results are consistent with the experiments on skinned cardiac preparations (Fabiato, 1983; Fabiato, 1985*b*). The increased period of release with decreased diastolic $[Ca^{++}]$ is explained by the lower degree of inactivation of the SR Ca^{++} channels and the reduced gradient for Ca^{++} release from the SR. The lower peak systolic $[Ca^{++}]$ can also be explained by the lower Ca^{++} gradient across the SR membrane. The level of Ca^{++} loading of the SR also determines the ease with which Ca^{++} can be released from the SR (Fabiato, 1983). Thus the steady state parameters that enter into the gating equations are expected to vary with the level of calcium loading. For our model this would translate into a variation in the threshold for Ca^{++} release from the SR. Since the dependence of the gating parameters on the Ca^{++} load of the SR is only known qualitatively, we performed most of our calculations using a fixed threshold for Ca^{++} release.

10 release sites per sarcomere were used since it was previously suggested by Fabiato (Fabiato, 1983, 1985*a*) that Ca^{++}-induced release of Ca^{++} from the SR may take place in a continuous fashion from the longitudinal SR. The velocities as predicted by the model were reduced by ~30% at high velocities and 300% at lower velocities if the peak $[Ca^{++}]$ remained fixed and the number of release sites was reduced to two per sarcomere.

In summary, our release functions were chosen as first order approximations of the more realistic model, which incorporates activation and inactivation kinetics. A more realistic model would incorporate these properties as well as the number, distribution, and permeability properties of the SR Ca^{++} channels as well as the degree of loading of the SR as a function of the stimulation history and diastolic $[Ca^{++}]$. Since little is known quantitatively about these processes, the model of Ca^{++} release was kept as simple as possible in order to test whether the essential ingredients (Ca^{++} diffusion and Ca^{++}-induced Ca^{++} release from the SR) can explain the experimentally observed range of propagation velocities.

RESULTS AND DISCUSSION

In obtaining the observed range of velocities from the simulation, four parameters appeared important: (*a*) the diastolic calcium concentration in the cytosol, (*b*) the rate of release of calcium from the SR, (*c*) the threshold for the release of calcium from the SR, and (*d*) the peak systolic calcium concentration. The latter three parameters depend crucially on the diastolic cytosolic $[Ca^{++}]$. For example, by increasing the diastolic calcium concentration we expect a decrease of the threshold for the release of calcium (Endo, 1985; Fabiato, 1985*b c*). This is a direct consequence of loading the SR. Furthermore, the rate of release of calcium from the SR will increase (Weiss and Morad, 1983). The period over which calcium release takes place will decrease as a result of more rapid inactivation of the release process due to higher cytosolic resting calcium levels (Fabiato, 1985*b*).

Fig. 1 demonstrates the effect of a varied release rate, the diastolic $[Ca^{++}]$, and the threshold for release of calcium by the SR. The top curve was obtained with a diastolic concentration of 1 μM and a threshold for release of calcium of 1.2 μM. As can be seen, varying the rate constant of calcium release has a profound effect on the velocity of propagation. The middle curve of Fig. 1 was obtained with a resting diastolic calcium level of 50 nM and a threshold for release of 1.2 μM. As can be seen, there is a drastic decrease of the velocity of propagation between the top and middle curves; this would be expected because much more time is required for the local calcium concentration to reach threshold level. The bottom curve of Fig. 1

corresponds to a diastolic calcium concentration of 50 nM and a threshold for release of 2 μM, which leads to a further decrease of the propagation velocity as compared with the middle curve in Fig. 1.

The effect of binding of calcium by calcium-binding molecules in the cell is shown in Fig. 2. This curve was obtained by varying the diastolic calcium concentration while the threshold for calcium release was kept at a fixed value above the resting diastolic calcium level (i.e., $[Ca^{++}]_{th} = 1.2\ [Ca^{++}]_{diastolic}$). As can be seen this only
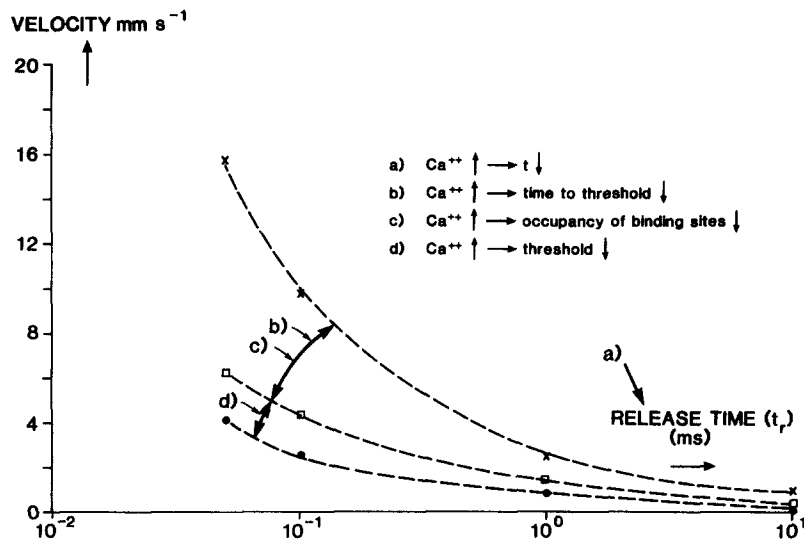

FIGURE 1. This graph shows the velocity of propagation vs. the logarithm of the release time. The release time is defined as twice the inverse of the release rate from the SR. The release time is directly dependent on the diastolic calcium concentration (a, see text for explanation). The top curve (open triangles) corresponds to a resting diastolic calcium concentration of 1 μM and a threshold for calcium release of 1.2 μM. The middle curve (open squares) results from conditions under which the resting diastolic calcium concentration is 50 nM while the threshold is 1.2 μM; the decrease of the propagation velocity at all release times results from the effect of the decreased calcium concentration on the time required to reach threshold as a result of both the larger difference between the diastolic and the trigger concentrations of calcium (b) and of the decreased occupancy of the binding sites (c). The bottom curves represent conditions under which the resting diastolic calcium concentration is 50 nM while the threshold is 2.0 μM. The decreased propagation velocity results directly from an increase of the difference between the diastolic and the trigger concentrations of calcium (d).

accounts for a twofold variation of the velocity of propagation. At lower velocities of propagation the modulating effect of calcium binding approaches threefold. If one assumes instantaneous binding of the available binding sites to calcium the above threefold decrease in the velocity of propagation is very close to the change in the apparent diffusion constant (Crank, 1975).

The effect of the diastolic calcium concentration on the velocity of propagation is shown in Fig. 3 for a threshold for calcium release of 1.2 μM. The top graph shows
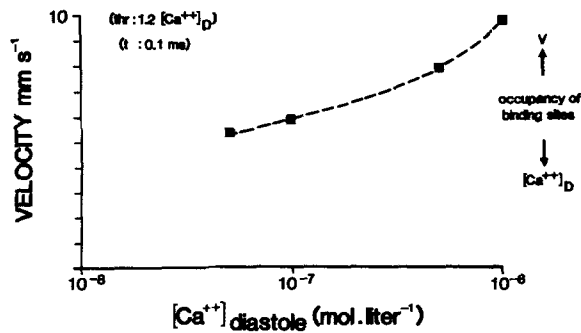
FIGURE 2. This figure shows the effect of the number of available binding sites on the velocity of propagation. The estimate was obtained by varying the threshold for calcium release in proportion to the resting diastolic concentration (i.e., threshold = 1.2 diastolic calcium concentration). The release time was fixed at 1 ms.

the results for a release rate of 0.2 ms^{-1}. As is expected, the velocity of propagation decreased with decreasing diastolic [Ca^{++}]. This is a consequence of an increase in the number of binding sites available for calcium binding as well as a larger change in [Ca^{++}] that is required to reach threshold for calcium release. The bottom graph of Fig. 3 depicts the variation of velocity of propagation for a release rate of 40 ms^{-1}. The velocity here is reduced for the same reasons as for a release rate of 0.2 ms^{-1}. By comparing Fig. 2 with the bottom graph of Fig. 3, it can be seen that binding accounts for $\sim$ 25% of the observed change in velocity between resting diastolic calcium levels of 1 μM and 50 nM.
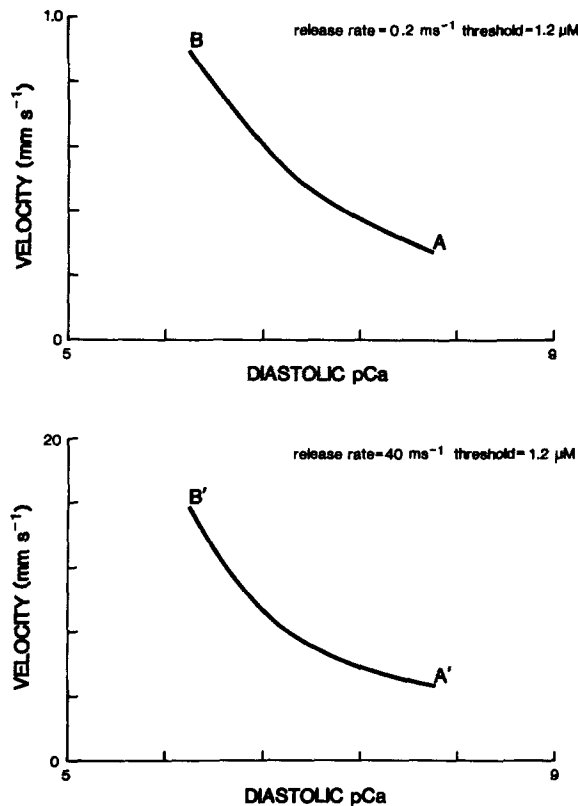

FIGURE 3. This figure shows graphs of the propagation velocity vs. diastolic pCa for two different release rates at a fixed threshold (1.2 μM). The top graph demonstrates the velocity of propagation vs. the diastolic pCa for a release rate of calcium from the SR of 0.2 ms^{-1}. The points labeled A and B correspond to a propagation velocity of 220 μm/s for a pCa = 7.3 and a velocity of 890 μm/s for a pCa = 6.0 respectively. The lower graph shows the velocity of propagation vs. pCa for a release rate of 40 ms^{-1}. Points A' and B' correspond to a velocity of propagation of 4.7 mm s^{-1} at a pCa = 7.3 and a velocity of 16 mm/s for a pCa = 6.0, respectively.

Fig. 4 shows the change in systolic [Ca^{++}] as a function of time averaged over three sarcomeres as the propagating calcium wave passes a fixed point in the preparation. The graphs labeled A, B, A', and B' in Fig. 4 correspond to those in Fig. 3. At high velocities the time-to-peak [Ca^{++}] is decreased. This is a direct consequence of the increased release rate of calcium from the SR. At all velocities the rate of rise starts slowly, which reflects the diffusion of calcium into the region from adjacent sarcomeres. Once the local [Ca^{++}] reaches the threshold for calcium release from the SR, the rate of rise of the local calcium is much more rapid.

The rate of decay of the calcium concentration is qualitatively similar for the four conditions shown in Fig. 4. At high release rates, however, the rate of decay is much faster for low resting diastolic calcium concentrations. This is a direct consequence of the large number of binding sites available for binding to released calcium, as
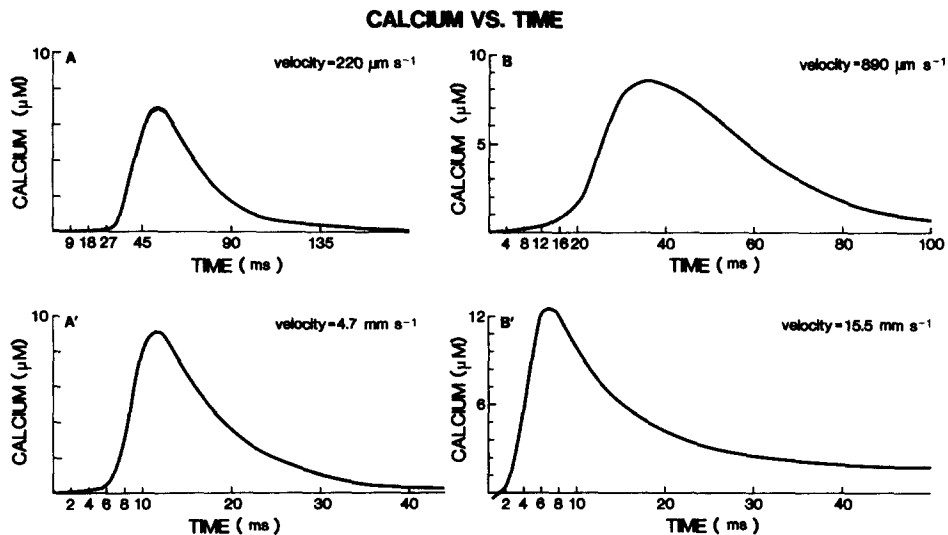
CALCIUM VS. TIME

FIGURE 4.   This figure shows the calcium concentration averaged over three sarcomeres as a function of time. The graphs labeled A, B, A', and B' correspond to the conditions as indicated in Fig. 3. As explained in the text, the transients are qualitatively similar to the transients observed experimentally using the calcium-sensitive luminescent protein aequorin.

well as the larger diffusion gradients under these conditions. At the higher diastolic [Ca^{++}] the rate of decay reflects the kinetics of the SR Ca^{++}-ATPase activity.

At lower release rates the decay curves are very similar, being a little faster for high diastolic [Ca^{++}]. Under conditions of slow release of calcium from the SR the local binding sites become saturated during the release period, and thereby do not contribute greatly to the decay of free calcium concentration. The decay rate is dominated by the kinetics of the SR Ca^{++}-ATPase.

Fig. 5 shows three-dimensional plots of the free calcium concentration in space and time. The plots labeled A, B, A', and B' correspond to the points in Fig. 3. At high release rates the wavelengths are long in relation to those at lower release rates. As can be seen, the propagating waves are characterized by a rather rapid rise of the

[Ca^{++}] at the leading front of the wave and a more gentle decline of the [Ca^{++}] behind the advancing crest. For higher propagation velocities the difference between the rising and falling edges of the propagating wave becomes much more pronounced. At low velocities the [Ca^{++}] is elevated over a distance of about 10 sarcomeres. At higher velocities the region in which the [Ca^{++}] is elevated is about 40 sarcomeres at low diastolic [Ca^{++}] and greater than 350 sarcomeres at high diastolic calcium concentrations. This is a direct consequence of the relatively low Ca^{++}-ATPase activity in the SR. Inclusion of the Na/Ca exchanger and Ca^{++}-ATPase in the sarcolemma would reduce the size of the region with an elevated
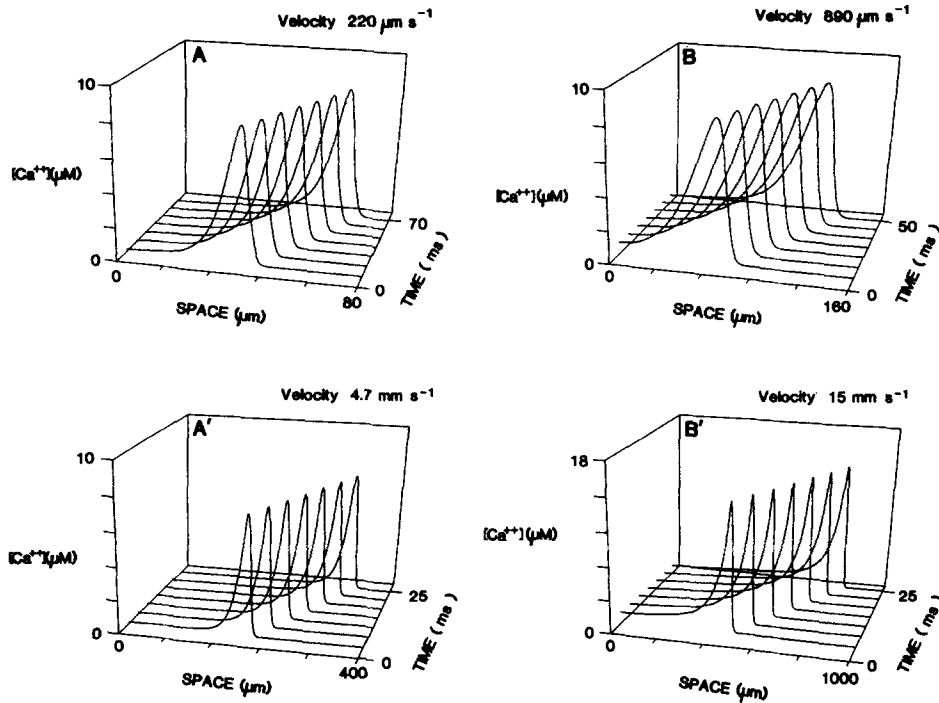

FIGURE 5. The calcium transients as a function of distance along the preparation at different times. The propagating nature of the calcium wave is evident from the figures. *A*, *B*, *A'*, and *B'* correspond to the conditions indicated in Fig. 3.

[Ca^{++}]. Furthermore, the wavelengths are shorter at low resting diastolic calcium concentrations for a given release rate.

The results of the simulation indicate that the release rate of calcium from the SR was the major determinant of the variation of the velocity of propagated contractions. The release rate of calcium was assumed to depend on the level of calcium loading of the SR, which in turn is related to the diastolic [Ca^{++}]. The variation of the rates of release of calcium by the SR with the level of calcium loading is supported by the kinetics of birefringence (Weiss and Morad, 1983) if one assumes that measurements of the second component of the birefringence signal reflects the calcium release process (Weiss and Morad, 1983). The shortest total release time used

in the simulations represents a total release period of 0.3 ms, which is in the same order of magnitude as the kinetics of the Na channel. We considered shorter release periods unlikely. The use of two release sites per sarcomere instead of 10, results in a slower propagation velocity, which has a larger impact at low velocities than high velocities. At high velocities of propagation, the reduction of the number of sites reduced the velocity by 30%. At low velocities this effect reduces the velocity by about a factor of 3. This effect is a result of the Ca^{++} sequestration by the SR.

An exponential rise and fall of release was chosen since no quantitative detailed kinetics are available for SR calcium release. In assuming an exponential character of the release rates we inherently assume that single first order processes control activation and inactivation of the release. The actual kinetics are much more complicated and depend on the gradient of calcium across the SR membrane and the kinetics of opening and closing of calcium channels in the SR. The dependence of the release of calcium on the rate of change of the calcium concentration and the level of calcium has been examined by Fabiato, but no kinetic scheme involving on and off rates for the binding of calcium to the regulatory sites of the channel is available so far (Fabiato, 1985b, c). Such a scheme does exist for skeletal SR but the applicability of this to cardiac SR is questionable (Smith et al., 1985; Meissner et al., 1986).

The use of a fixed time-independent threshold represents an approximation to the more detailed release kinetics measured by Fabiato (1985b). The simplified assumption of a threshold for opening calcium channels is useful if the effects of changes of cytosolic $[Ca^{++}]$ are slow compared with the kinetics of opening and closing of the channel itself. We have varied the threshold over a factor of two in this simulation; it is evident that larger variation of the apparent threshold will allow for an even larger variation of the predicted velocity propagation of the calcium wave.

Although we did not quantitatively study the relation between the propagation velocity of spontaneous contractions and the amplitude of the initiating regional contraction, we always observed that the amplitude of the regional contraction correlated with the velocity of the propagating contraction (Mulder et al., 1989). This implies that calcium loading of the trabeculae may have caused a concerted effect both on the initiating event (i.e., the regional spontaneous contraction) and the threshold and rate of the calcium release process, as well as on the occupancy of the binding sites (which affects diffusion and hence the time to reach threshold in an adjacent region). This correlation is consistent with the model of calcium-induced calcium release suggested by Fabiato (1985b, c). Based on the close correspondence of the observed propagation velocities (0.05–16 mm s^{-1}) and the propagation velocities predicted in our model (0.09–15 mm s^{-1}), we propose that the observed range of propagation velocities is consistent with the assumption that the propagation of a calcium wave is mediated by calcium-induced calcium release coupled by diffusion.

The authors would like to thank Academic Computer Services and Super-Computer Services at the University of Calgary for computing time on their Cyber-205.

REFERENCES

Allen, D. G., D. A. Eisner, and C. H. Orchard. 1984. Characterization of oscillation of intracellular calcium concentration in ferret ventricular muscle. *Journal of Physiology.* 352:113–128.

Allen, D. G., D. A. Eisner, J. S. Pirolo, and G. L. Smith. 1985. The relationship between intracellular calcium and contraction in calcium-overloaded ferret papillary muscles. *Journal of Physiology.* 364:169–182.

Allen, D. G., and S. Kurihara. 1980. Calcium transients in mammalian ventricular muscle. *European Heart Journal.* 19:5–15.

Allen, D. G., and S. Kurihara. 1982. The effects of muscle length on intracellular transients in mammalian cardiac tissue. *Journal of Physiology.* 327:79 P.

Bahinski, A., M. C. Capogrossi, S. R. Houser, and E. G. Lakatta. 1986. Multifocal spontaneous Ca^{++} release can trigger a spontaneous action potential in cardiac myocytes. *Journal of Physiology.* 371:196P. (Abstr.)

Cannell, M. B., and D. G. Allen. 1984. Model of calcium movements during activation in the sarcomere of frog skeletal muscles. *Biophysical Journal.* 45:913–925.

Capogrossi, M. C., A. A. Kort, H. A. Spurgeon, and E. G. Lakatta. 1986a. Single adult rabbit and rat cardiac myocytes retain the Ca^{++} and species-dependent systolic and diastolic contractile properties of intact muscle. *Journal of General Physiology.* 88:589–613.

Capogrossi, M. C., B. A. Suarez-Isla, and E. G. Lakatta. 1986b. The conduction of electrically stimulated twitches and spontaneous contractile waves in single cardiac myocytes. *Journal of General Physiology.* 88:615–633.

Capogrossi, M. C., and E. G. Lakatta. 1985. Frequency modulations and synchronization of spontaneous oscillations in cardiac myocytes. *American Journal of Physiology.* 248:H412–H418.

Coleman, A. W., J. R. Coleman, J. D. Griffin, J. K. Weltman, and K. M. Chapman. 1972. *Proceedings of the National Academy of Sciences.* 69:613.

Crank, J. 1975. The Mathematics of Diffusion. 2nd ed. Oxford Press, London.

Endo, M. 1985. Calcium release from sarcoplasmic reticulum. in: Current Topics in Membranes and Transport. Vol. 25, Ed. Shamoo, N., Academic Press, Orlando, FL. 181–230.

Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *American Journal of Physiology.* 245:C1–C14.

Fabiato, A. 1985a. Spontaneous versus triggered contractions of "calcium tolerant" cardiac cells from adult rat ventricle. *Basic Research in Cardiology.* 80(Suppl. 2):83–88.

Fabiato, A. 1985b. Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of skinned canine cardiac Purkinje cell. *Journal of General Physiology.* 85:247–289.

Fabiato, A. 1985c. Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *Journal of General Physiology.* 85:291–320.

Fabiato, A., and F. Fabiato. 1972. Excitation-contraction coupling of isolated cardiac fiber with disrupted or closed sarcolemmas. Calcium dependent cyclic and tonic contractions. *Circulation Research.* 31:293–307.

Fabiato, A., and F. Fabiato. 1975. Contractions induced by a calcium triggered release of calcium

from the sarcoplasmic reticulum of single skinned cardiac cells. *Journal of Physiology.* 249:469–495.

Gear, C. W. 1971. Numerical initial-value problems in ordinary differential equations. Prentice-Hall, Englewood Cliffs, NJ. 253 pp.

Gillis, J. M., D. Thomason, J. Lefevre, and R. H. Kretsinger. 1982. Parvalbumins and muscle relaxation: a computer study. *Journal of Muscle Research and Cell Motility.* 3:377–398.

Glitsch, H. G., and L. Potter. 1975. Spontaneous tension oscillation in guinea pig trabeculae. *Pflügers Archive des Physiologie.* 358:11–25.

Golovina, V. A., L. V. Rozenshtraukh, B. S. Solov'ev, A. I. Undrovinas, and G. G. Chernaya. 1986. Wavelike spontaneous contractions of isolated cardiomyocytes. *Biophysics.* 31:311–318.

Haynes, D. H., and A. Mandueno. 1987. Computer modelling of Ca^{++} pump function of Ca^{++}-Mg^{++}-ATPase of sarcoplasmic reticulum. *Physiological Reviews.* 67:244–284.

Kass, R. S., and R. W. Tsien. 1982. Fluctuations in membrane current driven by intracellular calcium in cardiac Purkinje fibers. *Biophysical Journal.* 38:259–269.

Kimura, S., J. Cameron, P. Kozlovski, A. Basset, and R. Myerburg. 1984. Delayed after depolarization and triggered activity induced in feline Purkinje fibers by α-adrenergic stimulation in the presence of elevated calcium levels. *Circulation.* 70:1074–1084.

Kort, A., and E. Lakatta. 1984. Calcium dependent mechanical oscillations occur spontaneously in unstimulated mammalian cardiac tissue. *Circulation Research.* 54:396–404.

Kruus, P. 1977. Liquids and Solutions: Structure and Dynamics. Marcel Dekker, New York. 240 pp.

Kushmerick, M. J., and R. J. Podolsky. 1969. Ionic mobility in muscle cells. *Science.* 166:1297–1298.

Lakatta, E. G., and D. L. Lappe. 1981. Diastolic scattered light fluctuation, resting force, and twitch force in mammalian cardiac muscle. *Journal of Physiology.* 315:369–394.

Matsuda, H., A. Noma, Y. Kurachi, and H. Irisawa. 1982. Transient depolarization and spontaneous voltage fluctuation in isolated single cells from guinea pig ventricles. Calcium-mediated membrane potential fluctuations. *Circulation Research.* 51:142–151.

Meissner, G., E. Darling, and J. Eudeth. 1986. Kinetics of rapid Ca^{++} release by sarcoplasmic reticulum. Effects of Ca^{++}, Mg^{++} and adenine-nucleotides. *Biochemistry.* 25:236–244.

Mulder, B. J. M., P. P. de Tombe, and H. E. D. J. ter Keurs. 1989. Spontaneous and propagated contractions in rat cardiac trabeculae. *Journal of General Physiology.* 93:943–961.

Ogawa, Y., N. Kurebayashi, A. Irimajiri, and T. Hanai. 1981. Transient kinetics for calcium uptake by fragmented sarcoplasmic reticulum from bullfrog skeletal muscle with reference to relaxation of living muscle. *Advances in Physiological Sciences.* 5:417–435.

Orchard, C. H., D. A. Eisner, and D. G. Allen. 1985. Oscillations of intracellular Ca^{++} in mammalian cardiac muscle. *Nature.* 304:735–738.

Phillies, G. D. T. 1974. Effects of intermolecular interactions on diffusion. I. Two component solutions. *Journal of Chemical Physiology.* 60:976–982.

Potter, J. D., S. P. Robertson, and J. D. Johnson. 1981. Magnesium and the regulation of muscle contraction. *Federation Proceedings.* 40:2653–2656.

Regirer, S. A., A. K. Tsaturyan, and G. G. Chernaya. 1986. Mathematical model of propagation of activated waves in isolated cardiac myocytes. *Biophysics.* 31:725–730.

Robertson, S. P., J. D. Johnson, and J. D. Potter. 1981. The time-course of Ca^{++} exchange with calmodulin, troponin, parvalbumin, and myosin in response to transient increases in Ca^{++}. *Biophysical Journal.* 34:559–569.

Smith, J. S., R. Coronado, and G. Meissner. 1985. Sarcoplasmic reticulum contains adenine nucleotide-activated calcium channels. *Nature.* 316:446–449.

ter Keurs, H. E., and B. Mulder. 1984. On the mechanism underlying triggered arrhythmias. A study of propagated after-contractions in rat cardiac trabeculae. *Journal of Physiology.* 353:59P. (Abstr.)

Wang, J. H. 1953. Tracer diffusion in ligands. IV. Self diffusion of calcium ion and chloride ion in aqueous calcium chloride solutions. *Journal of the American Chemical Society.* 75:1769–1770.

Weiss, R. E., and M. Morad. 1983. Birefringence signals in mammalian and frog myocardium. *Journal of General Physiology.* 82:79–117.

Wier, W. G., M. B. Cannell, J. R. Berlin, E. Marban, and W. J. Lederer. 1987. Cellular and subcellular inhomogeneity of $[Ca^{++}]_i$ in single heart cells revealed by FURA-2. *Science.* 235:325–328.

Wier, W., A. A. Kort, M. D. Stern, E. G. Lakatta, and E. Marban. 1983. Cellular calcium fluctuations in mammalian heart: direct evidence from noise analysis of aequorin signals in Purkinje fibers. *Proceedings of the National Academy of Sciences.* 80:7367–7371.

Wier, W. G., and D. T. Yue. 1986. Intracellular calcium transients underlying the short term force-interval relationship in ferret myocardium. *Journal of Physiology.* 376:507–530.

Wong, A. Y. K., A. Fabiato, and J. B. Bassingthwaite. 1987. Model of Ca release mechanism from the sarcoplasmic reticulum: Ca-mediated activation, inactivation and reactivation. *In* Electromechanical Activation, Metabolism and Perfusion of the Heart. S. Sideman and R. Bayer, editors. 281–295.

Yamaguchi, Y., Y. Komatisu, and H. Shimita. 1985. *In* Structure and Function of Sarcoplasmic Reticulum. S. Fleischer and Y. Tonomara, editors. Academic Press, Orlando, FL.

Yue, D. T. 1987. Intracellular $[Ca^{++}]$ related to rate of force development in twitch contraction of heart. *American Journal of Physiology.* 252:H760–770.