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MODEL OF CALCIUM-INDUCED CALCIUM RELEASE MECHANISM IN CARDIAC CELLS

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Abstract

A model with which to elucidate the mechanism of Ca^{2+} release from, and Ca^{2+} loading in the sarcoplasmic reticulum (SR) by Ca^{2+} current (I_{Ca}) in cardiac cells is proposed. The SR is assumed to be comprised of three functional subcompartments: (1) the main calcium store (MCS), which contains most of the calcium (both free and bound); (2) the releasable terminal (RT), which contains the calcium readily available for release; and (3) the longitudinal network of the SR (LSR), which sequesters and transfers the sarcoplasmic calcium to the RT. A rapid increase of the Ca^{2+} concentration at the outer surface of the SR (Ca_e) due to the fast component of I_{Ca} activates and inactivates this surface, inducing the release of Ca^{2+} from the RT to the sarcoplasmic space. The RT in turn is further activated and inactivated by a increase in the concentration of sarcoplasmic Ca^{2+} . The Ca^{2+} in the sarcoplasmic space is then sequestered by the LSR, leading to the reactivation of the RT. Further increase of Ca_e due to the slow component of I_{Ca} enhances the entry of Ca^{2+} into the MCS to be bound by the binding substance. The free Ca^{2+} released from the Ca-binding substance complex is transferred to the RT for subsequent release. The activation, inactivation and reactivation are Ca^{2+} -mediated and time-dependent. The proposed model yields simulation of the many events qualitatively similar to those observed experimentally in skinned cardiac cells.

Introduction

A transient increase of intracellular calcium is an important step coupling membrane excitation to muscular contraction. In mammalian cardiac muscle, calcium entry through the sarcolemma during the plateau of the action potential have been considered necessary for contraction. More than a decade ago, Bassingthwaigthe and Reuter (1972) postulated that the trans-sarcolemmal Ca^{2+} influx was insufficient for activating the myofilaments and that additional Ca^{2+} released from the internal stores was required.

Experiments on skinned cells (Fabiato and Fabiato, 1978; Fabiato, 1982) demonstrated that no stimulated trans-sarcolemmal influx of Ca^{2+} of any magnitude could activate the myofilaments directly. In isolated rat ventricular cells, the computed [total Ca^{2+}] was 7 μM and 10 μM during the initial 10 and 20 ms respectively of the fast Ca^{2+} current (Fabiato, 1983). This Ca^{2+} influx and the resting [Ca^{2+}] (7 μM) resulted in pCa values of 6.8 and 6.71 respectively. This meager rise in [free Ca^{2+}] is insufficient to induce any direct activation of the myofilaments.

The role of the trans-sarcolemmal Ca^{2+} seems to be to induce the release of Ca^{2+} from the intracellular Ca store—the sarcoplasmic reticulum (SR). The Ca^{2+} -induced release of Ca^{2+} had been demonstrated in skinned cardiac cells (Fabiato and Fabiato, 1975, 1977, 1978). Recent advances in single-channel conduction and gating experiments on canine (Rousseau *et al.*, 1986) and human cardiac SR (Holmberg and Williams, 1989) strongly support the concept of Ca^{2+} -induced Ca^{2+} release. These single-channel experiments did not elucidate the release mechanism or processes. However, Fabiato's experiments have suggested that the release mechanism is not an all-or-none process but is determined by three factors:

1. the level of preload of the SR with Ca^{2+} ;
2. the rate of change of trigger [free Ca^{2+}];
3. the level of [free Ca^{2+}] used as trigger.

Fabiato (1985b) demonstrated that in skinned Purkinje cells, the mechanism of Ca^{2+} -induced release of Ca^{2+} from the SR appeared to be via a channel with time- and Ca^{2+} -dependent activation and inactivation. Based on this concept, a preliminary model (Wong *et al.*, 1987) for Ca^{2+} -dependent Ca^{2+} release from the SR was proposed. Although the model fits well all experimental data tested, it does not include the mechanism involved in Ca^{2+} release from the SR by the fast component and Ca^{2+} loading in SR by the slow component of the trans-sarcolemmal Ca^{2+} influx (Fabiato, 1985c). Thus, the present model is an improved version in that it not only produces results quantitatively similar to the experimental observations, but also includes some processes not considered in the previous model.

This model formulation contains some features which have not been demonstrated experimentally, but which appear to be needed to provide behavior similar to the observed behavior of cardiac single cells and fiber bundle preparations. Thus, the model should be regarded as descriptive overall, and explicative in only certain well-defined features. The main innovative feature is the description of Ca^{2+} -dependent Ca^{2+} release, which is expressed in a fashion analogous to Hodgkin–Huxley kinetics, but the channel “conductance” is time- and [Ca^{2+}]-dependent rather than time- and voltage-dependent.

Overall Description of the Proposed Model

The schematic diagram shown in Fig. 1 represents a cardiac cell with intact sarcolemma. The SR comprises three subcompartments: (1) the main calcium store (MCS), which contains most of the calcium store in the SR and in which the calcium is predominantly bound; (2) the “releasable” terminal (RT), in which the calcium is mostly free and which

releases a fraction of its calcium content to the myofibrils to activate the contractile machinery; and (3) the longitudinal network (LSR), which sequesters the calcium from the sarcoplasmic space and transfers it to the RT.

To simulate experiments on skinned cardiac cells, the membrane in Fig. 1 is removed. At resting state, the calcium concentration at the outer surface of the SR (Ca_e) is low and this surface is completely inactivated. No calcium is released from the RT. When Ca_e increases rapidly, the outer surface of the SR is activated and inactivation of this surface is also initiated at a slower rate. Both the activation and inactivation are calcium (Ca_e)- and time-dependent, as described by Hodgkin–Huxley kinetics.

The activation of the SR outer surface triggers the release of calcium from the RT, resulting in an increase of calcium concentration in the sarcoplasmic space (Ca_{sp}). As the activation and inactivation of the RT is also Ca_{sp} - and time-dependent, more calcium release can be induced until inactivation becomes dominant. While Ca^{2+} is being released from the RT, [free Ca^{2+}] (Ca_f) in the MCS is increased due to the diffusion of Ca^{2+} from outside the SR and the dissociation of the Ca-binding substance complex. The free Ca^{2+} from the MCS and the LSR is transferred to the RT for subsequent release. The Ca^{2+} in the sarcoplasmic space is sequestered by an active process (J_{pump}). The decrease of Ca_{sp} results in the activation and reactivation of the RT.

Assumption and Formulation

(1) *Calcium concentration at the outer surface of the SR (Ca_e).* The calcium concentration at the outer surface of the SR is determined by:

- a. the rate and the amount of increase or decrease of Ca^{2+} from the external source (J_{in});
- b. the diffusion (J_D) of Ca^{2+} across the outer SR membrane into the MCS; and
- c. the calcium efflux (J_{ioCa}) from the MCS.

The rate of change of Ca_e is

$$dCa_e/dt = J_{in} + J_{ioCa} - J_D.$$

In experiments on skinned cells, the calcium source and sink can be considered very large. Therefore:

$$dCa_e/dt = J_{in}, \quad (1)$$

where J_{in} represents the rate of injection or aspiration of trigger Ca^{2+} .

(2) *Free calcium concentration in the MCS (Ca_f).* Calcium-binding substance exists in the SR (Solaro and Briggs, 1974). It is assumed that the free Ca^{2+} that enters the MCS binds to the free binding substance B (calsequestrin) by a simple first-order absorption, one Ca^{2+} molecule per site. The free calcium concentration in the MCS can be described in relation to the concentration of free binding substance B and bound Ca^{2+} (CaB) within the MCS:

$$\begin{aligned} dC_{a_f}/dt &= -K_1 \cdot B \cdot C_{a_f} + K_2 \cdot CaB + J_{net}, \\ dB/dt &= K_1 \cdot B \cdot C_{a_f} - K_2 \cdot CaB, \\ J_{net} &= J_D - J_{ioCa} - K_{fr} \cdot C_{a_f} / \tau_{fr}, \end{aligned} \quad (2)$$

where $J_D = D(\alpha) (Ca_e - Ca_f)$, $D(\alpha)$ is the diffusion coefficient, τ_{fr} is the time constant associated with the transfer of Ca_f to the RT, the K_{fr} is a constant of proportionality.

Binding is assumed at all times to be essentially at an equilibrium (Bassingthwaighe and Reuter, 1972), i.e.:

$$C_{a_f} \cdot B / CaB = K / K_2 = K_a, \quad (3)$$

where K_a is a dissociated constant. Taking the derivative of equation (3) and substituting in equation (2) gives:

$$\begin{aligned} dC_{a_f}/dt &= J_{net} \frac{(K_a + C_{a_f})^2}{(K_a + C_{a_f})^2 + B_t K_a}, \\ B_t &= B + CaB. \end{aligned} \quad (4)$$

(3) *Calcium concentration in the releasable terminal (Ca_{RT})*. The RT is assumed to differ only functionally from the MCS. The calcium content in the RT is free and is replenished by the free calcium transferred from the MCS and the LSR. The rate of release is determined by:

- i. the calcium gradient between the RT and the sarcoplasmic space;
- ii. the degree of activation and inactivation of the outer SR membrane, described by the kinetic parameters $p(t)$ and $q(t)$; and
- iii. the degree of activation and inactivation of the RT, which is described by the parameter $r(t)$, itself a function of Ca_{sp} .

The rate of change of Ca_{RT} is given by:

$$\begin{aligned} dC_{a_{RT}}/dt = & K_{fr} C_{a_f} / \tau_{fr} + K_{LSR} C_{a_{LSR}} / \tau_{LSR} \\ & - g_{Ca} \cdot p(t) \cdot q(t) \cdot r(t) (C_{a_{RT}} - C_{a_{sp}}) \\ & - J_{io}, \end{aligned} \quad (5)$$

where K_{fr} and K_{LSR} are constants, τ_{fr} and τ_{LSR} are time constants associated with the process of calcium transfer, g_{Ca} is the maximal rate of release, and J_{io} is the calcium efflux from the RT to the outside of the cell.

(4) *Calcium concentration in the sarcoplasmic space (Ca_{sp})*. The calcium concentration in the sarcoplasm is determined by the amount of calcium released from the SR, the uptake of calcium and the passive diffusion of calcium between the sarcoplasm and the outside of the cell. The rate of change of Ca_{sp} is:

$$\begin{aligned} dC_{a_{sp}}/dt = & g_{Ca} \cdot p(t) \cdot q(t) \cdot r(t) (C_{a_{RT}} - C_{a_{sp}}) \\ & - J_{pump} + D_e (Ca_e - C_{a_{sp}}), \end{aligned} \quad (6)$$

where J_{pump} is the uptake rate of the LSR and D_e is the diffusion coefficient.

(5) *Calcium concentration in the longitudinal sarcoplasmic reticulum* (Ca_{LSR}). The calcium content in the LSR depends on the Ca^{2+} uptake rate and the transfer of its content to the RT. This model assumes transmembrane transport to be the uptake mechanism. Kitazawa (1976) demonstrated that the Ca^{2+} uptake of fragmented chicken cardiac SR increased sigmoidally with free Ca^{2+} concentrations. At a certain free Ca^{2+} concentration, the Ca uptake increased exponentially with time and the initial uptake rate varies with the free Ca^{2+} concentration. The Ca uptake data suggests a process empirically described as:

$$J_{\text{pump}} = \frac{x(t)V_{\text{max}}\text{Ca}_{\text{sp}}^2}{(K_m + \text{Ca}_{\text{sp}}^2)} + \frac{f(t)V'_{\text{max}}\text{Ca}_{\text{sp}}}{K'_m + \text{Ca}_{\text{sp}}}$$

and

$$d\text{Ca}_{\text{LSR}}/dt = J_{\text{pump}} - K_{\text{LSR}}\text{Ca}_{\text{LSR}}/\tau_{\text{LSR}}, \quad (7)$$

where V_{max} , V'_{max} are maximum pump rates and K_m , K'_m are the concentrations at which the half-maximal pump rate occurs; K_{LSR} is a constant; τ_{LSR} , a time constant, is related to the apparent diffusion coefficient.

Both $x(t)$ and $f(t)$ are kinetic parameters describing the “conductance” of the channels of the RT surface. As $x(t)$ is slow-varying and its value is significant only at relatively high Ca_{sp} , Ca^{2+} uptake by the LSR starts near the end of Ca^{2+} release. The second term in J_{pump} accounts for the removal of Ca^{2+} during the relaxation phase.

(6) *Activation, inactivation and reactivation*. The activation and inactivation of the outer SR surface are characterized by two parameters— p and q . These parameters are governed by the first-order kinetic equation:

$$\begin{aligned} dy/dt &= [\alpha_y(1-y) - \beta_y y], \quad y=p, q; \\ y_{\infty} &= \alpha_y/(\alpha_y + \beta_y), \\ \tau_y &= 1/(\alpha_y + \beta_y). \end{aligned}$$

The rate constants α_y , β_y are functions of Ca_e . Thus, the steady-state parameters p_{∞} , q_{∞} and the time constants τ_p , τ_q are functions of Ca_e . To explain the experimental observations that the release and accumulation of Ca^{2+} are influenced by the rate (α) of change of Ca_e , it is necessary to assume that τ_p is inversely proportional to, and τ_q proportional to, the rate of change of Ca_e . Thus:

$$\begin{aligned} D(\alpha) &= D\alpha_0/\alpha, \\ \tau_p(\alpha, \text{Ca}_e) &= \alpha_0\tau_p(\text{Ca}_e)/\alpha, \quad (8) \\ \tau_q(\alpha, \text{Ca}_e) &= \alpha\tau_q(\text{Ca}_e)/\alpha_0; \end{aligned}$$

$$\text{Ca}_e = \text{Ca}_o[1 - \exp(-\alpha t)], \quad (9)$$

where Ca_0 is the maximal calcium concentration injected or aspired and α_0 is a certain rate constant chosen as standard. Equation (8) indicates that τ_p , τ_q are linearly and inversely proportional to the rate of change of Ca_e respectively. When dCa_e/dt is high, the outer SR surface is rapidly activated, but slowly inactivated. At low rate, the opposite occurs.

The activation, inactivation and reactivation of the RT is described by the parameter $r(t)$. However, $r(t)$ is comprised of four parameters: d, f, X , and x , such that:

$$\begin{aligned} r(t) &= d(t)f(t) - \lambda \bar{X}(t)x(t), \\ y(t) + \tau_y dy/dt &= y_\infty \quad y = d, f, \\ d\bar{X}/dt &= \alpha_{\bar{X}} \Delta pCa_e [(1 - X) - \beta_{\bar{X}} \bar{X}], \\ \Delta pCa_e &= 7.2 - pCa_e, \\ dx/dt &= \eta(pCa_e) [\alpha_x(1 - x) - \beta_x x], \\ \eta(pCa_e) &= \exp[0.6(pCa_e - 6.4)]. \end{aligned}$$

The steady-state parameters $d_\infty, f_\infty, x_\infty$ and the time constants τ_d, τ_f and τ_x are functions of Ca_{sp} ; X and τ_X are functions of the trigger calcium concentration (Ca_e). The parameter $r(t)$ should be considered as a variable that varies with Ca_{sp} and Ca_e , rather than as a Hodgkin–Huxley-type kinetic parameter. The formulations of the kinetic equations for X and x are similar to those used by Standen and Stanfield (1982).

(7) *Calcium efflux.* Calcium efflux is assumed to take place at two locations within the SR: the MCS and the RT. At the MCS, free Ca^{2+} is extruded across the SR membrane by a Ca^{2+} – Ca^{2+} exchange process (Wong and Bassingthwaighte, 1981):

$$J_{ioCa} = \phi(Na_o) [Ca_f / (K_1 + Ca_f)] [Ca_e / (K_2 + Ca_e)],$$

where $\phi(Na_o)$ is a function of $[Na^+]_o$ in the oppositional space or outside the SR, and K_1, K_2 are constants.

In the present model, the RT is replenished by free Ca^{2+} from the LSR and the MCS. When high $[Ca^{2+}]$ outside the cell is maintained, there will be a continuous production of free Ca^{2+} in the MCS and a continuous transfer of Ca^{2+} to the RT. If there were no Ca^{2+} extrusion from the RT to the outside of the cell, the RT would be loaded infinitely with Ca^{2+} —an impossible situation.

To prevent an unreasonable overloading of RT, a Ca^{2+} extrusion mechanism is assumed. This mechanism is described as:

$$J_{io} = K_{RT} [Ca_e / (K_3 + Ca_e)] F(Ca_{RT}).$$

The function $F(Ca_{RT})$ should be such that J_{io} is insignificant when Ca_{RT} is equal or below its resting value (14 μ M) but increases otherwise. An exponential function of the form:

$$F(Ca_{RT}) = 1.0 / \{1 + \exp[2(18 - Ca_{RT})]\}$$

seems to give the most satisfactory results.

(8) *Time constant associated with the calcium transfer.* The two time constants τ_{fr} and τ_{LSR} associated with the transfer of Ca_f and Ca_{LSR} to the RT are assumed to be inversely proportional to the apparent diffusion coefficient for Ca. Based on the study of Safford and Bassingthwaite (1977), the apparent diffusion coefficient for Ca_f is related to the degree of binding of Ca^{2+} by the binding substance. Thus:

$$\tau_{fr} \propto [(K_a + Ca_f)^2 + B_t K_a] / (K_a + Ca_f)^2.$$

The rate of transfer of Ca^{2+} from LSR to RT is again assumed to be related to $[Ca^{2+}]$ in the LSR. This is based on the assumption that some degree of binding occurs in the LSR and that the apparent diffusion coefficient for Ca_{LSR} varies nonlinearly with Ca_{LSR} . Therefore:

$$\tau_{LSR} \propto [K'_a + Ca_{LSR}]^2 + B'_t K'_a + Ca_f)^2.$$

Numerical Parameters

All model responses in this study were simulated with the same set of kinetic parameters, time constants and the following numerical constants:

$$\begin{aligned} \text{Resting } pCa_e, pCa_f, pCa_{sp}, pCa_{LSR} &= 7.4, \\ \text{Resting } Ca_{RT} &= 14\mu M, D = 0.005/ms, D_e = 0.0001/ms, \\ K_{fr} &= 0.005/ms, K_{LSR} = 0.005/ms, K_{RT} = 1.0\mu M/ms, \\ K_a &= 0.14\mu M, K'_a = 4.0\mu M, B_t = B'_t = 50\mu M, \\ K_1 = K_2 = K_3 &= 10\mu M, \sqrt{K_m} = 10\mu M, K'_m = 100\mu M, \\ \phi_{Ca} &= 0.15/ms, V_{max} = 0.5\mu M/ms, \\ \phi(Na_o) &= 0.5\mu M/ms, \lambda = 5.0. \end{aligned}$$

The above numerical values were chosen to produce an optimal performance of the model. The only variable in all simulations was the trigger $[Ca^{2+}]$ at the outer surface of the SR, described by equation (9).

Kinetic Parameters and Time Constants

Figure 2 depicts the steady-state parameters as functions of trigger pCa or sarcoplasmic pCa. The time constants, as functions of pCa, are shown in Fig. 3. These functions are chosen after trial and error so that the model produces results qualitatively similar to those observed experimentally.

Cyclic Release

Cyclic release of Ca from the SR of skinned cardiac cells of rat (Fabiato and Fabiato, 1975) and Purkinje cells (Fabiato, 1985b) occurs when the trigger Ca^{2+} above a critical level is maintained outside the cell. The amplitude and frequency of the cyclic release depends on the trigger Ca concentration. The cyclic release of Ca was simulated with the present model.

With a trigger $[Ca]$ of $1 \mu M$ ($pCa = 6.0$), the peak sarcoplasmic Ca (Ca_{sp}) is $2.62 \mu M$ with a cycle period of 11.6 s (Fig. 4a). At trigger $pCa = 5.4$, the peak Ca_{sp} is $5.27 \mu M$ and the cycle period is 6.8 s (Fig. 4b). At supraoptimal trigger Ca ($pCa = 5.2$), both peak Ca_{sp} ($4.38 \mu M$) and cycle period (5.25 s) are reduced (Fig. 4c). The simulation is in agreement with the experimental observations on skinned ventricular cell of rat (Fig. 8 of Fabiato and Fabiato, 1975).

Effect of Concentration and the Rate of Increase of Trigger Ca (Ca_e) on Ca Release

The bell-shaped relationship between the trigger pCa and peak sarcoplasmic Ca transient (Fig. 5) is mainly due to the activation p and the inactivation q of the outer SR surface. This is equivalent to the relation of peak Ca current and voltage. The effect of the rate of increasing trigger Ca on Ca release is indicated by the value of α [see equation (9)]. A higher value of α means a faster rate of increase.

The higher the rate of increase of trigger Ca , the greater the peak sarcoplasmic Ca , similar to the peak tension and trigger pCa relation shown by Fabiato (1985b). These are the consequences of the assumption that the time constants of activation (τ_p) and inactivation (τ_q) are functions of α (equation (8)). When trigger Ca is increased slowly (α is small), the outer SR surface is slowly activated, but rapidly inactivated, resulting in a reduction of the value of $p \cdot q$.

Rate of Inactivation and of Removal of Inactivation

Fabiato's experiments (1985b, Fig. 18) to demonstrate the rate of inactivation and of removal of inactivation were mimicked. The model response, shown in Fig. 6, is qualitatively similar to the experimental observations. The curve in Fig. 6a can be expressed as:

$$Ca = Ca_c + (Ca_m - Ca_c)[1 - \exp(-\tau/T)], \quad (10)$$

where Ca_c is the control peak Ca_{sp} ; Ca_m is the maximal peak Ca_{sp} ; τ is the delay from injection pCa 7.9 to the next triggering; T is the time constant of the exponential curve.

According to the present model scheme, it can be speculated that Ca_m reflects the magnitude of X_∞ and T , the time constant, τ_X at $pCa_e = 7.9$. The speculation is based on the release expression: $p \cdot q \cdot r (Ca_{RT} - Ca_{sp})$, $r = d \cdot f - X \cdot x$. At $pCa_e = 7.2$, $p \cdot q \approx 0$, $d \cdot f \approx 0$, $X \cdot x \approx 0$ but slowly decreases toward zero in 24 s or longer. Therefore, r is negative and the releasable terminal is inactivated. When pCa_e of 7.9 is introduced at a delay (τ) from the next trigger, $X(\tau)$ at the end of τ is:

$$\bar{X}(\tau) = \bar{X}_\infty(7.9) - [\bar{X}_0(7.2)] \exp[-\tau/\tau_{\bar{X}}(7.9)],$$

where $\bar{X}_0(7.2)$ is the value of X just before $pCa_e = 7.9$ is introduced.

In the present model, $\bar{X}_0(7.2) \approx 0$, and x , being very slow-varying, does not vary much within τ . Thus:

$$r \propto \bar{X}_\infty(7.9)[1 - \exp(-\tau/\tau_{\bar{X}}(7.9))].$$

As \bar{X}_∞ is negative, r has a positive value. The degree of activation of the releasable terminal increases exponentially with a time constant = $\tau_{\bar{X}}(7.9)$. To show that the time constant (T) in Fig. 6a is equal to $\tau_{\bar{X}}(7.9)$, Ca in equation (10) was computed (dotted curve) with $Ca_c = 2.51 \mu\text{M}$, $(Ca_m - Ca_c) = 1.20 \mu\text{M}$ and $T = \tau_{\bar{X}}(7.9) = 0.903 \text{ s}$, a value used in the present model.

The curve in Fig. 6b can be described as:

$$Ca = Ca_c + (Ca_m - Ca_c) \exp(-\tau/T), \quad (11)$$

where τ is the delay from reinjection of pCa 7.2 to the next triggering, and T is the time constant; Ca_m is the peak Ca_{sp} at $\tau = 0$.

When pCa 7.2 is reinjected after τ seconds, the value of \bar{X} at the end of τ is:

$$\bar{X}(\tau) = \bar{X}_\infty(7.2) - [\bar{X}_\infty(7.2) - \bar{X}_0(4, 7.9)] \exp[-\tau/\tau_{\bar{X}}(7.2)],$$

where $\bar{X}_0(4, 7.9)$ is the value of \bar{X} at the end of 4 s at pCa = 7.9.

As $\bar{X}(7.2) = 0$, $\bar{X}(\tau) = \bar{X}_0(4, 7.9) \exp[-\tau/\tau_{\bar{X}}(7.2)]$ indicates that before the next triggering, the degree of activation of the releasable terminal decays exponentially with a time constant of $\tau_{\bar{X}}(7.2)$. The dotted curve in Fig. 6b is described by equation (11) with $Ca_c = 2.51 \mu\text{M}$, $(Ca_m - Ca_c) = 1.20 \mu\text{M}$ and $T = \tau_{\bar{X}}(7.2) = 1.43 \text{ s}$, which is the value chosen in this model.

Simulation of Ca^{2+} Current

To simulate the fast component of Ca^{2+} current, Fabiato (1985c) injected trigger Ca^{2+} at pCa 5.0 in 10 ms and mixed with a volume of solution at pCa 7.0 injected at a slow rate. The slow component was made by mixing pCa 5.0 and pCa 7.0 solution at a slower rate. In the present study, the fast component was simulated by increasing the [free Ca^{2+}] from pCa 7.4 to pCa 5.0 in 10 ms (i.e. $\alpha = 0.6$ in equation (9)), which decayed to either pCa 6.4 or pCa 6.0 in 40 ms ($\alpha = 2.0$), and lasted for 200 ms. The [free Ca^{2+}] was slowly increased to a final pCa of 5.6 at two rates: $\alpha = 0.01$ for 300 ms and $\alpha = 0.025$ for the next 300 ms. This [free Ca^{2+}] was rapidly reduced to resting pCa level (e.g. 7.4). The total duration of fast and slow components was 810 ms.

Potentiation of Subsequent Ca^{2+} Transient by the Slow Component

Figure 7 shows the effect of adding a slow component to the trigger Ca^{2+} on the subsequent sarcoplasmic Ca^{2+} transient (Ca_{sp}). The first trigger Ca^{2+} (i.e. third pulse), comprising of fast and slow components, does not potentiate the sarcoplasmic Ca^{2+} , but increases the [free Ca^{2+}] sequestered by LSR are then transferred to the releasable terminal and become available for subsequent release.

Positive and Negative Staircase

An increase of frequency of stimulation by trigger Ca^{2+} with fast component only results in a negative staircase. In Fig. 8a, the first sarcoplasmic Ca^{2+} (Ca_{sp}) transient is triggered by a fast component after a long rest period. Subsequent stimulation at one per 6 s produces decreasing Ca^{2+} transient. In the absence of slow component, the $[\text{free Ca}^{2+}]$ production in MCS (Ca_{f}) is minimal. The higher frequency of stimulation reduces the time for the releasable terminal to be replenished by the $[\text{Ca}^{2+}]$ from MCS and LSR, resulting in a progressive reduction of calcium content in this compartment. In contrast, trigger Ca^{2+} with fast and slow components elicits a positive staircase (Fig. 8b) at the same frequency of stimulation (one per 6 s). The calcium content of the releasable terminal progressively increases due to the increase of $[\text{Ca}^{2+}]$ in the MCS.

Effect of Resting $[\text{free Ca}^{2+}]$ on the Subsequent Calcium Release

The initial conditions in Fig. 9a,b are identical. The trigger Ca^{2+} consists of a fast component only; a 10 ms pCa 5.0 decays rapidly to pCa 6.0 in 40 ms and lasts for 800 ms. The first sarcoplasmic Ca^{2+} transient is elicited by the trigger Ca^{2+} after a long rest period; it has a peak value of 3.1 μM . When the resting $[\text{Ca}^{2+}]$ during the interval between stimuli is lowered to $\text{pCa}_e = 7.8$ and stimulation frequency is increased (one per 6 s), the subsequent sarcoplasmic Ca^{2+} transient is potentiated (Fig. 9a). However, at a resting $[\text{free Ca}^{2+}]$ of $\text{pCa}_e = 7.4$, higher stimulation frequency produces a reduced Ca^{2+} transient in the next beat.

Fabiato (1985c) reasoned that when the resting $[\text{free Ca}^{2+}]$ was higher, the negative staircase elicited was caused by a progressive reduction in the degree of refilling of the SR during the interval between tension transients resulting from the increase of frequency. According to the model response, the potentiated Ca^{2+} transient at higher resting $[\text{free Ca}^{2+}]$ is not due to the loading of the releasable terminal. In fact, the calcium content in this compartment is reduced. The potentiation is caused by the elevated degree of reactivation of the releasable terminal ($r = d \cdot f - \lambda X \cdot x$).

At resting $\text{pCa}_e = 7.8$, X_{∞} is more negative, resulting in a larger positive value of r during the interval between stimuli. At $\text{pCa}_e = 7.4$, X_{∞} is less negative. The releasable terminal at the end of the resting period (6 s) has a smaller value of r and thus less Ca^{2+} is released in the subsequent beat.

Discussion

The present model has demonstrated that trans-sarcolemmal Ca^{2+} influx, such as Ca^{2+} current, can trigger Ca^{2+} release from and load the sarcoplasmic reticulum in cardiac cells. The demonstration is made possible by assuming that the activation, inactivation and the reactivation of the SR membrane is Ca^{2+} -mediated and time-dependent.

In many aspects, the proposed model is phenomenological as some of the assumptions do not seem to have a molecular basis. For example, it is difficult to explain how the rate of change of trigger Ca^{2+} affects τ_p , τ_q , the diffusion of Ca^{2+} into the MCS, or how trigger $[\text{Ca}^{2+}]$ can influence τ_x , which is primarily a function of sarcoplasmic $[\text{Ca}^{2+}]$. Another

objectionable feature is J_{io} , the calcium efflux from the RT to the outside of the cell. J_{io} can be eliminated by (1) making the free Ca^{2+} transferred from LSR to MCS instead of to RT. By modifying the numerical values of parameters of constants in Fig. 2, the free Ca^{2+} in RT can be limited to about 18 μM (Adler *et al.*, 1985). (2) Considering the MCS as the compartment which can store, release and sequester Ca^{2+} , then LSR is not necessary.

In the above two cases, it is necessary to modify or adjust the parameters and constants so that the amount of free Ca^{2+} produced in MCS is equal to the amount of Ca^{2+} sequestered, otherwise the free $[Ca^{2+}]$ in RT cannot be maintained. In other words, it is necessary to assume a high free Ca^{2+} in the MCS during resting state, as in the other E-C coupling models (Cannell and Allen, 1984; Adler *et al.*, 1985). If the free Ca^{2+} in the MCS were high, calcium would be extruded continuously across the SR membrane to the outside diffusion. High resting free Ca^{2+} within the MCS could be maintained only if the SR membrane was nondiffusible at the resting condition, or some “calcium pump” similar to the Na-K pump was assumed. However, these assumptions do not seem reasonable.

The releasable terminal, being part of the SR, does contain relatively high free Ca^{2+} ; this calcium is not extruded by diffusion. The separation of the SR into a “main calcium store” and the Releasable Terminal is for the convenience of mathematical formulation. One should perceive the MCS and RT together as one single compartment (i.e. SR), in which the resting free Ca^{2+} is high and calcium is extruded by a Ca-Ca exchange mechanism.

Cannell *et al.* (1987) reported the effect of depolarization duration on the time course of calcium transient (indicated by the fluorescent Ca^{2+} indicator fura-2) in single rat cardiac cells. They suggested that Ca release might be mediated by Calcium-Induced Calcium Release (CICR) and modulated directly by changes in voltage. An alternative scheme of Ca^{2+} release from the SR based on CICR and a voltage-dependent mechanism (Hilgemann and Noble, 1987) should be considered. However, in a review article, Fabiato (1985d) cited studies which demonstrated no change of SR potential during Ca^{2+} release induced by Ca^{2+} or caffeine, or no Ca^{2+} release induced by change of potential in single skinned cardiac cells. The effect of depolarization duration on calcium transient is equivalent to the effect of decreasing trigger $[Ca^{2+}]$ at different phases of Ca^{2+} transient (Fabiato, 1985b) and can be explained by the present model. The release parameter $r(t) = (f \cdot d - \lambda X \cdot x)$ is dependent on the sarcoplasmic calcium (Ca_{sp}) or intracellular Ca^{2+} and trigger Ca^{2+} (Ca_e) concentration. In a cardiac cell with sarcolemma, repolarization at different phases of the Ca^{2+} transient alters Ca^{2+} influx into the appositional space, changing Ca_e and $X(t)$. Subsequently, $r(t)$ is altered, affecting the amount of Ca^{2+} released and intracellular Ca^{2+} concentration.

Studies by Winegrad (1979, 1982) showed no evidence for different sites for Ca^{2+} release and accumulation in cardiac muscle. Experiments on skinned cardiac cells (Fabiato, 1981, 1983) suggest that the longitudinal SR is capable of both accumulating and releasing Ca^{2+} . A recent study by Fabiato (1985c) suggests a mechanism through which Ca^{2+} release and accumulation could take place with only a functional compartment caused by the time and Ca^{2+} dependence of activation and inactivation of Ca^{2+} release from the SR and of the Ca^{2+} accumulation into the SR.

The present model appears to be in conflict with the above concept as the release and accumulation of Ca^{2+} occurs at different sites or compartments (RT and LSR). However, the kinetic parameters $f(t)$ and $x(t)$ are involved in Ca^{2+} release and uptake. Furthermore, the LSR transfers free Ca^{2+} directly to RT for subsequent release. Arbitrarily, both RT and LSR could be lumped together as one functional unit capable of releasing and accumulating Ca^{2+} .

The model is consistent with the concept that the trans-sarcolemmal Ca^{2+} influx loads the SR with Ca^{2+} when it is slow and occurs during the refractory period of the process of Ca^{2+} -induced release of Ca^{2+} (Fabiato, 1985c). The refractory period in the model is determined by the release expression $(d \cdot f - \lambda X \cdot x)$. After the fast component of the Ca^{2+} influx triggers the release of Ca^{2+} , $x(t)$ increases and $f(t)$ decreases, making $(d \cdot f - \lambda X \cdot x)$ negative and model refractory. Within the refractory period, the slow component of the influx increases the $[\text{Ca}^{2+}]$ outside the SR. Subsequently, $[\text{Ca}^{2+}]$ enters the MCS, increasing its free Ca^{2+} content.

In many aspects, the kinetics of the proposed Ca release mechanism are similar to those of cardiac action potential (Beeler and Reuter, 1977). The parameters p , q , d and f can be considered equivalent to the activation and inactivation of inward currents, and $X \cdot x$ to the outward current. The rate of Ca released from releasable terminal, given as:

$$g_{\text{Ca}} \cdot p \cdot q \cdot (d \cdot f - \lambda \bar{X} \cdot x)(\text{Ca}_{\text{RT}} - \text{Ca}_{\text{sp}}),$$

differs little conceptually from the kinetics of ionic current, $I = g(E - E_r)$, in which g is the conductance and $(E - E_r)$ the driving force. The first term of the Ca release equation is equivalent to conductance, the second term to the driving force.

The expression for J_{pump} , despite its empirical nature, has its physiological function. As $X(t)$ is slow-varying and its value is small when Ca^{2+} is being released, Ca uptake is not significant, thus allowing the increase of sarcoplasmic Ca^{2+} at a faster rate to activate the myofilaments. Near the end of release, when $x(t)$ has become relatively large and the sarcoplasmic Ca^{2+} is high, the Ca uptake is accelerated. This is in agreement with the experimental observation (Fabiato, 1985b) that Ca uptake starts at the end of the release.

The delay of Ca uptake is physiologically significant. If release and uptake occurred simultaneously, more Ca^{2+} had to be released than necessary in order to be sufficient to activate the myofilaments; energy expenditure would be greater to keep the uptake process operating all the time.

An alternative and realistic scheme for J_{pump} is:

$$J_{\text{pump}} = K_{\text{ATP}} [\text{ATP}] [\text{Ca}_{\text{sp}}]^2 - K_{\text{ADP}} [\text{P}_i] [\text{ADP}] [\text{Ca}_{\text{LSR}}]^2.$$

In this equation, ATP promotes Ca uptake while ADP and P_i inhibits it. If ATP is depleted early there is an accumulation of P_i and ADP, for example during moderate or severe hypoxia (Nayler *et al.*, 1979), and the Ca accumulation by the LSR and the transport of Ca

to the releasable terminal will be depressed. However, the variation of local concentrations of ATP, P_i and ADP with time on a beat-to-beat basis is not clear.

Despite the fact that the “steady state” kinetic parameters and the time constants are “guessed” or speculated, the model is able to simulate many events qualitatively similar to those observed experimentally (Fabiato, 1985c). Perhaps the model can be refined if the kinetic parameters are determined more realistically. The amplitudes and rate of rise of aequorin light data vs trigger pCa of the Fabiato’s experiments (1985a,b) on skinned cells can provide clues for estimating p , q , d , f and their associated time constants. X , \bar{x} and τ_x can be estimated from the periodicity and amplitude of the cyclic release.

The present model can be applied directly to cardiac cells with intact sarcolemma by replacing the space at the outer surface of the SR with the oppositional space (APS). Thus equation (1) becomes:

$$dCa_c/dt = J_{in} + J_{ioCa} - (J_{out} + J_D).$$

J_{in} is the transmembrane calcium influx during a depolarization such as the calcium current. J_{out} is the transmembrane calcium efflux from APS to the extracellular space. The efflux is probably due to Ca–Na exchange and/or calcium pump. Both J_D and J_{ioCa} have the same meaning as defined in equation (1).

A small number of experiment observations on skinned cells cannot be simulated by the present model. This is because many mechanistic and molecular details of Ca^{2+} release and accumulation are still lacking in this study. Nevertheless, the model appears to confirm the hypothesis that the activation, inactivation and reactivation of Ca^{2+} release and accumulation processes are calcium-mediated and time-dependent, analogous in principle to Hodgkin–Huxley channel regulation by voltage.

Conclusion

A model has been proposed to describe the mechanism of calcium-induced calcium release from cardiac sarcoplasmic reticulum (SR). Based on the assumption that the activation, inactivation and reactivation of the SR calcium channels are Ca^{2+} -mediated and time-dependent, the model satisfactorily explains (a) how an increase of [free Ca^{2+}] outside the SR membrane induces a release of Ca^{2+} from the lumen of the SR, (b) why this phenomenon is graded rather than all-or-none, and (c) why trans-sarcolemmal Ca^{2+} influx such as Ca^{2+} current can trigger Ca^{2+} release from and also load the SR.

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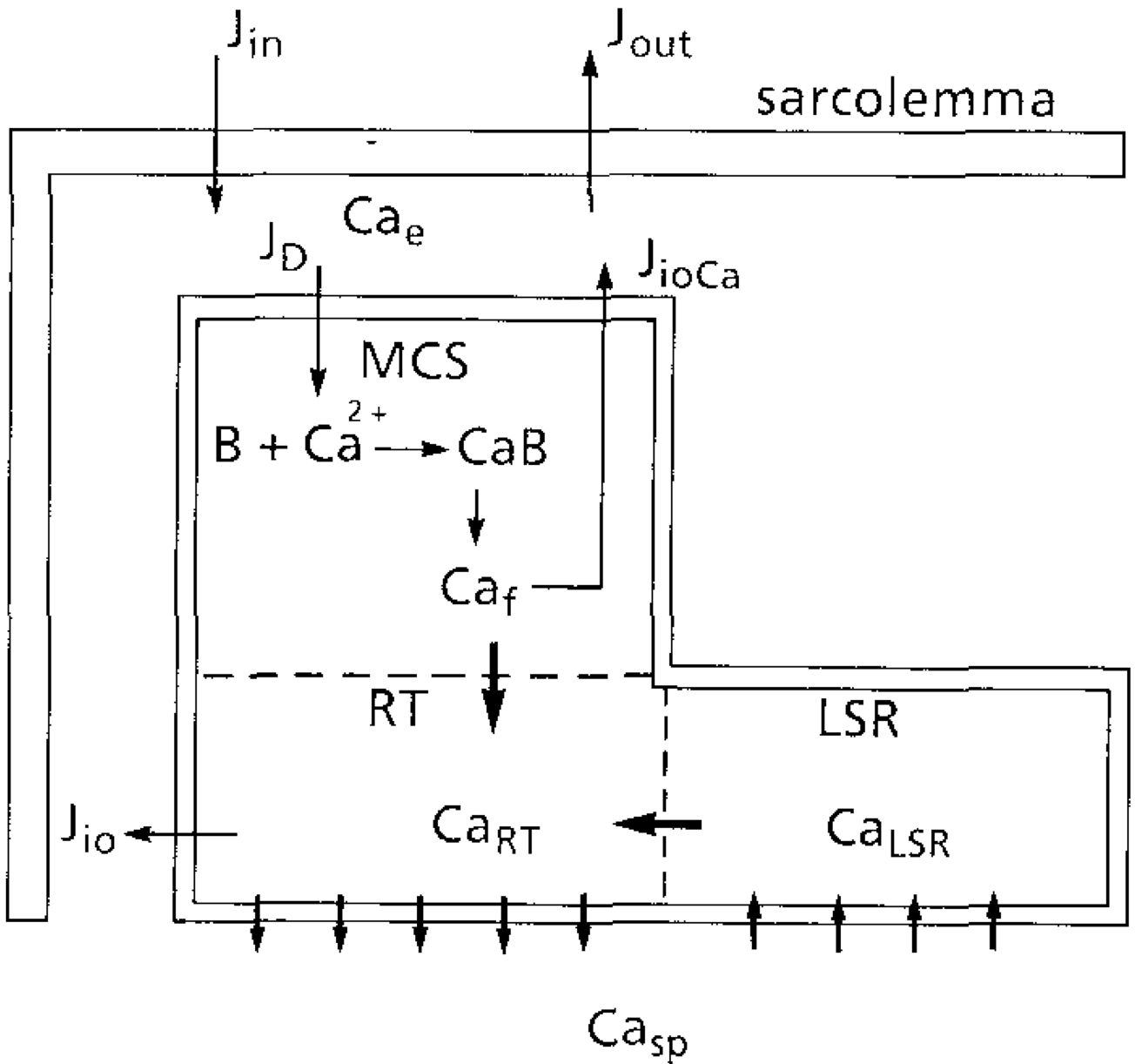


Figure 1.

Schematic diagram of the model. The sarcoplasmic reticulum (SR) comprises of: (1) Main Calcium Store (MCS) into which Ca^{2+} entering by diffusion is bound by the binding substance B; (2) a Releasable Terminal (RT) which releases Ca^{2+} to the sarcoplasmic space; (3) the longitudinal SR which sequesters Ca^{2+} from the sarcoplasmic space. Free Ca from MCS (Ca_f) and LSR (Ca_{LSR}) are transferred to RT with time constants τ_{fr} and $\tau_{LSR}J_{in}$ and J_{out} are the trans-sarcolemmal influx and efflux respectively. J_{ioCa} and J_{io} are Ca–Ca exchange between Ca_e and Ca_{RT} . Ca_e is the $[Ca^{2+}]$ between sarcolemma and SR; Ca_{sp} is the sarcoplasmic Ca^{2+} .

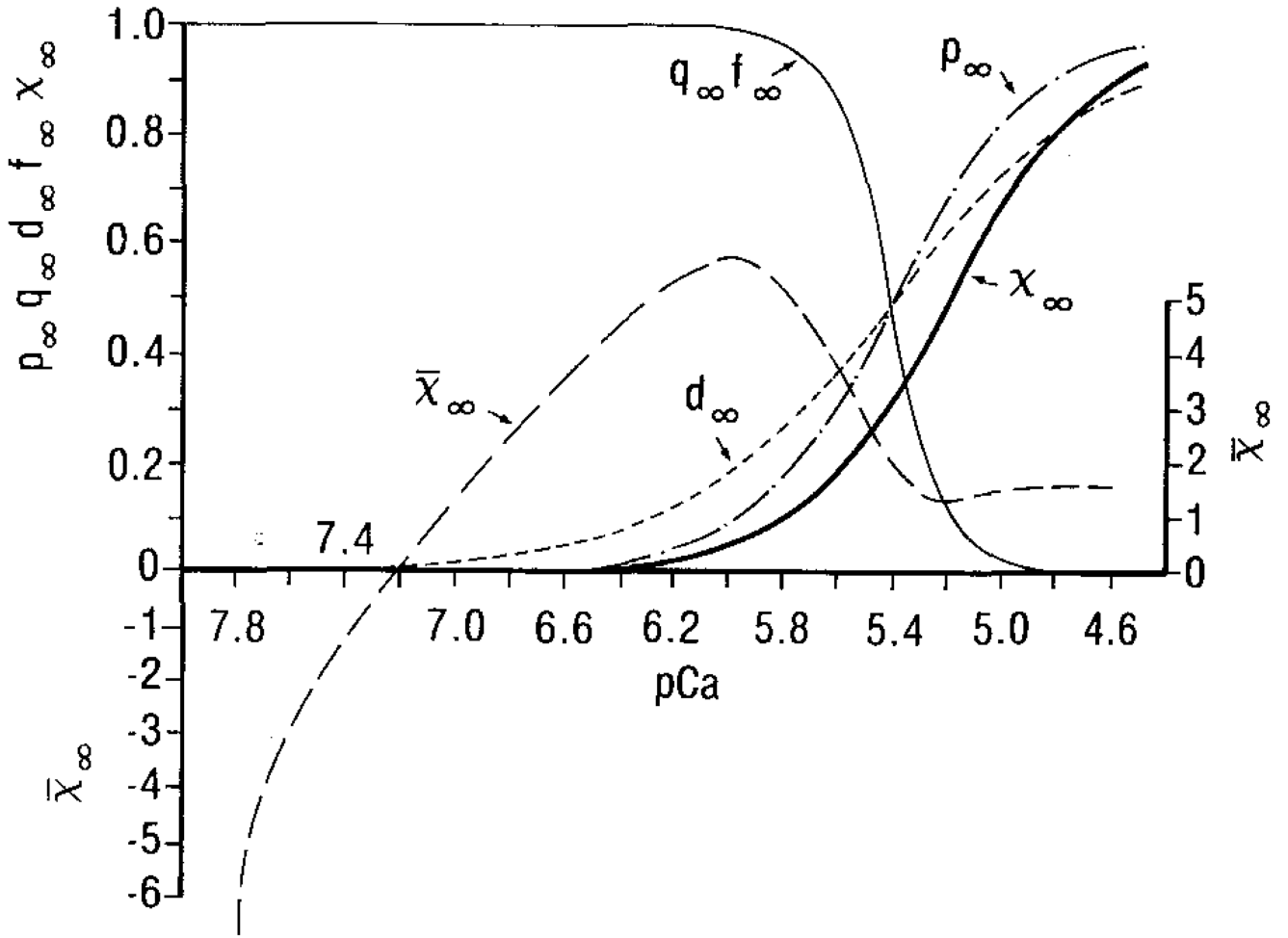


Figure 2. The steady-state values for the activation (q_∞) and inactivation (p_∞, λ_x) parameters as functions of pCa at the outer SR surface. The activation (d_∞) and inactivation (f_∞, x_∞) parameters for the RT are functions of the pCa of the sarcoplasmic space.

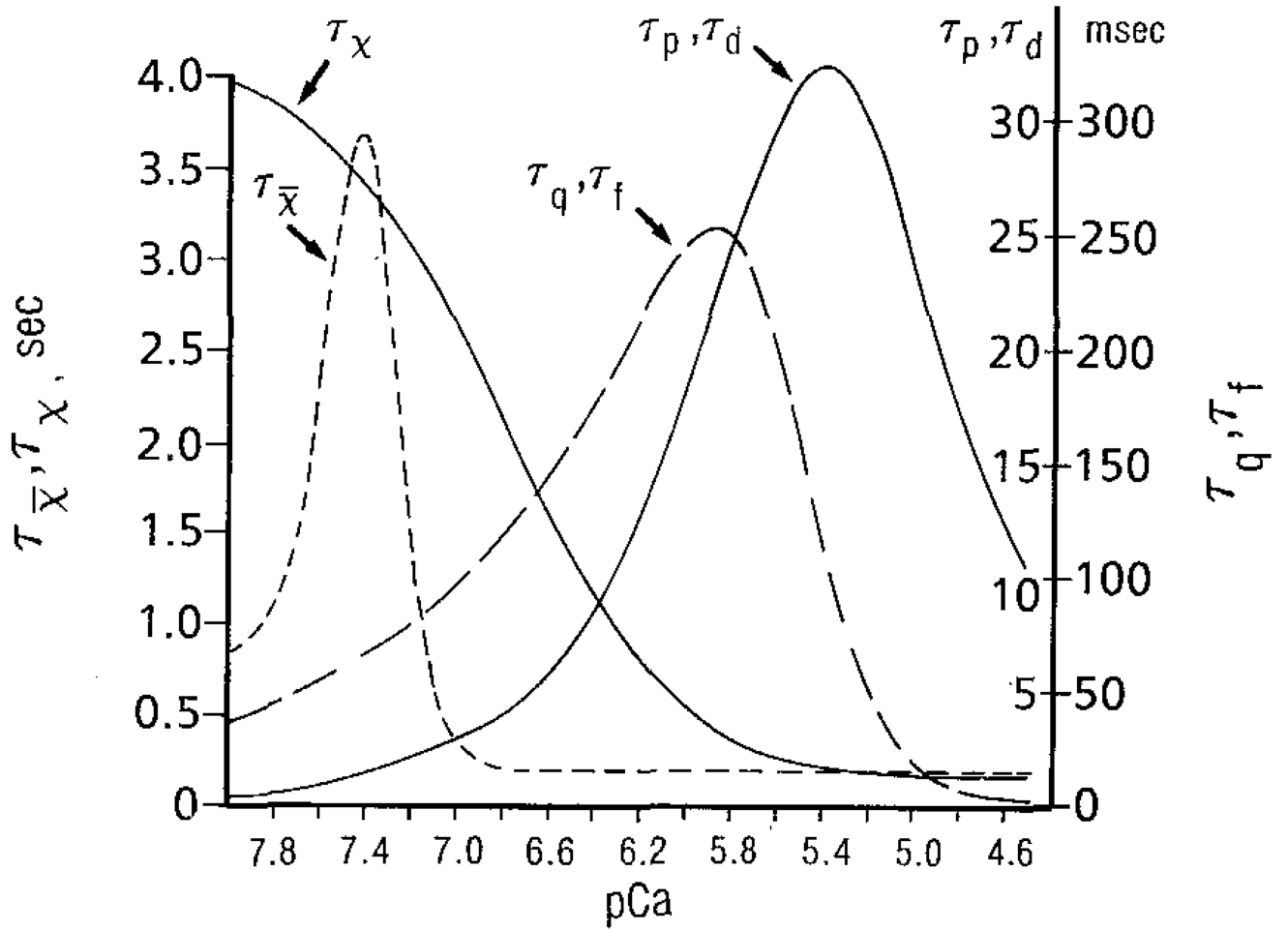
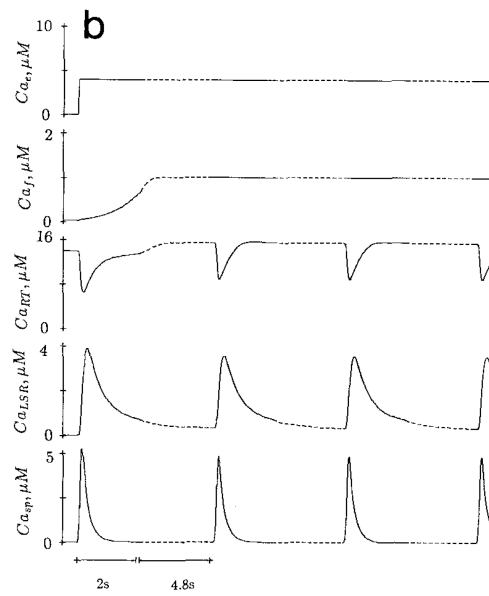
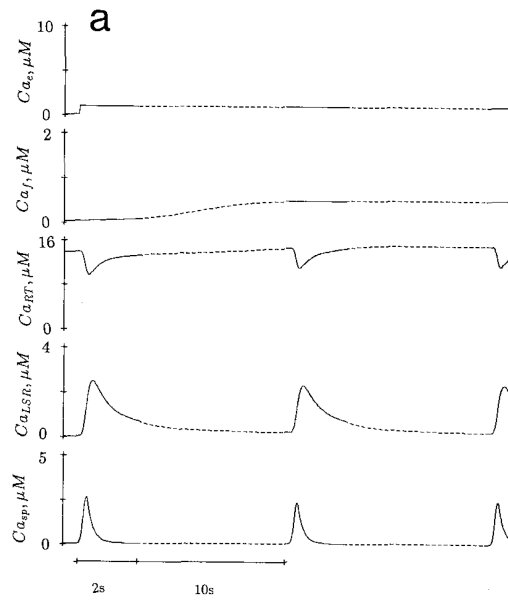


Figure 3.

Time constants τ_p , τ_q , and $\tau_{\bar{x}}$ are functions of the pCa of the outer SR surface; τ_f , τ_d , and τ_x are functions of the pCa of the sarcoplasmic space.



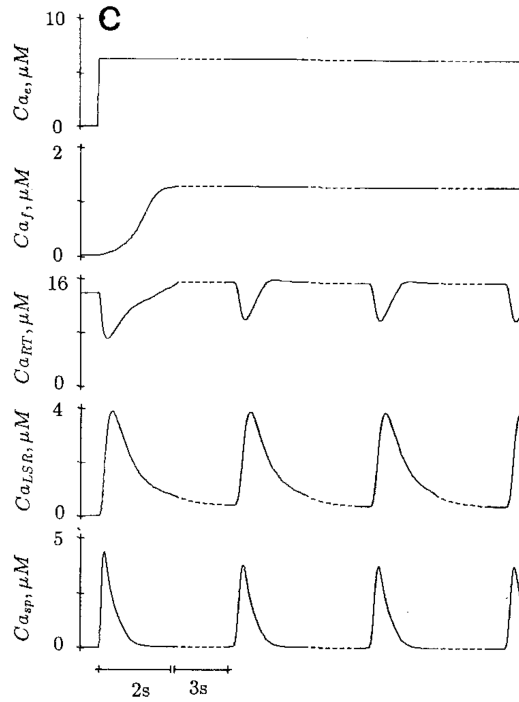


Figure 4. Simulated cyclic release of Ca at constant trigger Ca^{2+} (Ca_e). The cycle period is 11.6 s at pCa_e 6.0 (a), 6.8 s at pCa_e 5.4 (b) and 5.25 s at pCa_e 5.2 (c). The change of time scale (---) should be noted.

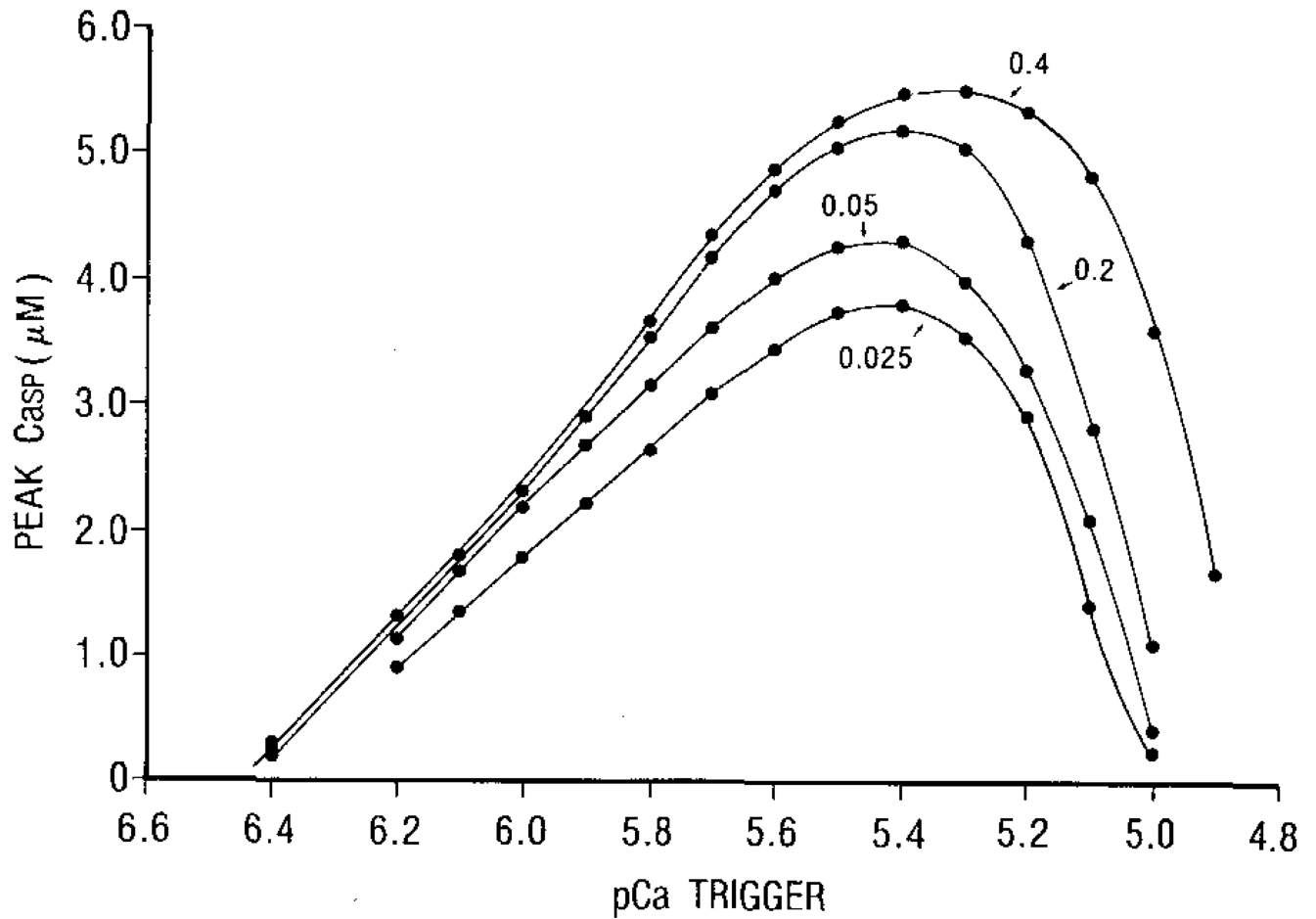


Figure 5.

The relation between the trigger pCa, the rate of rise of trigger PCa and the peak Ca_{sp} in the sarcoplasmic space. The numbers indicate the value of rate constant (α) in $Ca_e = Ca_0[1 - \exp(-\alpha t)]$. The larger the number, the faster the increase of Ca_e .

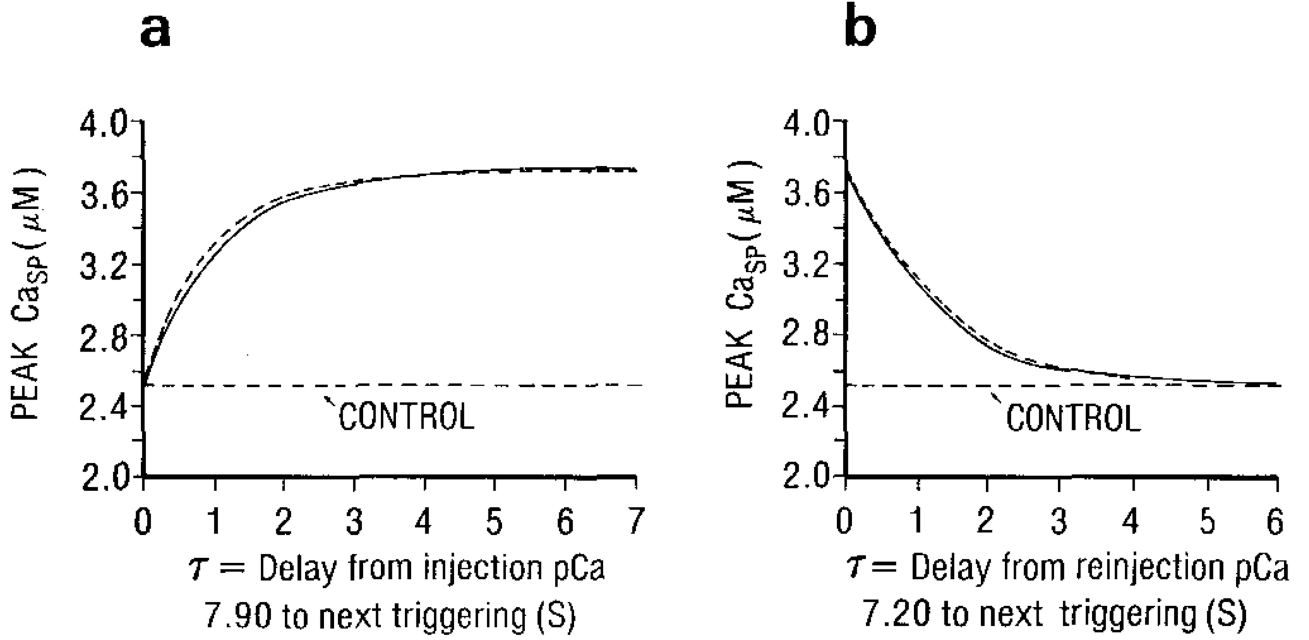


Figure 6. The simulated protocols (upper panels) and the model’s results (lower panel) demonstrate the rate of removal of inactivation (a) and the rate of inactivation (b). The duration of trigger Ca at pCa 6.0 is 0.5 s. The dotted curve in (a) is described by equation (10) with $T = \tau_X(7.9) = 0.903$ s, and in (b) by equation (11) with $T = \tau_X(7.9) = 1.43$ s.

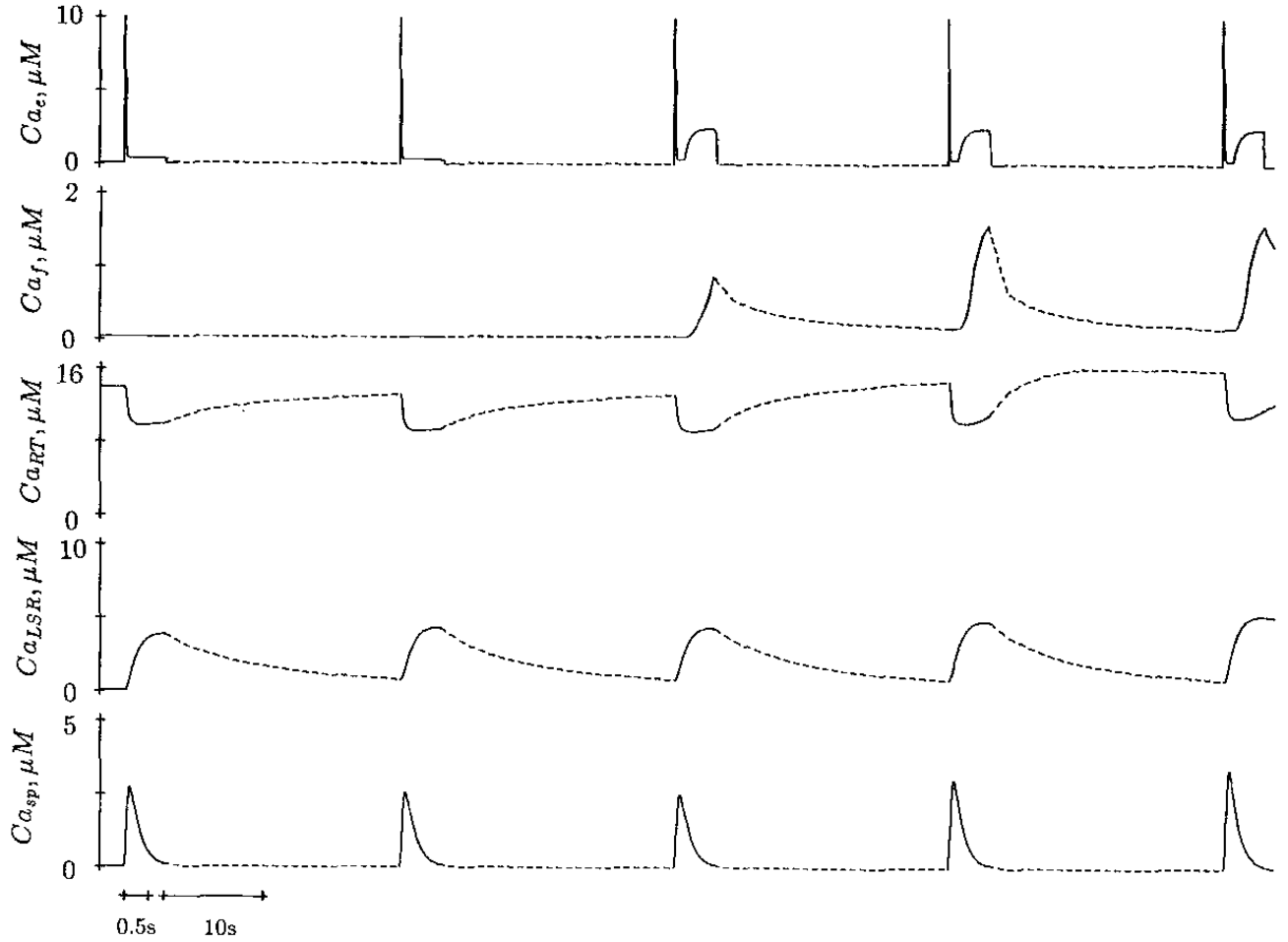


Figure 7.

Demonstration of the potentiation of subsequent Ca transient by the slow component of trigger Ca. The first two trigger Ca pulses (uppermost panel) consist of fast component only. The third pulse, comprising of fast and slow component, does not modify the corresponding sarcoplasmic Ca transient immediately. The fourth and fifth trigger Ca pulses potentiate the subsequent transient. Note the change of time scale (---).

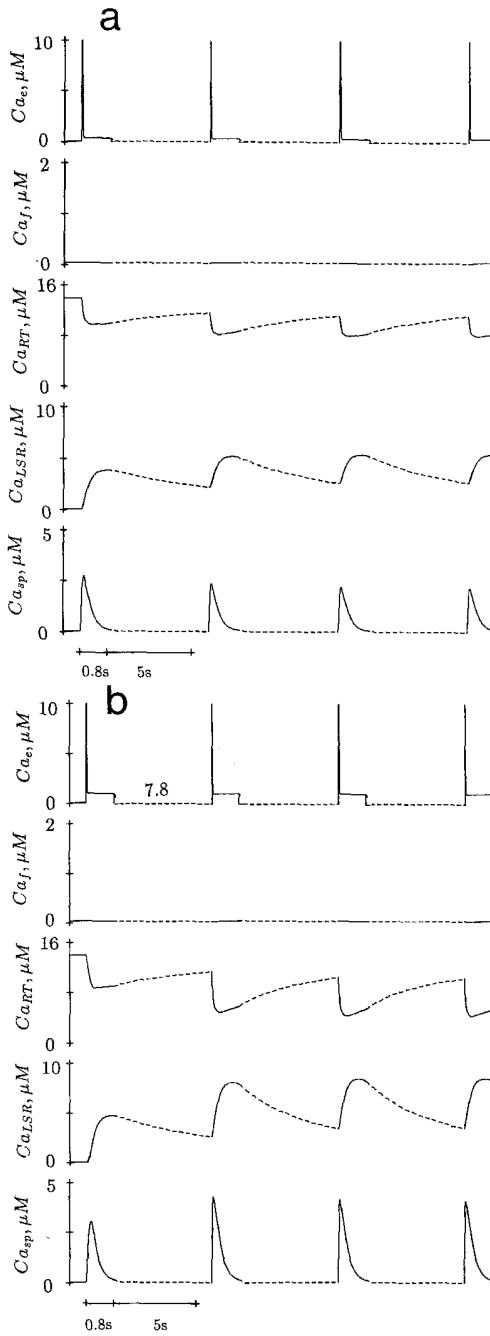


Figure 8. Negative staircase (a) is elicited by trigger Ca^{2+} with fast component only, and stimulated at a frequency of one per 6 s. Positive staircase (b) is caused by trigger Ca^{2+} with fast and slow components, stimulated at the same frequency. Change of time scale is indicated by the dotted line.

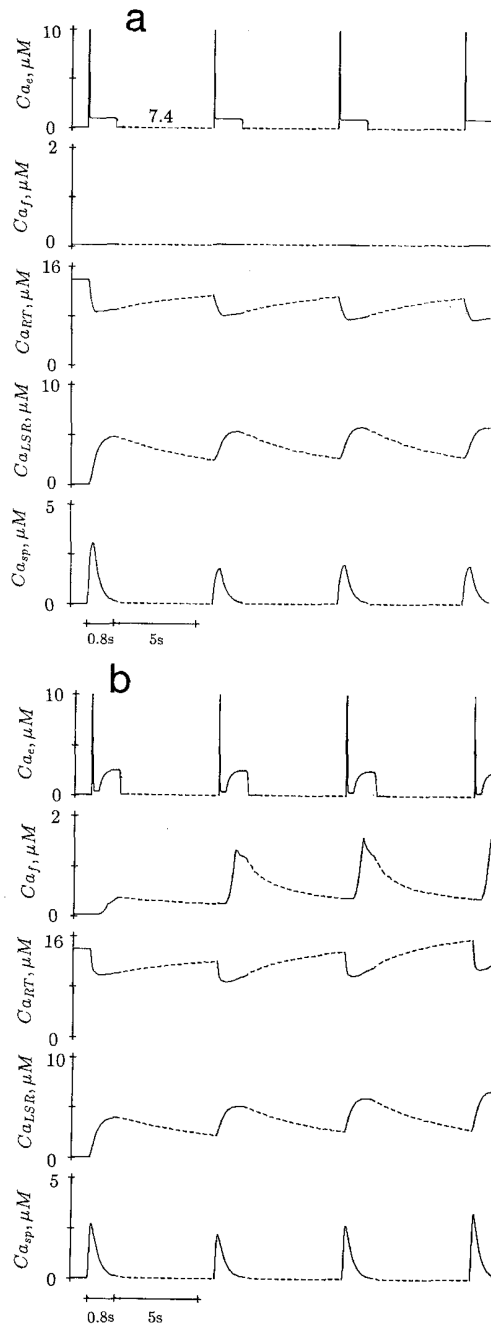


Figure 9. Effect of resting pCa_e during the interval between two stimuli on subsequent Ca^{2+} transient. The initial resting pCa_e is 7.4. After the first Ca^{2+} transient, the resting pCa_e is changed to 7.8. Dotted lines indicate smaller time scale.