Model for the Regulation of Ca^{2+} Release from the Sarcoplasmic Reticulum in Heart Muscle

Tsukasa TAMEYASU

Department of Physiology, St. Marianna University, Kawasaki, 216–8511 Japan

Abstract: To elucidate the essential features of Ca^{2+} release from the terminal sac (TS) of the sarcoplasmic reticulum (SR) in heart muscle, a model for Ca^{2+} release from the TS was constructed based on the mechanism of Ca^{2+} -induced Ca^{2+} -release by assuming a small TS volume. For numerical computation, we divided the cytoplasm in a half sarcomere into 50 compartments, one end of which faced the TS and determined the open probability of the Ca^{2+} release channel in the TS. Ca^{2+} moves from compartment to compartment by simple diffusion and is taken up from each compartment by the tubular SR via Ca^{2+} -ATPase. Simulation based on one-dimensional diffusion of Ca^{2+} showed that TS Ca^{2+} release is smoothly graded over a wide range of levels of Ca^{2+} influx when the TS is

moderately loaded with Ca^{2+} , and it occurs in an all-or-none manner when the TS is heavily loaded with Ca^{2+} . The graded TS Ca^{2+} release was generated with both local and uniform Ca^{2+} influx into the cytoplasm. The propagation of the Ca^{2+} wave was simulated by the use of a model consisting of several longitudinally consecutive sarcomeres with TS heavily loaded with Ca^{2+} . Mechanical alternans, characterized by an alternation of small and large twitches, was also simulated by applying Ca^{2+} influx at an appropriate interval during which Ca^{2+} was replenished to the TS at a constant rate. Thus the present model reflects several important aspects of TS Ca^{2+} release in heart muscle. [Japanese Journal of Physiology, 52, 57–68, 2002]

Key words: heart muscle, sarcoplasmic reticulum, Ca^{2+} wave, mechanical alternans, Ca^{2+} -induced Ca^{2+} -release.

In mammalian heart muscle, a small amount of Ca^{2+} influx (I_{Ca}) through the L-type Ca^{2+} channel during depolarization of the sarcolemma causes a large amount of Ca^{2+} release from the terminal sac (TS) of the sarcoplasmic reticulum (SR) via the mechanism of Ca^{2+} -induced release of Ca^{2+} (CICR) [1, 2]. In principle, CICR leads to an all-or-none type TS Ca^{2+} release, since Ca^{2+} released from the TS raises the free Ca^{2+} concentration in the cytoplasm ($[\text{Ca}^{2+}]_{\text{cy}}$), causing a further release of Ca^{2+} from the TS. Such regenerative TS Ca^{2+} release has been thought to underlie the “ Ca^{2+} wave” in which TS Ca^{2+} release occurs over many sarcomeres in succession [3–7]. Physiologically, however, TS Ca^{2+} release is smoothly graded over a wide range of I_{Ca} [8, 9]. To account for the graded TS Ca^{2+} release, Fabiato [1] suggested a time- and Ca^{2+} -

dependent inactivation of TS Ca^{2+} release. However, subsequent studies with isolated ryanodine receptors (Ca^{2+} release channels) suggested that inactivation is, if present, too slow to account for the pattern of physiological TS Ca^{2+} release [10–13].

Alternatively, another idea, local control theory, assumes that I_{Ca} transiently raises the cytoplasmic $[\text{Ca}^{2+}]$ near the Ca^{2+} release channels in the TS facing the cytoplasmic side of the L-type Ca^{2+} channels at the “diad” [14–16]. The local control theory assumes that to grade the TS Ca^{2+} release depending on I_{Ca} and to prohibit regenerative TS Ca^{2+} release, the Ca^{2+} release channels open at high levels of cytoplasmic $[\text{Ca}^{2+}]$ that are attained only by I_{Ca} . However, this assumption makes it difficult to interpret the Ca^{2+} wave straightforwardly in terms of this theory, since the

Received on June 29, 2001; accepted on November 21, 2001

Correspondence should be addressed to: Tsukasa Tameyasu, Department of Physiology, St. Marianna University School of Medicine, 2–16–1 Sugao, Miyamae-ku, Kawasaki, 216–8511 Japan. Tel and Fax: +81–44–977–3915, E-mail: t2tame@marianna-u.ac.jp

$[\text{Ca}^{2+}]$ triggering TS Ca^{2+} release is equal to or lower than the maximum $[\text{Ca}^{2+}]_{\text{cy}}$ attained by Ca^{2+} release from other TSs. A further weak point of this theory is that the TS continues to release Ca^{2+} for a substantial period of time after the termination of I_{Ca} [17].

Recent experiments in which local changes of $[\text{Ca}^{2+}]_{\text{cy}}$ were visualized by the use of a laser-scanning confocal microscope made it possible to detect a spontaneous, very small amount of TS Ca^{2+} release under certain conditions. A unitary Ca^{2+} release called a “ Ca^{2+} spark” (or “ Ca^{2+} quark”) may represent Ca^{2+} release from a single Ca^{2+} release channel or a few channels acting in concert [17–21]. It is uncertain, however, how such a unit is recruited in graded TS Ca^{2+} release or whether each unit releases a fixed amount of Ca^{2+} [22–24]. Therefore a macroscopic model based on CICR is still helpful for understanding the TS Ca^{2+} release in heart muscle.

So far, the volume of the TS has not been seriously considered when TS Ca^{2+} release in heart muscle is being interpreted. It is important to consider the smallness of the effective TS volume relative to the volume of the cytoplasm [25] in the case of TS Ca^{2+} release, since the amount of Ca^{2+} in the small TS will decrease very rapidly upon the release of Ca^{2+} . In the present study, taking the small TS volume and $[\text{Ca}^{2+}]$ gradient in the cytoplasm [26, 27] into account and assuming a regenerative TS Ca^{2+} release, a model for TS Ca^{2+} release in heart muscle was constructed based on CICR. A numerical simulation based on the present model showed that a Ca^{2+} release from TS moderately loaded with Ca^{2+} is smoothly graded with a change of I_{Ca} , whereas the TS heavily loaded with Ca^{2+} releases Ca^{2+} in an all-or-none manner, enabling the propagation of the Ca^{2+} wave. The graded TS Ca^{2+} release was produced with either local or uniform I_{Ca} to the cytoplasm. Furthermore, mechanical alternans, characterized by an alternation of small and large twitches and produced experimentally by stimulating heart muscle at high frequency from rest at low extracellular $[\text{Ca}^{2+}]$ [28–30], was simulated based on the present model. The effects of changing the values of parameters used in the present study were also examined. A preliminary account of the present model has been presented at an international symposium [31].

MODELING

The Ca^{2+} gradient is formed from the cytoplasmic surface of the TS toward the middle of the sarcomere [26]. TS Ca^{2+} release is regulated by $[\text{Ca}^{2+}]$ at the cytoplasmic surface of the TS ($[\text{Ca}^{2+}]_{\text{tsl}}$). Since the open

probability of the Ca^{2+} release channel is reported to rise with an increase of $[\text{Ca}^{2+}]$ with the Hill coefficient $n=1.05$ [13], a first order reaction was used for the on–off switch of the Ca^{2+} release channel as follows:

where k_{on} and k_{off} are on and off rate constants, respectively. Then,

$$\begin{aligned} d[\text{Ca-channel}]/dt \\ = k_{\text{on}}[\text{Channel}][\text{Ca}^{2+}] - k_{\text{off}}[\text{Ca-channel}]. \end{aligned} \quad (2)$$

Ca^{2+} in the TS lumen is assumed to exist in an ionic form at a concentration expressed as $[\text{Ca}^{2+}]_{\text{tsl}}$. The rate of TS Ca^{2+} release is proportional to the number of Ca^{2+} release channels in the open state, which is obtained by multiplying the total number of Ca^{2+} release channels and the open probability. The rate of TS Ca^{2+} release, or the rate of decrease of $[\text{Ca}^{2+}]_{\text{tsl}}$ in another expression, is also proportional to the difference of $[\text{Ca}^{2+}]$ across the TS membrane ($[\text{Ca}^{2+}]_{\text{tsl}} - [\text{Ca}^{2+}]_{\text{tso}}$). Therefore,

$$d[\text{Ca}^{2+}]_{\text{tsl}}/dt = -\alpha[\text{Ca-channel}]([\text{Ca}^{2+}]_{\text{tsl}} - [\text{Ca}^{2+}]_{\text{tso}}), \quad (3)$$

where α is a constant concerned with conversion from the quantity to the concentration of Ca^{2+} .

Bassani *et al.* [32] reported that more than 90% of Ca^{2+} released from the TS is taken up by the tubular SR through Ca^{2+} -pumps and the rest mainly by the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger. For simplicity, we assumed that all the Ca^{2+} released from the TS is taken up by the tubular SR. The velocity of the Ca^{2+} uptake (v) is described by the following equation,

$$v = V_{\text{max}}([\text{Ca}^{2+}])^n / \{ (K_m)^n + ([\text{Ca}^{2+}])^n \}, \quad (4)$$

where V_{max} is the maximum uptake velocity, K_m the $[\text{Ca}^{2+}]$ at $v=(1/2)V_{\text{max}}$, and n the Hill coefficient.

SIMULATION

For the present purposes, a one-dimensional diffusion of Ca^{2+} along the length of the sarcomere is assumed. The cytoplasm in a half sarcomere is divided into 50 compartments, which are numbered sequentially from the level of the Z-line toward the middle of the sarcomere so that each compartment is 20-nm long (Fig. 1). The first compartment faces the TS, so that the opening of the Ca^{2+} release channels in the TS is regulated by $[\text{Ca}^{2+}]$ in the first compartment ($[\text{Ca}^{2+}]_1$). Then,

Fig. 1. Schematic drawing of sarcomere (upper) and compartmentalization of a half sarcomere for simulation (lower). TS, terminal sac; T-tubule, transverse tubule; Z, Z-line. Lower, large arrows show movement of Ca^{2+} . For details, see text.

$$d[\text{Ca}^{2+}]_1/dt = (V_1/V_{\text{ts}})d[\text{Ca}^{2+}]_{\text{tsl}}/dt - \beta([\text{Ca}^{2+}]_1 - [\text{Ca}^{2+}]_2), \quad (5)$$

where subscripts designate compartments, β is a constant related to the diffusion of Ca^{2+} and V_1 and V_{ts} the volumes of the first compartment and the TS, respectively. The value of β was determined by the use of formulae derived for diffusion in a finite system with simple geometry (see APPENDIX).

Ca^{2+} in the 2nd–50th compartments is taken up by the Ca^{2+} pump in the tubular SR at a rate of v (Eq. 4). Ca^{2+} taken up by the tubular SR is not available for the ongoing Ca^{2+} release. For the i compartment ($1 < i < 50$), therefore,

$$d[\text{Ca}^{2+}]_i/dt = \beta([\text{Ca}^{2+}]_{i+1} + [\text{Ca}^{2+}]_{i-1} - 2[\text{Ca}^{2+}]_i) - v. \quad (6)$$

For the 50th compartment,

$$d[\text{Ca}^{2+}]_{50}/dt = \beta([\text{Ca}^{2+}]_{49} - [\text{Ca}^{2+}]_{50}) - v. \quad (7)$$

A numerical computation was made for $[\text{Ca}^{2+}]$ in each compartment for every unit of time ($10 \mu\text{s}$), using Eqs. 2–7 with the parameters listed in Table 1 unless otherwise stated. TS Ca^{2+} release is initiated by introducing I_{Ca} with a time course similar to that during an action potential in rat ventricular muscle [33], i.e., I_{Ca} increases linearly during the initial 5 ms, followed by a linear decrease to zero level during the subsequent 20 ms (Fig. 2A). Unless otherwise stated, such I_{Ca} with a triangular shape was introduced uniformly to all compartments. The value of I_{Ca} is expressed as an integral of I_{Ca} per compartment during

the initial 25 ms.

RESULTS

$[\text{Ca}^{2+}]$ gradient in sarcomere

Figure 2 shows an example of Ca^{2+} release from TS moderately loaded with Ca^{2+} (i.e., 1 mM) caused by I_{Ca} of $0.625 \mu\text{M}$. The changes of $[\text{Ca}^{2+}]_{\text{tsl}}$, $[\text{Ca}^{2+}]_1$, and mean $[\text{Ca}^{2+}]$ in the cytoplasm ($[\text{Ca}^{2+}]_{\text{av}}$) given by averaging $[\text{Ca}^{2+}]$ in the 1st–50th compartments are shown in Fig. 2B, along with the change of $[\text{Ca}^{2+}]_{\text{av}}$ without TS Ca^{2+} release (asterisk). This graph shows clearly that TS Ca^{2+} release occurs regeneratively after the termination of I_{Ca} . The distribution of $[\text{Ca}^{2+}]$ along the compartments after the onset of I_{Ca} is shown in Fig. 2C and D, revealing a steep $[\text{Ca}^{2+}]$ gradient from the 1st to 50th compartments around the peak of the c1 curve, i.e., during the initial several tens of ms, in agreement with a previous report [26].

Effects of parameters

The main simulations of TS Ca^{2+} release were made by using the parameters (standards) listed in Table 1. The standards were selected by trial and error to obtain satisfactory simulations. For an understanding of the degree of contribution of each of the parameters to the results, it is worthwhile to show some examples of TS Ca^{2+} release when the value of one of the parameters is changed around the standard. For this purpose, a TS Ca^{2+} release with 1 mM $[\text{Ca}^{2+}]_{\text{tsl}}$ at I_{Ca} of $0.625 \mu\text{M}$ under the condition in which the value of any one of the parameters is varied around the standard is shown in the following.

k_{on} and k_{off} . As shown in Fig. 3A, an increase of k_{on} from the standard of 1.5 to $2.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ greatly increased TS Ca^{2+} release, but k_{on} below $1.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ caused no substantial TS Ca^{2+} release. A reduction of k_{off} from the standard of 1.0 to $0.5 \times 10^4 \text{ s}^{-1}$ increased TS Ca^{2+} release, but k_{off} above $1.5 \times 10^4 \text{ s}^{-1}$ caused no substantial TS Ca^{2+} release (Fig. 3B).

V_{max} and K_{m} . Figure 4A shows the effect of changing V_{max} on TS Ca^{2+} release. Little TS Ca^{2+} release occurred when V_{max} was one order of magnitude lower than the standard, and nearly all the Ca^{2+} was released from the TS when V_{max} was one order of magnitude higher than the standard. As K_{m} increased from 0.14 to $0.70 \mu\text{M}$ in $0.14\text{-}\mu\text{M}$ steps, the TS Ca^{2+} release increased gradually as shown in Fig. 4B.

β . Since β is the rate for diffusion from one compartment to another, an increase of β causes a rapid loss of Ca^{2+} from the first compartment by diffusion to the second compartment. This results in a rapid lowering of the $[\text{Ca}^{2+}]_1$, leading to a small amount of

Fig. 2. (A) Time course of I_{Ca} . Rate (left-hand scale) and total amount of I_{Ca} (right-hand scale) are shown. (B) An example of the changes of $[\text{Ca}^{2+}]_{\text{tsl}}$, $[\text{Ca}^{2+}]_1$, and $[\text{Ca}^{2+}]_{\text{av}}$. The change of $[\text{Ca}^{2+}]_{\text{av}}$ without any TS Ca^{2+} release is also shown (asterisk). In this and subsequent figures, the left-hand scale is for $[\text{Ca}^{2+}]_1$, indicated by c1, and $[\text{Ca}^{2+}]_{\text{av}}$, indi-

cated by av (bold curve), and the right-hand scale is for $[\text{Ca}^{2+}]_{\text{tsl}}$, indicated by tsl. (C) and (D) $[\text{Ca}^{2+}]$ gradient along compartments during TS Ca^{2+} release shown in B. The $[\text{Ca}^{2+}]$ gradient from 5 to 50 ms after the onset of I_{Ca} is shown in C and from 55 to 100 ms in D in 5 ms steps.

Table 1. Parameters used for simulation and their reported values.

Parameter	This study	Reported value
k_{on} ($\text{M}^{-1} \text{s}^{-1}$)	1.5×10^8	2×10^8 [13]
k_{off} (s^{-1})	1.0×10^4	2×10^2 [13]
V_{max} (mM s^{-1})	0.05	0.21 [39]
K_{m} (μM)	0.28	0.28
n	2	2
α	0.001	
β	0.24	
D ($\text{cm}^2 \text{s}^{-1}$)	4.2×10^{-7}	5×10^{-6} [40]
$V_{\text{ts}}/V_{\text{cy}}$	5×10^{-3}	3.5×10^{-3} [26]
Unit of time (s)	1.0×10^{-5}	
$[\text{Ca}^{2+}]_{\text{av}}$ at rest (M)	1.0×10^{-8}	

TS Ca^{2+} release. Thus an increase of β led to a small TS Ca^{2+} release and vice versa (Fig. 5A).

TS volume. The volume of TS (V_{ts}) relative to that of the cytoplasm (V_{cy}) greatly affected the TS Ca^{2+} release. An increase of $V_{\text{ts}}/V_{\text{cy}}$ caused an increase of the total amount of TS Ca^{2+} release (Fig. 5B). The augmentation of TS Ca^{2+} release because of an increase of $V_{\text{ts}}/V_{\text{cy}}$ was accompanied by a slowing of the

TS Ca^{2+} release, as indicated by a late peak in the $[\text{Ca}^{2+}]_{\text{av}}$. Such a marked slowing of the TS Ca^{2+} release is unique to the change in TS volume.

I_{Ca} and $[\text{Ca}^{2+}]_{\text{tsl}}$

Figure 6A shows an example of the effect of changing the magnitude of I_{Ca} on TS Ca^{2+} release. As shown in this figure, a decrease of I_{Ca} from 1.0 to 0.375 μM by 0.125- μM steps with moderate $[\text{Ca}^{2+}]_{\text{tsl}}$ (1 mM) resulted in a gradual reduction of TS Ca^{2+} release. On the other hand, as $[\text{Ca}^{2+}]_{\text{tsl}}$ was decreased from 1.4 to 0.6 mM by 0.2-mM steps at moderate I_{Ca} (0.625 μM), TS Ca^{2+} release decreased gradually (Fig. 6B). With $[\text{Ca}^{2+}]_{\text{tsl}} \leq 0.8$ mM, $[\text{Ca}^{2+}]_{\text{av}}$ decreased monotonically after a termination of I_{Ca} . Thus the TS Ca^{2+} release varies depending on both I_{Ca} and $[\text{Ca}^{2+}]_{\text{tsl}}$ within certain ranges.

Figure 6C summarizes the effects of I_{Ca} and $[\text{Ca}^{2+}]_{\text{tsl}}$ on TS Ca^{2+} release. With moderate $[\text{Ca}^{2+}]_{\text{tsl}}$, ranging from 0.8 to 1.1 mM, as shown in the lower panel of Fig. 6C, TS Ca^{2+} release increased with an increase of I_{Ca} . The upper panel in Fig. 6C shows the TS Ca^{2+} release near the origin of the graph in the lower panel with an expanded horizontal scale. The

Fig. 3. Effects of (A) k_{on} and (B) k_{off} on TS Ca^{2+} release. k_{on} was 1.0, 1.5, and $2.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ in A, and k_{off} was changed from 0.5 to $2.5 \times 10^4 \text{ s}^{-1}$ by $0.5 \times 10^4 \text{ s}^{-1}$ steps in B from bottom to top for the c1 and av curves and in the reverse order for the tsl curves.

Fig. 5. Effects of (A) β and (B) V_{ts}/V_{cy} on TS Ca^{2+} release. $\beta = 0.12, 0.24, 0.36, \text{ and } 0.48$ in A and $V_{ts}/V_{cy} = 1/100, 1/150, 1/200, 1/250, \text{ and } 1/300$ in B from top to bottom for the c1 and av curves and in the reverse order for the tsl curves.

resting $[\text{Ca}^{2+}]$ in each compartment was always set at 10^{-8} M in the present simulation for technical reasons; therefore the changes of $[\text{Ca}^{2+}]$ below 10^{-8} M because of I_{Ca} were neglected. This resulted in substantial TS Ca^{2+} release whenever $[\text{Ca}^{2+}]_i$ once rose over 10^{-8} M , giving rise to a sudden increase of TS Ca^{2+} release in curves near the origin of the abscissa in the upper panel of Fig. 6C.

Characteristics of $[\text{Ca}^{2+}]_{av}$ transient

To characterize the simulated TS Ca^{2+} release for a comparison with the experimental observations, the time course of the change of $[\text{Ca}^{2+}]_{av}$ ($[\text{Ca}^{2+}]_{av}$ transient) resulting from TS Ca^{2+} release was examined with various values of $[\text{Ca}^{2+}]_{tsl}$ and I_{Ca} . At a given I_{Ca} , the magnitude of $[\text{Ca}^{2+}]_{av}$ transient increased with an increase of $[\text{Ca}^{2+}]_{tsl}$ (Fig. 7). In the range of $[\text{Ca}^{2+}]_{tsl}$ where regenerative TS Ca^{2+} release is significant, i.e.,

Fig. 4. Effects of (A) V_{max} and (B) K_m on TS Ca^{2+} release. $V_{max} = 0.005, 0.05, \text{ and } 0.5 \text{ mM s}^{-1}$ in A, and K_m was changed from 0.14 to $0.70 \mu\text{M}$ by $0.14 \mu\text{M}$ steps in B from bottom to top for the c1 and av curves and in the reverse order for the tsl curves.

Fig. 6. Effects of I_{Ca} and $[Ca^{2+}]_{tsl}$ on TS Ca^{2+} release. **A:** $[Ca^{2+}]_{tsl}=1.0$ mM. **B:** $I_{Ca}=0.625$ μM. I_{Ca} was changed from 0.375 to 1.0 μM by 0.125-μM steps in A and $[Ca^{2+}]_{tsl}$ from 0.6 to 1.4 mM by 0.2-mM steps in B from bottom to top for the c1 and av curves and in the reverse order for the tsl curves. **C:** Summary of effects of I_{Ca} and $[Ca^{2+}]_{tsl}$ on TS Ca^{2+} release. Ordinate, total amount of TS Ca^{2+} release ex-

pressed as % of $[Ca^{2+}]_{tsl}$. Abscissa, I_{Ca} . $[Ca^{2+}]_{tsl}$ is indicated on the left side of each curve in mM. The graph in the upper panel shows the very small I_{Ca} range near the origin in the lower panel with an expanded horizontal scale. The step increase at $I_{Ca}=0.003$ μM is due to technical reasons in the simulation. For details, see text.

with the time to the peak of $[Ca^{2+}]_{av}$ transient longer than 40 ms in Fig. 7A, the time to the peak of the $[Ca^{2+}]_{av}$ transient was relatively insensitive to the magnitude of $[Ca^{2+}]_{av}$ transient with a given $[Ca^{2+}]_{tsl}$ and decreased with an increase of I_{Ca} (Fig. 7A). The rate of increase of the $[Ca^{2+}]_{av}$ transient obtained by dividing the peak value by the time to peak in the $[Ca^{2+}]_{av}$ transient increased with an increase of either $[Ca^{2+}]_{tsl}$ or I_{Ca} (Fig. 7B). This result qualitatively resembles previously reported experimental results [34].

Localized I_{Ca}

The foregoing simulation was made based on the assumption that I_{Ca} raises $[Ca^{2+}]$ in every compartment uniformly. Since the local control theory claims that I_{Ca} raises the $[Ca^{2+}]$ only in the diadic cleft sandwiched by the sarcolemma and the TS membrane, the effect of localized I_{Ca} on TS Ca^{2+} release was examined by introducing I_{Ca} to limited compartments in the present model. In the case of local I_{Ca} , Ca^{2+} moves very rapidly by diffusion from the compartments into

which I_{Ca} was introduced compared to other compartments without I_{Ca} . Therefore $[Ca^{2+}]_i$, which is the main determinant of TS Ca^{2+} release, should be increased at a rate higher than that of a loss of Ca^{2+} to initiate effective TS Ca^{2+} release. Figure 8 shows the response of the TS with $[Ca^{2+}]_{tsl}=1$ mM to I_{Ca} introduced only to the 1st compartment in A and to the 1st–10th compartments in B (see also Fig. 6A, in which I_{Ca} was introduced uniformly to all compartments). Both the time course and magnitude of TS Ca^{2+} release were comparable for the three kinds of I_{Ca} application (cf. Figs. 8A, B and 6C). I_{Ca} ranged from 3.125 to 18.75 μM in Fig. 8A, from 0.3125 to 1.875 μM in Fig. 8B and from 0.375 to 1.0 μM in Fig. 6C. This means that I_{Ca} throughout the cytoplasm, i.e., to all 50 compartments, is 3.125–18.75 μM in Fig. 8A and B and 18.75–50 μM in Fig. 6C. Thus localized I_{Ca} is more effective at inducing TS Ca^{2+} release than uniform I_{Ca} is in the present model.

Fig. 7. Relation of (A) time to peak and (B) rate of increase of $[Ca^{2+}]_{av}$ transient to peak $[Ca^{2+}]_{av}$. Each curve was obtained by varying $[Ca^{2+}]_{tsl}$ from 0.8 to 1.4 mM by 0.1-mM steps. Peak $[Ca^{2+}]_{av}$ increased with an increase of $[Ca^{2+}]_{tsl}$. $I_{Ca}=0.25$ (○), 0.5 (△), and 0.75 μ M (□). The rate of increase of $[Ca^{2+}]_{av}$ was obtained by dividing the peak $[Ca^{2+}]_{av}$ by the time to the peak.

Mechanical alternans

Mammalian heart muscle exhibits mechanical alternans, which is characterized by an alternation of large and small twitches when the muscle is stimulated at a proper frequency [28]. To examine if a similar mechanical alternans is produced in rat papillary muscle, we isolated papillary muscle from the rat right ventricle and recorded the twitch forces by using a technique similar to that described previously [34]. Mechanical alternans was produced when rat papillary muscle was stimulated at a high frequency from rest, especially with low extracellular $[Ca^{2+}]$, as shown in Fig. 9A.

For a simulation of mechanical alternans with the present model, the rate of replenishment of Ca^{2+} in the TS must be supplied. Since short-term mechanical restitution is approximated by a monoexponential function [35], a replenishment of Ca^{2+} in the TS was computed by using the following equation with the simple rate constant r ,

Fig. 8. Effect of localized I_{Ca} on TS Ca^{2+} release. I_{Ca} was given only to the 1st compartment in A and the 1st–10th compartments in B. I_{Ca} was changed from 3.125 to 18.75 μ M by 3.125- μ M steps in A and from 0.3125 to 1.875 μ M by 0.3125- μ M steps in B from bottom to top for the c1 and av curves and in the reverse order for the tsl curves.

$$[Ca^{2+}]_{tsl,t} = ([Ca^{2+}]_{tsl,t=\infty} - [Ca^{2+}]_{rem})(1 - e^{-rt}) + [Ca^{2+}]_{rem}, \quad (8)$$

where $[Ca^{2+}]_{tsl,t}$ and $[Ca^{2+}]_{tsl,t=\infty}$ are $[Ca^{2+}]_{tsl}$ at time t and infinity, respectively, and $[Ca^{2+}]_{rem}$ is $[Ca^{2+}]$ at the beginning of replenishment. Figure 9B shows two examples of the change of $[Ca^{2+}]_{av}$ resulting from a TS Ca^{2+} release triggered by low I_{Ca} at frequencies similar to those used for the force records shown in Fig. 9A. An alternation of the magnitude of the $[Ca^{2+}]_{av}$ transient is more prominent at higher trigger frequency (right-hand record in Fig. 9B), as in the force records shown in Fig. 9A (right-hand record).

Figure 9C shows the dependence of TS Ca^{2+} release on $[Ca^{2+}]_{tsl}$ at three different values of I_{Ca} . TS Ca^{2+} release rises steeply as $[Ca^{2+}]_{tsl}$ increases over a critical level in a sigmoid manner; the lower the I_{Ca} , the steeper the curvature. An alternation of the magnitude of the $[Ca^{2+}]_{av}$ transient, such as shown in the right-hand record in Fig. 9B, is produced when $[Ca^{2+}]_{tsl}$ varies back and forth, crossing the critical level from one $[Ca^{2+}]_{av}$ transient to another. This oc-

Fig. 9. Simulation of mechanical alternans. **A:** Experimental force records. Isolated rat papillary muscle was stimulated at 0.7 (left) or 1.0 Hz (right) from rest at 0.3 mM extracellular $[Ca^{2+}]$. **B:** Computed $[Ca^{2+}]_{av}$ transients. Changes of $[Ca^{2+}]_{tsl}$ (upper) and $[Ca^{2+}]_{av}$ (lower). Initial $[Ca^{2+}]_{tsl} = 1.4$ mM. $l_{Ca} = 0.25$ μM is applied at 0.89 (left) and 1.0 Hz (right) to trigger $[Ca^{2+}]_{av}$ transients. Ca^{2+} replenishment was computed by the use of Eq. 8 with $[Ca^{2+}]_{tsl, t=\infty} = 1.4$ mM and $r = 0.89 s^{-1}$. **C:** Relationship between $[Ca^{2+}]_{tsl}$ and TS Ca^{2+} release. Ordinate, TS Ca^{2+} release expressed as amount of decrease of $[Ca^{2+}]_{tsl}$. $l_{Ca} = 0.25$ (○), 0.5 (△) and 0.75 μM (□). Dotted line shows 100% release of Ca^{2+} .

curs in a certain range of interval between $[Ca^{2+}]_{av}$ transients. When the interval is above this range, an alternation of the magnitude of $[Ca^{2+}]_{av}$ transient becomes unclear (left-hand record in Fig. 9B). Note a similar dependence of the form of variation of the magnitude of twitches (Fig. 9A) and $[Ca^{2+}]_{av}$ transients (Fig. 9B) on their frequency.

Ca^{2+} wave

During a propagation of the Ca^{2+} wave, Ca^{2+} released from a TS diffuses over adjacent sarcomeres and triggers a Ca^{2+} release from other TSs, leading to successive Ca^{2+} release from separated TSs. For a simulation of the Ca^{2+} wave, five 2-μm-long sarcomeres with TSs at both ends of each sarcomere were aligned in series (upper inset in Fig. 10). Figure 10 shows Ca^{2+} waves with different values of $[Ca^{2+}]_{tsl}$, in which a Ca^{2+} release from the leftmost TS is initiated by introducing l_{Ca} to the 1st–5th compartments.

Figure 10A, in which $[Ca^{2+}]_{tsl} = 1.8$ mM in all TSs, shows that Ca^{2+} release from the leftmost TS induces Ca^{2+} release from adjacent TSs (2nd and 3rd TSs) 2 μm away from it; the 2nd and 3rd TSs then induce Ca^{2+} release from the 4th and 5th TSs, and so on, with some delay, without changing the amount of Ca^{2+} release from each TS. The same occurred with $[Ca^{2+}]_{tsl} = 1.5$ mM, but with a lower velocity of propagation of the $[Ca^{2+}]_{av}$ transient (Fig. 10A and B). A further decrease of $[Ca^{2+}]_{tsl}$ to, for example, 1.0 mM greatly reduced the magnitude of the $[Ca^{2+}]_{av}$ transient, resulting in a termination of the propagation of the $[Ca^{2+}]_{av}$ transient among sarcomeres (Fig. 10C). This agrees with the experimental observation that the Ca^{2+} wave occurs when TS is heavily loaded or overloaded with Ca^{2+} .

Fig. 10. Simulation of $[Ca^{2+}]$ wave. For simulation, 5 sarcomeres (V–Z), each of which consisted of 100 compartments with TS at both ends (A–J), were aligned longitudinally with no barrier between them (**upper inset**). **A:** $[Ca^{2+}]_{tsl} = 1.8$ mM in all TSs. Ca^{2+} release from the left-most TS (A) is initiated by giving $I_{Ca} = 30 \mu M$ to the 1st–5th compartments during the initial 0.1 ms in the sarcomere (V). Ca^{2+} release occurs from TS (A–J) in order with some delay. $[Ca^{2+}]_{av}$ in sarcomeres (V–Z) (bold curves) rises biphasically because of delayed Ca^{2+} release from TSs at both ends of the sarcomere. **B:** $[Ca^{2+}]_{tsl} = 1.5$ mM in all TSs. Note the longer delay between $[Ca^{2+}]_{av}$ transients than in A. **C:** $[Ca^{2+}]_{tsl} = 1.5$ mM in TS (A) and 1.0 mM in the others. Note the negligibly small $[Ca^{2+}]_{av}$ transients in the sarcomeres (W–Z).

DISCUSSION

Graded response of TS. The present simulation showed that the amount of TS Ca^{2+} release varied in a graded manner with a variation of I_{Ca} when TS was moderately loaded with Ca^{2+} . The time course of the $[Ca^{2+}]_{av}$ change simulated in this paper resembles that reported previously (Fig. 7, compared with Fig. 5 in Tameyasu *et al.* [34]). The graded TS Ca^{2+} release was reproduced with both local and uniform I_{Ca} (Figs. 6C and 8). So the localization of I_{Ca} is not a critical factor for graded TS Ca^{2+} release in the present model. In cardiac cells, only a small part of the transverse tubule is attached via the TS forming the diad, with the remaining part of the tubule exposed to the cytoplasm [36]. Therefore it is possible that I_{Ca} through parts of the transverse tubule other than the diad contributes to triggering CICR from the TS by raising $[Ca^{2+}]$ in the bulk cytoplasm, besides the effect of I_{Ca} through the transverse tubule at the diad. I_{Ca} through the surface membrane may also raise $[Ca^{2+}]$ in the bulk cytoplasm in the sarcomeres facing the surface membrane, contributing to the triggering of CICR from the TS. The distribution and density of the L-type Ca^{2+} release channels would determine the importance of I_{Ca} in areas other than the diad for triggering CICR from TS.

In the present model, the rate of Ca^{2+} release from TS is regulated by both $[Ca^{2+}]_{tsl}$ and $[Ca^{2+}]_i$ in a complex manner. The rate of reduction of $[Ca^{2+}]_{tsl}$ during TS Ca^{2+} release is affected greatly by the volume of TS. In regard to TS volume, Page and McAllister [25] have obtained a value of 0.0035 for V_{ts}/V_{cy} from morphometric measurements. Taking the condition of sectioning of TS for an electron microscopy into account, a more reasonable value for V_{ts}/V_{cy} may be about 0.0045 [37]. A value of 0.005 for V_{ts}/V_{cy} used in the present study is close to this value. A decrease of the V_{ts}/V_{cy} value from 0.005 slightly reduced TS Ca^{2+} release, and its increase especially to more than 0.01 not only prolonged but also greatly enhanced TS Ca^{2+} release (Fig. 5B). In the present model, which does not assume any inactivation of the Ca^{2+} release channels, the larger the TS volume, the greater the total amount of TS Ca^{2+} release. The graded nature of TS Ca^{2+} release is eventually lost at a large TS volume in such models as the present one. In this connection, Stern [14] has shown theoretically that TS Ca^{2+} release occurs in an all-or-none manner in such classic common pool models that do not assume either inactivation of the Ca^{2+} release channels or Ca^{2+} gradient in the cytoplasm. His conclusion may be altered if both the smallness of TS volume and the Ca^{2+} gradient in the

cytoplasm are taken into consideration.

Ca²⁺ wave. The graded nature of the TS response to I_{Ca} disappears when $[Ca^{2+}]_{tsl}$ is increased in the present model. That is, the curvature of the TS Ca²⁺ release/store vs. I_{Ca} is steeper with higher $[Ca^{2+}]_{tsl}$, giving rise to an all-or-none type response of TS loaded heavily with Ca²⁺ (Fig. 6). These characteristics of the TS Ca²⁺ release are the basis for the Ca²⁺ wave, since the Ca²⁺ wave terminates in sarcomeres with the TS moderately loaded with Ca²⁺ (Fig. 10C). The velocity of the Ca²⁺ wave would be determined mainly by the delay in the Ca²⁺ release between TSs separated by the distance between Z-bands, i.e., about 2 μ m. If this is really so, the present model predicts that the rate of increase of $[Ca^{2+}]_{av}$ transient and the speed of the resulting contraction at the sarcomere level are smaller in the Ca²⁺ wave than the physiological twitch, since the $[Ca^{2+}]_{av}$ transient in a sarcomere is affected by the Ca²⁺ released from the TSs at both ends of the sarcomere (Fig. 10); this is shown by a hump in the rising phase of the $[Ca^{2+}]_{av}$ transient. This agrees with our previously reported findings [7]. On the other hand, Cheng *et al.* [5] reported that the $[Ca^{2+}]$ transient in the Ca²⁺ wave rises as rapidly as that in the physiological TS Ca²⁺ release. This discrepancy may be explained if Cheng *et al.* observed the $[Ca^{2+}]$ in the I-band region, i.e., in the region near the TS, since it is possible that individual TSs release Ca²⁺ in the Ca²⁺ wave at a rate quite similar to the physiological rate, as suggested by the present model (cf. the time course of the change of $[Ca^{2+}]_i$ between Figs. 6B and 10B).

Ca²⁺ in TS. For simplicity, it is assumed in the present model that all Ca²⁺ in the TS exists in the ionic state, though there may be Ca²⁺-binding proteins such as calsequestrin in the TS [38]. When heart muscle is stimulated from rest at a high frequency under conditions in which the recruitment of Ca²⁺ to the TS is slow, an initial large $[Ca^{2+}]_{av}$ transient is followed by a much smaller one [34]. This suggests that only a small amount of Ca²⁺, if any, is in the TS immediately after the preceding TS Ca²⁺ release. In other words, there is a rapid equilibrium between bound forms of Ca²⁺, if present, and free Ca²⁺ in the TS. Therefore it is reasonable to assume that Ca²⁺ exists functionally in the ionic form in the TS.

Inactivation of Ca²⁺ release channel (ryanodine receptor). Fabiato [1] reported that in rat ventricular cells, the TS Ca²⁺ release in response to a given trigger $[Ca^{2+}]$ is diminished by the pretreatment of the cell with a high $[Ca^{2+}]$ solution for a very short period and suggested that time- and Ca²⁺-dependent inactivation of the TS Ca²⁺ release occurs. However,

subsequent studies showed that the inactivation, if present, is too slow to account for the physiological TS Ca²⁺ release [10–13]. In Fabiato's experiments, to expose the cell to high $[Ca^{2+}]$ for a very short period, a high $[Ca^{2+}]$ solution was applied to the cell through a pipette, followed by sucking with another pipette after a very short delay. Since the present simulation suggests that the response of the TS to I_{Ca} changes markedly with a small change of $[Ca^{2+}]_{tsl}$ (cf. TS Ca²⁺ release/storage with $[Ca^{2+}]_{tsl}=1.0$ and 1.1 mM at $I_{Ca}=0.5$ μ M in Fig. 6C), one possibility is that in Fabiato's experiments, a brief application of the high $[Ca^{2+}]$ solution to the cell induced TS Ca²⁺ release without substantially raising cytoplasmic $[Ca^{2+}]$ because of strong buffering of Ca²⁺ by EGTA. Therefore this topic should be reexamined by the use of different techniques.

Mechanical alternans. Mechanical alternans characterized by alternating amplitudes of $[Ca^{2+}]_{av}$ transient and the resulting twitch is evoked in heart muscle when it is stimulated from rest at high frequency, especially at low extracellular $[Ca^{2+}]$ (Fig. 9A) [28, 30]. The successful simulation of mechanical alternans in the present study suggests a new possibility for the origin of the mechanical alternans; i.e., mechanical alternans may be due to a sigmoid increase of the amount of TS Ca²⁺ release with an increase of the Ca²⁺ content in the TS.

Relation to local control theory. The local control theory assumes that TS releases Ca²⁺ in response to such the high $[Ca^{2+}]$ as formed only by I_{Ca} through the sarcolemmal L-type Ca channels facing the TS membrane [14–16]. Thus the local control theory does not predict regenerative TS Ca²⁺ release, i.e., TS Ca²⁺ release terminates when I_{Ca} stops. In contrast, the present model predicts regenerative Ca²⁺ release from the TS. The prediction of both regenerative and graded TS Ca²⁺ release is unique to the present model.

Conclusions. This study was not intended to provide a perfect simulation of some special features of the TS Ca²⁺ release, but rather to provide a general model based on CICR for understanding various aspects of the TS Ca²⁺ release in heart muscle. The model used in this study gave interpretations of both graded and all-or-none type responses of the TS without ad hoc assumptions. Therefore the present model is helpful for understanding the regulation of heart muscle contraction, though it makes some oversimplified assumptions, such as ignoring Ca²⁺ binding proteins in the cytoplasm and in the small k_{off} . It will be worthwhile, by extending the present model, to construct a more sophisticated model that will include in-

teraction between the Ca^{2+} and Ca^{2+} binding proteins in the cytoplasm and that will use more realistic parameter values.

The author is grateful to Ms. Megumi Yamada for her help in preparing the manuscript.

REFERENCES

1. Fabiato A: Time and calcium dependence of activation and inactivation of calcium induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J Gen Physiol* 85: 247–289, 1985
2. Fabiato A: Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J Gen Physiol* 85: 291–320, 1985
3. Takamatsu T and Wier WG: Calcium waves in mammalian heart: quantification of origin, magnitude, waveform and velocity. *FASEB J* 4: 1519–1525, 1990
4. Williams Da, Delbridge LM, Cody SH, Harris PJ, and Morgan TO: Spontaneous and propagated calcium release in isolated cardiac myocytes viewed by confocal microscopy. *Am J Physiol* 262 (Cell Physiol 31): C731–C742, 1995
5. Cheng H, Lederer MR, Lederer WJ, and Cannell MB: Calcium sparks and $[\text{Ca}^{2+}]$ waves in cardiac myocytes. *Am J Physiol* 270 (Cell Physiol 39): C148–C159, 1996
6. Lukyanenko V, Subramanian S, Györke I, Wiesner TF, and Györke S: The role of luminal Ca^{2+} in the generation of Ca^{2+} waves in rat ventricular myocytes. *J Physiol (Lond)* 518: 173–186, 1999
7. Tameyasu T, Shimada M, and Sugi H: Intracellular $[\text{Ca}^{2+}]$ transients in Ca^{2+} wave in single rat ventricular myocyte. *Jpn J Physiol* 51: 187–191, 2001
8. Cannel MB, Berlin JR, and Lederer WJ: Effect of membrane potential changes on the calcium transient in single rat cardiac muscle cells. *Science* 238: 1419–1423, 1987
9. Beuckelmann DJ and Wier WG: Mechanism of release of calcium from sarcoplasmic reticulum of guinea-pig cardiac cells. *J Physiol (Lond)* 405: 233–255, 1988
10. Rousseau E, Smith JS, Henderson JS, and Messner G: Single channel and $^{45}\text{Ca}^{2+}$ flux measurements of the cardiac sarcoplasmic reticulum calcium channel. *Biophys J* 50: 1009–1014, 1986
11. Györke S and Fill M: Ryanodine receptor adaptation: control mechanism of Ca^{2+} -induced Ca^{2+} release in heart. *Science* 260: 807–809, 1993
12. Lamb GD, Fryer MW, and Stephenson DG: Ca^{2+} -induced Ca^{2+} release in response to flash photolysis. *Science* 263: 986–987, 1994
13. Schiefer A, Meissner G, and Isenberg G: Ca^{2+} activation and Ca^{2+} inactivation of canine reconstituted cardiac sarcoplasmic reticulum Ca^{2+} -release channels. *J Physiol (Lond)* 489: 337–348, 1995
14. Stern MD: Theory of excitation-contraction coupling in cardiac muscle. *Biophys J* 63: 497–517, 1992
15. Wier WG, Egan TM, López-López JR, and Balke CW: Local control of excitation-contraction coupling in rat heart cells. *J Physiol (Lond)* 474: 463–471, 1994
16. Stern MD, Song L-S, Cheng H, Shem JSK, Yang HT, Boheler KR, and Rios E: Local control models of cardiac excitation-contraction coupling: a possible role for allosteric interactions between ryanodine receptors. *J Gen Physiol* 113: 469–489, 1999
17. Cannell MB, Cheng H, and Lederer WJ: The control of calcium release in heart muscle. *Science* 268: 1045–1049, 1995
18. Cheng H, Lederer WJ, and Cannell MB: Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* 262: 740–744, 1993
19. Lipp P and Niggli E: Submicroscopic calcium signals as fundamental events of excitation-contraction coupling in guinea-pig cardiac myocytes. *J Physiol (Lond)* 492: 31–38, 1996
20. Lipp P and Niggli E: Fundamental calcium release events revealed by two-photon excitation photolysis of caged calcium in guinea-pig cardiac myocytes. *J Physiol (Lond)* 508: 801–809, 1996
21. Collier ML, Thomas AP, and Berlin JR: Relationship between L-type Ca^{2+} current and unitary sarcoplasmic reticulum Ca^{2+} release events in rat ventricular myocytes. *J Physiol (Lond)* 516: 117–128, 1999
22. Cannell MB and Soeller C: Mechanisms underlying calcium sparks in cardiac muscle. *J Gen Physiol* 113: 373–376, 1999
23. Schneider MF: Ca^{2+} sparks in frog skeletal muscle: generation by one, some, or many SR Ca^{2+} release channels? *J Gen Physiol* 113: 365–371, 1999
24. Schrokova N, Gonzalez A, Kirsh WG, Rios E, Pizzaro G, Stern DM, and Cheng H: Calcium sparks: release packets of uncertain origin and fundamental role. *J Gen Physiol* 113: 377–384, 1999
25. Page E and McAllister LP: Quantitative electron-microscopic description of heart muscle cells: application to normal, hypertrophied and thyroxine-stimulated hearts. *Am J Cardiol* 31: 172–181, 1973
26. Isenberg G, Etter EF, Wendt-Gallitelli M-F, Schiefer A, Carrington WA, Tuft RA, and Fay FS: Intracellular $[\text{Ca}^{2+}]$ gradients in ventricular myocytes revealed by high speed digital imaging microscopy. *Proc Natl Acad Sci USA* 93: 5413–5418, 1996
27. Peskoff A and Langer GA: Calcium concentration and movement in the ventricular cardiac cell during an excitation-contraction cycle. *Biophys J* 74: 153–174, 1998
28. Lab MJ and Lee JA: Changes in intracellular calcium during mechanical alternans in isolated ferret ventricular muscle. *Circ Res* 66: 585–595, 1990
29. Kihara Y and Morgan JP: Abnormal Ca^{2+} handling is the primary cause of mechanical alternans: study in ferret ventricular muscles. *Am J Physiol* 261: H1746–H1755, 1991
30. Tameyasu T: Origin of twitch oscillation in rat ventricular muscle. *Jpn J Physiol* 49 (Suppl): S63, 1999 (abstract)
31. Tameyasu T: Regulation of contraction in heart muscle: a Ca^{2+} gradient model for the Ca^{2+} -induced Ca^{2+} -release of the sarcoplasmic reticulum. In: *Mechanisms of Work Production and Work Absorption in Muscle*, ed. Sugi H and Pollack GH, Plenum Press, New York, pp 209–218, 1998
32. Bassani JWM, Bassani RA, and Bers DM: Relaxation in

- rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. *J Physiol (Lond)* 476: 279–293, 1994
33. Bouchard RA and Giles CWR: Effects of action potential duration on excitation-contraction coupling in rat ventricular myocytes. Action potential voltage-clamp measurements. *Circ Res* 76: 790–801, 1995
34. Tameyasu T, Shimada M, and Sugi H: Effect of extracellular $[Ca^{2+}]$ on Ca^{2+} release from sarcoplasmic reticulum in rat ventricular myocytes. *Jpn J Physiol* 51: 169–176
35. Wier WG and Yue DT: Intracellular calcium transients underlying the short-term force–interval relationship in ferret ventricular myocardium. *J Physiol (Lond)* 376: 507–530, 1986
36. Fawcett DW and McNutt NS: The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. *J Cell Biol* 42: 1–45, 1969
37. Moravec CS and Bond M: Calcium is released from the junctional sarcoplasmic reticulum during cardiac muscle contraction. *Am J Physiol* 260: H989–H997, 1991
38. Jorgensen AO, Shen AC-Y, and Campbell KP: Ultrastructural localization of calsequestrin in adult rat atrial and ventricular muscle cells. *J Cell Biol* 101: 257–268, 1995
39. Balke CW, Egan TM, and Wier WG: Processes that remove calcium from the cytoplasm during excitation-contraction coupling in rat heart cells. *J Physiol (Lond)* 474: 447–462, 1994
40. Kushmerick MJ and Podolsky RJ: Ion mobility in muscle cells. *Science* 166: 1297–1298, 1969
41. Riggs DS: Transfer of substances by simple diffusion. In: *The Mathematical Approach to Physiological Problems*, The M.I.M. Press, Cambridge, UK, pp 173–179, 1967

APPENDIX

Formulation for diffusion in a finite system

Factor β , related to a diffusion of Ca^{2+} between compartments in Eqs. 5 and 6, was determined by using formulae derived for diffusion in a finite system with simple geometry, as shown in Fig. 11 [41]. Here it is assumed that the diffusion gradient is parallel to the x axis. Then, according to Fick's law of diffusion,

$$(dQ_s/dt)_x = D \cdot A \cdot (-dC_s/dx), \quad (A1)$$

where $(dQ_s/dt)_x$ is the quantity of solute S diffusing per unit of time across a plane with area A at a distance x from the initial boundary where $x=0$, D is the diffusion coefficient of S and $-dC_s/dx$ is the decrease in concentration per unit increase in x . The integral form of Eq. A1 is

$$C_{s,x,t} = (C_{s,0}/2) \left[1 - \frac{2}{\sqrt{\pi}} \int_0^y \exp(-y^2) dy \right], \quad (A2)$$

where $y = x/2\sqrt{Dt}$, $C_{s,x,t}$ is the concentration of

Fig. 11. Diffusion in a finite system. Solute diffuses from the left to the right compartment (arrow). ± 1 shows unit length. For details, see APPENDIX.

S at point x at time t , and $C_{s,0}$ is the concentration of S in the original solution. The expression $(2/\sqrt{\pi}) \int_0^y \exp(-y^2) dy$ is the error function and is symbolized by “ $\text{erf}(y)$.” Equation A2 is then rewritten as

$$C_{s,x,t} = (C_{s,0}/2) \{ 1 - \text{erf}(x/2\sqrt{Dt}) \}. \quad (A3)$$

As t approaches infinity, $x/2\sqrt{Dt}$ approaches 0, $\{ 1 - \text{erf}(x/2\sqrt{Dt}) \}$ approaches unity, and $C_{s,x,t}$ approaches $C_{s,0}/2$. Therefore,

$$C_{s,x,\infty} = C_{s,0}/2 \quad (A4)$$

Combining Eqs. A3 and A4 by eliminating $C_{s,0}$ and rearranging, the following is obtained.

$$1 - (C_{s,x,t}/C_{s,x,\infty}) = \text{erf}(x/2\sqrt{Dt}). \quad (A5)$$

The fraction $C_{s,x,t}/C_{s,x,\infty}$ is the fraction of the final equilibrium concentration at point x , which has been attained at time t . For any particular $C_{s,x,t}/C_{s,x,\infty}$, the numerical value for $x/2\sqrt{Dt}$ is obtained from a table of the error function.

Now consider the diffusion of Ca^{2+} from compartment i to ii. Since $[Ca]_{ii,t=\infty} = (1/2)([Ca^{2+}]_{i,t=0} - [Ca^{2+}]_{ii,t=0})$,

$$1 - [Ca^{2+}]_{ii,t} / \{ ([Ca^{2+}]_{i,t=0} - [Ca^{2+}]_{ii,t=0}) / 2 \} = \text{erf}(x/2\sqrt{Dt}). \quad (A6)$$

Then,

$$[Ca^{2+}]_{ii,t} = (1/2)([Ca^{2+}]_{i,t=0} - [Ca^{2+}]_{ii,t=0}) \times \{ 1 - \text{erf}(x/2\sqrt{Dt}) \}. \quad (A7)$$

The increment of $[Ca]_{ii}$ after a unit of time is computed by the use of Eq. A7. Comparing Eq. A7 with Eq. 7 in the text, $\beta = (1/2) \{ 1 - \text{erf}(x/2\sqrt{Dt}) \}$. From a table of the error function, $\beta = 0.24$ gives $x/2\sqrt{Dt} = 0.48$. When x is taken as the distance between the centers of adjacent compartments, i.e., 20 nm, and unit time = 10^{-5} s, then $D = 4.2 \times 10^{-7}$ cm²/s.