Model of Calcium Diffusion, Binding and Membrane Transport in the Sarcomere of Frog Skeletal Muscle

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Abstract. A model of calcium distribution in the sarcomere during activation of contraction was developed. It allows for diffusion and binding of calcium ions to various sarcoplasmic binding sites in the three dimensional spatial coordinate system. The model was used to analyze the influence of kinetic characteristic of binding processes on the temporal and spatial distribution of calcium in the sarcomere during activation of contraction by the action potential and by rectangular depolarizing pulses. The hypothesis concerning the calcium release control in the membrane of terminal cisternae was tested.

Key words: Mathematical model — Skeletal muscle — Calcium release — Calcium distribution — Calcium binding

Introduction

Excitation of the surface membrane of frog skeletal muscle cell spreads inside the fibre along the T-system. Depolarization of the tubular membrane controls the release of calcium from the terminal cisternae (TC) into the sarcoplasm. Calcium diffuses and binds to various sites in the sarcomere. The uptake of calcium to the sarcoplasmic reticulum (SR) by the calcium pump results in relaxation of the muscle and its return to the resting state. The steady state activity of the calcium pump compensates for small calcium leaks from the SR and maintains the resting concentration of calcium in the sarcomere.

From the point of view of contraction the most important calcium-binding sites in skeletal muscle are on troponin, parvalbumin, calsequestrin and Ca-ATPase. Calcium binding to troponin, attached to the thin filaments, allows an interaction of actin and myosin. The cyclic pattern of this interaction is the basis of the mutual sliding of the thin and thick filaments resulting in contraction. Parvalbumin is a soluble protein found in the sarcoplasm. It takes a substantial part in the calcium redistribution only for long lasting depolarization or tetanus

(Baylor et al. 1983). Calsequestrin is a low-affinity protein localized in the TC (Jorgensen et al. 1979). It allows to maintain large supplies of calcium in the TC without high concentrations of free calcium. Binding of calcium to the Ca-ATPase in the SR membrane (Jorgensen et al. 1979) is a part of its uptake from the sarcoplasm.

Calcium is compartmentalized in the sarcomere and even during the activation of contraction it is not distributed homogenously in the sarcomere. In view of this, the appropriate description requires that calcium distribution has to be treated in time and spatial coordinates. A similar approach was used by Cannell and Allen (1984), who modelled the calcium binding in the sarcomere after activation by action potential. We developed a new model with the purpose

a) to check a more adequate mechanism of the calcium pump;
b) to study the effects of changes of the chosen parameters on calcium distribution in the sarcomere following activation by the action potential;
c) to model various modes of activation of contraction; and
d) to test some hypotheses concerning the control of the calcium release.

Model and Methods

The model describes only one half of the sarcomere because of its symmetry about the M line. Considering calcium distribution, the sarcomere can be divided into three parts (Fig. 1): the myofibrilar space (MS; occupying 85% of the fiber volume), the longitudinal sarcoplasmic reticulum (LSR; 5% of the fiber volume), and the terminal cisternae (TC; 4% of the fiber volume). The volume fractions were taken from Mobley and Eisenberg (1975). The remaining 6% of the fiber volume are dispersed throughout the whole fiber (and are not located between the SR and the MS as suggested by Cannell and Allen (1984)). Omitting this part from the model does not affect the calcium distribution pattern.

The length of the TC in longitudinal direction is about one fifth of the sarcomere length (Peachey 1965). Applying discretization, the TC was divided into several longitudinal elements, and calcium release has been localized but in the last one (Fig. 1), the site of the tubulo-reticular junction (Franzini-Armstrong and Nunzi 1983; Meissner et al. 1988).

The calcium distribution is generally described by the parabolic partial differential equation (Crank 1975)

$$\partial C/\partial t = D \,.\, \nabla^2 C - \mathrm{F}(C, t),$$

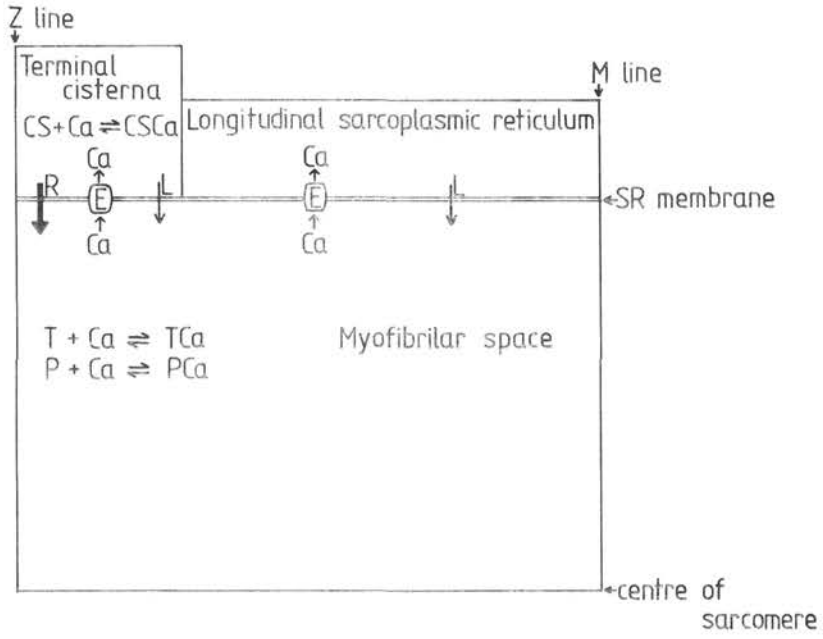

Fig. 1. Diagram of calcium distribution in the frog half sarcomere. Calcium (Ca) is present in the TC either as free ions or bound to calsequestrin (CS). Activating the fibre, calcium is released (R) into the myofibrilar space. There calcium diffuses and binds to the T-sites (T), the P-sites (P) and the Ca-ATPase (E). The enzyme Ca-ATPase provides calcium reuptake to the SR. Calcium diffuses through the longitudinal sarcoplasmic reticulum to the terminal cisterna. There is also a constant leak of calcium from the SR (*L*). Applying a numerical method the half sarcomere has been divided radially (vertically on the figure) into 11 elements and longitudinally (horizontally on the figure) into 22 elements, so that element (1,1) is in the right lower corner and element (11,22) in the left upper corner. The longitudinal sarcoplasmic reticulum and the terminal cisterna represented radially only one row of elements (the last one). Moreover, longitudinally the terminal cisterna consisted of four elements (the last four elements).

where $C = C(x, t)$ is the free calcium concentration, and D is the diffusion coefficient. The first term on the right side represents calcium diffusion and the other one describes sources and sinks of calcium.

The flux of calcium released from the TC into the sarcoplasm by the action potential is described by the phenomenological equation (Cannell and Allen 1984)

$$\partial C_x/\partial t = P_{max} \cdot [1 - \exp(-t/t_{on})] \cdot \exp(-t/t_{off}) \cdot A_{tc} \cdot (C_{tc} - C_x),$$

where P_{max} is the coefficient of maximal permeability of the TC membrane for calcium, t_{on}, t_{off} are time constants of the increase or decrease of the TC membrane permeability, respectively, A_{tc} is the TC membrane surface area, and C_{tc}, C_x are free calcium concentrations in the last TC element or in the MS element nearest to the TC, respectively.

Calcium leak from the SR into the sarcoplasm is represented by the equation

$$\partial C_{sr}/\partial t = -P_1 \cdot A_{sr} \cdot (C_{sr} - C_{ms}),$$

where P_1 is the SR membrane permeability for calcium, and A_{sr} is the SR membrane surface area.

Similarly as in the model of Cannell and Allen (1984), the troponin sites were divided according to their binding kinetics into low affinity sites (T-sites) and high affinity sites (which together with the parvalbumin sites make up the P-sites). Calcium competes with magnesium for binding to the P-sites. Calcium binding to the T-sites, the P-sites and calsequestrin is described, in general, by the kinetic equation

$$S + Ca \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} SCa,$$

where k_{on}, k_{off} are the on or off rate constants for calcium binding to the site S, respectively.

The corresponding differential equations are

$$\partial C/\partial T = k_{off} \cdot [SCa] - k_{on} \cdot C \cdot [S]$$
$$\partial [SCa]/\partial t = -\partial C/\partial t,$$

where the square brackets denote the respective substance concentrations. Similar equations hold for magnesium binding to the P-sites.

The total concentration of any site S is constant

$$L_s = [S] + [SCa] \qquad (L_P = [P] + [PCa] + [PMg]).$$

The equation in the parentheses expresses the competition between calcium and magnesium binding to the P-sites.

Calcium binding to the Ca-ATPase and its uptake to the SR are described by the kinetic equations (Inesi 1981)

$$E + Ca_{ms} \underset{k1_{off}}{\overset{k_{on}}{\rightleftharpoons}} ECa$$

Table I. Model parameters (according to Cannell and Allen 1984)

D	$0.7\ \mu m \cdot ms^{-1}$	(Wang 1953)
t_{on}	1 ms	(Kovács et al. 1979)
t_{off}	5 ms	(Kovács et al. 1979)
A_{tc}	$0.54 \times 10^{15}\ \mu m^2 \cdot l^{-1}$	(Mobley and Eisenberg 1975)
A_{sr}	$1.48 \times 10^{15}\ \mu m^2 \cdot l^{-1}$	(Mobley and Eisenberg 1975)
Half sarcomere dimension		
Radius	$0.5\ \mu m$	(Peachey 1965)
Length	$1.1\ \mu m$	(Peachey 1965)
T-sites		
L_t	$140\ \mu mol \cdot l^{-1}$	(Ebashi et al. 1969)
k_{on}	$0.12\ \mu mol^{-1} \cdot l \cdot ms^{-1}$	(Robertson et al. 1981)
k_{off}	$0.12\ ms^{-1}$	(Canell and Allen 1984)
P-sites		
L_p	$940\ \mu mol \cdot l^{-1}$	(Gosselin-Rey and Gerday 1977)
With calcium		
k_{on}	$0.25\ \mu mol^{-1} \cdot l \cdot ms^{-1}$	(Robertson et al. 1981)
k_{off}	$0.001\ ms^{-1}$	(Robertson et al. 1981)
With magnesium		
k_{on}	$6.6 \times 10^{-5}\ \mu mol^{-1} \cdot l \cdot ms^{-1}$	(Robertson et al. 1981)
k_{off}	$0.006\ ms^{-1}$	(Robertson et al. 1981)
Calsequestrin		
L_c	$31\ mmol \cdot l^{-1}$	(MacLennan and Wong 1971)
k_{on}	$6 \times 10^{-6}\ \mu mol^{-1} \cdot l \cdot ms^{-1}$	(Cannell and Allen 1984)
k_{off}	$0.005\ ms^{-1}$	(Cannell and Allen 1984)
Resting concentrations		
Free MS calcium	$0.06\ \mu mol \cdot l^{-1}$	(Coray et al. 1980)
Free SR calcium	$1.5\ mmol \cdot l^{-1}$	(Somlyo et al. 1981)
Free MS magnesium	$3.3\ mmol \cdot l^{-1}$	(Hess et al. 1982)

Table II. Additional model parameters

Concentrations of Ca-binding sites in the respective compartments:		
T-sites	240 μmol . l^{-1}	
P-sites	1.24 mmol . l^{-1}	
Calsequestrin	52 mmol . l^{-1}	
Ca-ATPase	2 mmol . l^{-1}	
Concentrations of membrane area in the respective compartments:		
LSR	29.6×10^{15} μm^2 . l^{-1}	
TC	13.5×10^{15} μm^2 . l^{-1}	
Ca-ATPase characteristic:		
L_e	100 μmol . l^{-1}	(Endo 1977)
k_{on}	0.35 μmol^{-1} . l . ms^{-1}	(Inesi 1981)
$k1_{off}$	0.4 ms^{-1}	(Inesi 1981)
$k2_{off}$	0.025 ms^{-1}	(Inesi 1981)
k_{to}	0.005 ms^{-1}	(Martonosi and Beeler 1983)

$$ECa + Ca_{ms} \underset{k2_{off}}{\overset{k_{on}}{\rightleftharpoons}} ECa_2 \xrightarrow{k_{to}} E + 2Ca_{sr},$$

where E is the enzyme Ca-ATPase, ECa, ECa_2 are complexes of Ca-ATPase with one or two calcium ions, respectively, k_{on} is the on rate constant for calcium binding to Ca-ATPase, $k1_{off}$, $k2_{off}$ are the off rate constants for calcium binding to Ca-ATPase, and k_{to} is its turnover. Upon binding the first calcium ion, the affinity of the enzyme increases and after the second calcium ion has been bound, the calcium pair is transferred into the SR (Inesi 1981). The differential equations corresponding to this kinetics are

$$\partial C_{ms}/\partial t = -k_{on} \cdot C_{ms} \cdot ([E] + [ECa]) + k1_{off} \cdot [ECa] + k2_{off} \cdot [ECa_2]$$
$$\partial C_{sr}/\partial t = 2 \cdot k_{to} \cdot [ECa_2]$$
$$\partial [ECa]/\partial t = k_{on} \cdot C_{ms} \cdot ([E] - [ECa]) - k1_{off} \cdot [ECa] + k2_{off} \cdot [ECa_2]$$
$$\partial [ECa_2]/\partial t = k_{on} \cdot C_{ms} \cdot [ECa] - (k2_{off} + k_{to}) \cdot [ECa_2]$$
$$L_e = [E] + [ECa] + [ECa_2],$$

where L_e, unlike L_s, denotes the total concentration of the Ca-ATPase and not that of the calcium sites on it.

The Ca-ATPase is uniformly distributed throughout the LSR and TC membranes, except the part of the TC membrane in apposition with the T-tubule (27% of the TC membrane — Mobley and Eisenberg (1975)). The absence of Ca-ATPase in this region was shown by Franzini-Armstrong and Nunzi (1983).

The sarcomere is axially symmetric, what allows to reduce the spatial dimension to 2 using cylindrical coordinates. The equations have been solved by the Peaceman-Rachford's method (Twitzell 1984). The initial conditions were the physiological intracellular concentrations of free calcium and magnesium (Table I) and the solution of the equilibrium equations for calcium binding. The boundary condition was zero calcium flux from the half sarcomere.

The parameters of the model were (except P_1 and P_{max}) experimentally measured values shown in Tables I and II. The value of parameter P_1 was taken 1×10^{-5} $\mu m \,.\, ms^{-1}$ to balance the resting uptake of the calcium pump. For activation by the action potential the parameter P_{max} was 0.3 $\mu m \,.\, ms^{-1}$ to obtain similar time courses of the free calcium concentration at the TC as reported by Cannell and Allen (1984). For activation by the depolarizing pulse this parameter was 3.5×10^{-2} $\mu m \,.\, ms^{-1}$ so that the maximal calcium flux (6 $\mu mol \,.\, l^{-1} \,.\, ms^{-1}$) raised the free calcium concentration at the TC to 5 $\mu mol \,.\, l^{-1}$.

Calcium binding site concentrations were included in the model, in terms of mol/(kg H_2O of the corresponding part of the sarcomere) according to the procedure of Baylor et al. (1983) (their new values are given in Table II). The surface areas of the LSR and TC membranes were expressed as the concentrations in the respective compartments (in the LSR or TC, respectively) (Table II).

Calcium release from the TC has not been well understood as yet. Simultaneous activation and inactivation make this process difficult to study. The calcium flux elicited by the action potential can be phenomenologically described by double-exponential course (Fig. 2*D*). However the action potential is too short to allow to study inactivation; owing to this we tried to model the calcium release after long depolarizing pulse (Fig. 4*D*). We assumed inactivation to be dependent on the free calcium concentration. The feedback maintains a definite free calcium concentration at the TC (5 $\mu mol \,.\, l^{-1}$) during the pulse.

Results

The time courses of the model solution upon activation by the action potential are shown in Fig. 2. The coefficient P_{max} (see Model and Methods) was adjusted to fit the values of the free calcium concentrations on both sides of the TC membrane as presented by Cannell and Allen (1984).

In view of the fact that experimentally measured values of parameters cover a certain interval (rather than being single numbers) we analyzed the effects of variation of some parameters on the calcium distribution in the half sarcomere. The following changes were considered (Fig. 3):

— decrease of the diffusion coefficient, D, to 0.525 $\mu m \,.\, ms^{-1}$ (curves 2 — only the free calcium concentration in the MS shows sensitivity to this change),

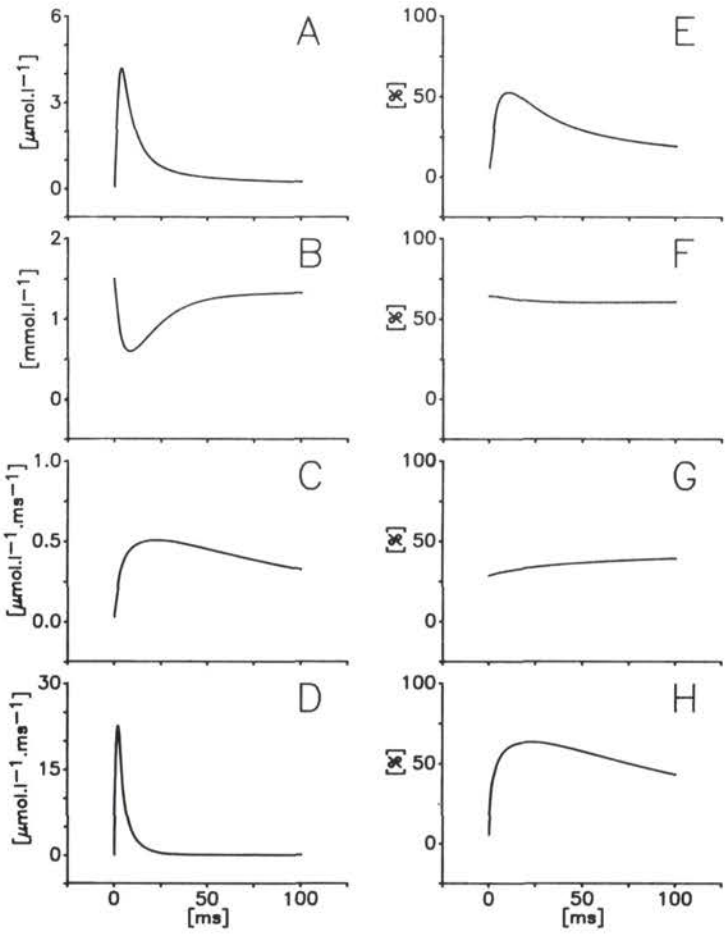

Fig. 2. The time courses of the model variables upon activation by the action potential: (*A*) Free calcium concentration in the MS; (*B*) Free calcium concentration in the SR; (*C*) Calcium pumping into the SR; (*D*) Calcium release from the TC; (*E*) Saturation of the T-sites by calcium in the MS; (*F*) Saturation of calsequestrin by calcium in the TC; (*G*) Saturation of the P-sites by calcium in the MS; and (*H*) Saturation of the Ca-ATPase by calcium in the SR. The time scale is the same throughout.

— decrease of the T-sites concentration, L_t, to 70 μmol.l^{-1} (curves 3 — the free calcium concentration in the MS and saturations of the Ca-ATPase and T-sites are sensitive to this change),

— decrease of k_{off} for calcium binding to the T-sites to 0.024 ms^{-1} (at the same time the dissociation constant K_D increased five times) (curves 4 — only the saturation of the T-sites is substantially affected),

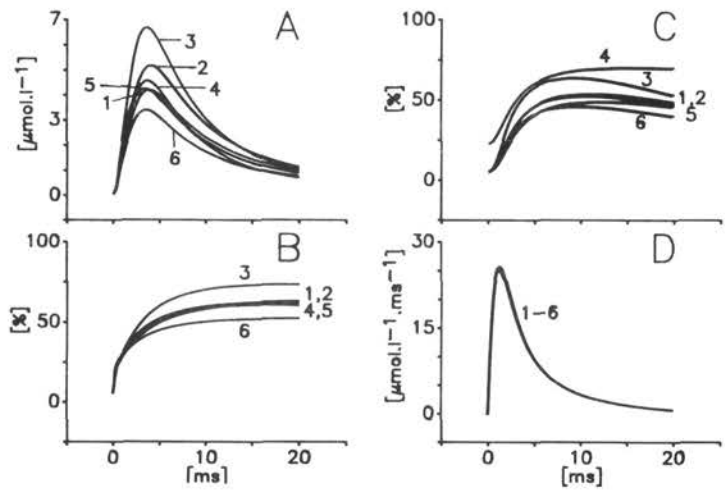

Fig. 3. The effects of changes of some parameters on the time courses of the model variables: (*A*) Free calcium concentration in the MS; (*B*) Saturation of the Ca-ATPase by calcium in the SR; (*C*) Saturation of the T-sites by calcium in the MS; and (*D*) Calcium release from the TC. The courses correspond to the following parameter changes (cf. with Table I.): (1) the original parameters, (2) $D = 0.525\ \mu m \,.\, ms^{-1}$, (3) $L_t = 70\ \mu mol \,.\, l^{-1}$, (4) k_{off} (T-sites) $= 0.024\ ms^{-1}$, (5) k_{on} (T-sites) $= 0.06\ \mu mol^{-1} \,.\, l \,.\, ms^{-1}$, k_{off} (T-sites) $= 0.06\ ms^{-1}$ and (6) $L_e = 200\ \mu mol \,.\, l^{-1}$. The time scale is the same throughout.

— decrease of k_{on} and k_{off} for calcium binding to the T-sites to $0.06\ \mu mol^{-1} \,.\, l \,.\, ms^{-1}$ or $0.06\ ms^{-1}$, respectively (the constant K_D remained unchanged) (curves 5 — without any significant effect), and
— increase of the Ca-ATPase concentration, L_e, to $200\ \mu mol \,.\, l^{-1}$ (i. e. $400\ \mu mol \,.\, l^{-1}$ of Ca-binding sites) (curves 6 — the free calcium concentration in the MS and the saturation of the Ca-ATPase sites, and partially of the T-sites, are affected).

The other time courses (cf. Fig. 3 with Fig. 2) were not significantly influenced by the above variations.

Due to better defined experimental conditions and to the possibility of their variation the activation of calcium release by a rectangular depolarizing pulse is more informative than the activation by the action potential. We present the time courses for pulses of various lengths (Fig. 4). The time course of the free calcium concentration in the MS (Fig. 4*A*) is in good qualitative agreement with the experimentally measured curves obtained from calcium binding to Arsenazo III (Palade and Vergara 1982).

To check certain hypotheses concerning the calcium distribution in the sarcomere during contraction the radial averages (or the longitudinal profiles) of some variables were recorded (Fig. 5). The profiles of the saturation of the

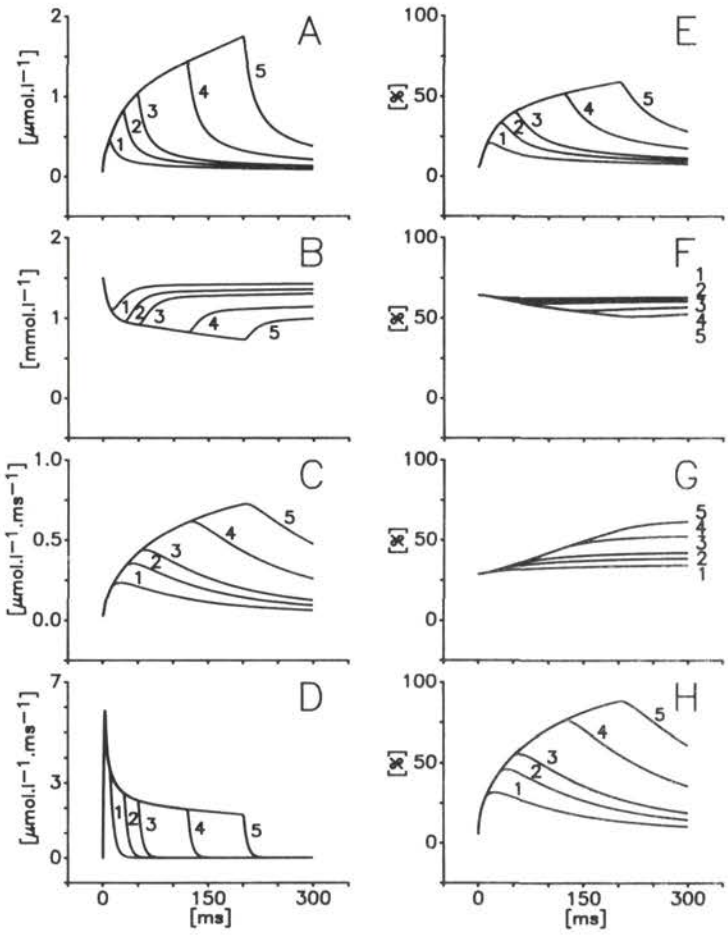

Fig. 4. The time courses of solutions of the model activated by depolarizing pulses of various lengths: (1) 10 ms, (2) 30 ms, (3) 50 ms, (4) 120 ms, and (5) 200 ms. (*A*) Free calcium concentration in the MS; (*B*) Free calcium concentration in the SR; (*C*) Calcium pumping into the SR; (*D*) Calcium release from the TC; (*E*) Saturation of the T-sites by calcium in the MS; (*F*) Saturation of calsequestrin by calcium in the TC; (*G*) Saturation of the P-sites by calcium in the MS; and (*H*) Saturation of the Ca-ATPase by calcium in the SR. The time scale is the same throughout.

T-sites (Fig. 5*B*), and particularly of the Ca-ATPase (Fig. 5*C*), clearly show the movement of the saturating front during activation of contraction in longitudinal direction from the Z line to the M line, i. e. towards the centre of the sarcomere (Poledna 1989a). The reason for the better indication of this movement by the saturation of the Ca-ATPase are different spatial distributions of

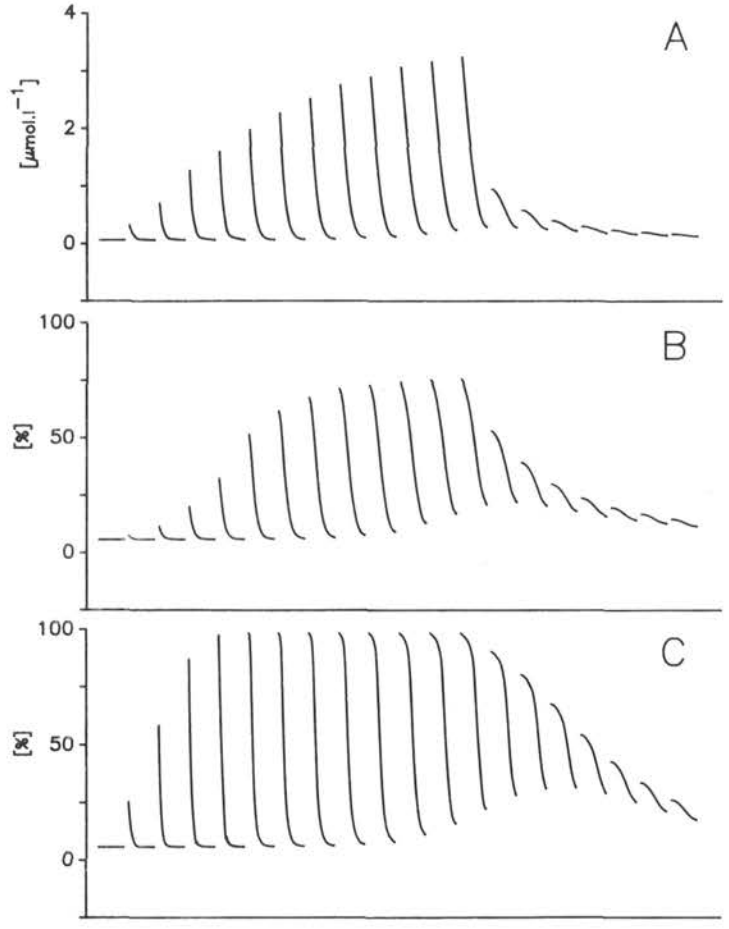

Fig. 5. Longitudinal profiles: (*A*) Free calcium concentration in the MS; (*B*) Saturation of the T-sites by calcium in the MS; and (*C*) Saturation of the Ca-ATPase by calcium at intervals (from the left to the right): 0, 0.5, 1.3, 2.5, 4.2, 9, 14, 20, 30, 40, 60, 80, 100, 120, 140, 170, 210, 260, 320, and 400 ms. The figure shows the response to 100 ms pulse. The Z line is on the left end of every profile and the M line is on the right one.

the T-sites and of the Ca-ATPase. While the T-sites are uniformly distributed throughout the sarcomere, the Ca-ATPase is localized in the SR membrane, and its saturation profiles hence are not radially averaged (in contrast to the T-site profiles). The same profiles also show a decrease of the calcium binding sites occupancy near the Z line simultaneously with an increase near the M line soon

Table III. Dissociation constants of Ca-binding

Ca-ATPase		
(the first Ca ion)	1.14 μmol . l^{-1}	(Inesi 1981)
(the second Ca ion)	0.07 μmol . l^{-1}	(Inesi 1981)
Arsenazo III	15—60 μmol . l^{-1}	(Scarpa et al. 1978)
(relaxation time constant	2.8 ms	(Scarpa et al. 1978))
Antipyrylazo III	60—400 μmol . l^{-1}	(Scarpa et al. 1978)
(relaxation time constant	0.18 ms	(Scarpa et al. 1978))

after the end of the pulse. This could correspond to a redistribution of the occupied sites (Poledna 1989b) in early phases of relaxation in longitudinal direction.

Discussion

A number of models concerning calcium distribution in skeletal muscle have been developed, serving various purposes. They describe calcium binding either to several proteins as a response to an increased free calcium concentration at twitch (Robertson et al. 1981), or to troponin using experimental records of Antipyrylazo III calcium transients of voltage clamped fibres at the threshold of contraction (Kovács et al.1987). Experimental records of a calcium-dye complex were also used to estimate either calcium binding to troponin and parvalbumin sites at both types of activation (Baylor et al. 1983), or the calcium release flux in the voltage-clamp conditions (Melzer et al. 1987). Other models represent calcium movement during contraction and relaxation, considering longitudinal calcium diffusion and binding (Poledna 1989a,b).

The calcium distribution in the sarcomere during contraction is a process occuring in three-dimensional space and in time. Earlier models of this process left a part of the information, mainly concerning radial calcium diffusion and its spatial distribution, to become lost. The three-dimensional model presents a more realistic picture of the calcium movement (see e. g. Cannell and Allen 1984). Our model is in good agreement with the earlier models and unlike them, it allows to study dynamical changes of calcium binding to Ca-ATPase sites during contraction-relaxation cycle. Processes of calcium redistribution in the sarcomere (e. g. the contributions of surface membrane, of other Ca-binding sites, etc.), which, owing to physiological concentrations of ions and the time scale used, have no effect on the presented results, have been neglected.

Experimentally measured values of the maximal free calcium concentration in the myoplasm show considerable dispersion, depending on the experimental method used: 2.9 μmol.l^{-1} (Baylor et al. 1982), 5.25 μmol.l^{-1} (Miledi et al. 1977; Parker 1979) and 7 μmol.l^{-1} (Blinks et al. 1978). The maximum, 4.2 μmol.l^{-1}, of free calcium in the MS, calculated by our model, is in the experimentally estimated interval 2—10 μmol.l^{-1} (Baylor et al. 1982).

The mechanism of calcium binding to Ca-ATPase and its turnover has been rather simplified (see Inesi 1981). However the turnover of the calcium pump (see Table II) is a good time characteristic of the process of calcium uptake from the MS. Another simplifying assumption concerning this process is that of the sufficient amount of ATP for the pump activity. The calcium uptake from the MS has been assumed to be independent of ATP concentration.

The variations of the model parameters have shown that it is predominantly the amount of fast binding sites (T-sites, Ca-ATPase) and their rate constants that decisively contribute to the changes in calcium distribution.

The exact calcium release control mechanism is not known as yet. So far, only calcium flux from the SR was modelled, corresponding to depolarization of the tubular membrane by the action potential (Cannell and Allen 1984) or by a rectangular pulse (Melzer et al. 1987). The exponential time course of the calcium permeability change in response to step changes of tubular voltage is likely a characteristic of gating kinetics. This time course is prevailing at short depolarizations by the action potential. For the long lasting depolarization there is a distinct inactivation phase. We tested the hypothesis concerning the feedback nature of this inactivation (Poledna 1989a). Free calcium concentration maintained near the TC (5 μmol.l^{-1}) during the pulse was estimated based on the model of Poledna (1989a) and also with respect to the maximal free calcium concentration in the sarcoplasm (1—2 μmol.l^{-1}, Melzer et al. 1987; Kovács et al. 1987). This value depends on the amplitude of the activation pulse. The model results gave a very good agreement with experimentally measured values.

The passive SR membrane permeability for calcium (P_l) employed in the model is higher than that measured experimentally (3.1×10^{-7} μm.ms^{-1}, Feher and Briggs 1982). It was adjusted to maintain the intracellular free calcium concentration on a constant level in the resting steady state (see Model and Methods).

Table III gives a comparison of dissociation constants of calcium binding to Ca-ATPase, Arsenazo III and Antipyrylazo III. The interference of calcium binding to Ca-ATPase with calcium binding to the dyes seems to be of little importance. The dissociation constant of calcium binding to Arsenazo III is closer to the actual free calcium concentrations in the MS during contraction than the respective constant for Antipyrylazo III. A comparison of the relaxa-

tion time constants (Table III) implicates that calcium binding to Arsenazo III monitors changes of the free calcium concentration in the sarcoplasm, while calcium binding to Antipyrylazo III describes the process of the calcium release from the TC. This conclusion is supported by comparing our results (Fig. 4*A*, *D*) with the experimentally measured courses of calcium binding to both dyes studied (Palade and Vergara 1982).

The shapes of the longitudinal profiles (Fig. 5) of calcium binding suggest that the boundary between the regions with saturated and free fast binding sites is sharp. This is in accordance with the hypothesis that contraction of the intact muscle fibre is proportional to the extension of the region with the calcium saturated troponin regulatory sites (Poledna 1989a,b).

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