

Relation of Muscle Cell Contraction and Calcium Distribution in Sarcomere

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Abstract. A model of activation of muscle contraction has been proposed. It is based on calcium diffusion and binding to specific regulatory sites in a sarcomere. Calcium ions activate interactions of contractile proteins and thus the generation of force. The model quantifies the relation between calcium released from intracellular stores and the elicited force.

Key words: Ca distribution in sarcomere — Activation of muscle contraction

Introduction

Muscle contraction is a highly specialized process converting chemical form of energy into mechanical work. The molecular basis of contraction is a cyclic attachment and detachment of heads of myosin filaments to actin filaments, resulting in their relative shift. Both types of filaments are structurally organized in a sarcomere. A muscle cell is morphologically and functionally designed to effectively control this process (Zachar 1971).

A voltage change on the surface membrane spreads inside a cell by a system of transversally invaginated tubules. They form a structurally distinct apposition with an intracellular membrane system, the sarcoplasmic reticulum, surrounding a sarcomere. An electrical signal in the tubular membrane is transformed and transmitted to control calcium release and reuptake by the sarcoplasmic reticulum. Calcium ions regulate the actin-myosin interaction. At rest, the intracellular concentration of calcium ions is very low, of the order of 10^{-7} mol/l, and binding of myosin heads to actin filaments is prevented by the troponin-tropomyosin complex. The occupation of two specific calcium binding sites on troponin allows a conformation of the regulatory complex and removes its inhibitory effect. Calcium, released from the sarcoplasmic reticulum, diffuses in the sarcoplasm, binds to regulatory proteins and is sequestered by the sarcoplasmic reticulum via Ca ATPase. The sarcoplasm contains also parvalbumin, a calcium buffering system.

The released calcium enters a complicated chain of interactions with intracellular structures. The dynamics of these interactions is given by the binding kinetic of different target proteins and by diffusion due to spatial distribution of these proteins. A possibility, how to infer implication of these processes for functional characteristics of the muscle cell is a mathematical description of the system. Most of our recent knowledge about the calcium movement in a muscle cell has been gained from measurements with calcium-sensitive dyes. Dyes applied into the sarcoplasm compete with other binding sites and influence the physiological response. Attempts have been done to model calcium movement in the muscle cell and to study mutual interactions between calcium binding sites in a sarcomere, including optical probes (Robertson et al. 1981; Baylor et al. 1983; Cannell and Allen 1984; Melzer et al. 1987). Contraction is the natural indicator of calcium concentration in the sarcoplasm. However, the relation between calcium released into the sarcoplasm and contraction remains unknown. The aim of this paper is to present a model of calcium distribution in the sarcomere and of the corresponding force elicited by the activated actin-myosin interaction. The model is easily solved and presented in a simple form to allow experimental verification.

Model and Results

The cycle of calcium release from terminal cisternae of the sarcoplasmic reticulum, its diffusion in the sarcoplasm with binding to regulatory sites, and reuptake by the sarcoplasmic reticulum is almost exclusively confined to the space of a single sarcomere. Hence the model of one half-sarcomere (owing to the symmetry) represents the behaviour of the entire muscle cell, when sarcomeres are synchronized e.g. under voltage clamp conditions. The main target of calcium released from terminal cisternae into the sarcoplasm is troponin C.

Troponin molecules have specific calcium binding sites with a very high binding rate ($10^{+8} \text{ mol}^{-1}\text{s}^{-1}$) (Johnson et al. 1981). Their concentration in the sarcoplasm is about 0.24 mmol/l (Baylor et al. 1983). Similar concentration (Baylor et al. 1983) and binding rate (Inesi 1981) have specific binding sites on calcium ATP-ase of the sarcoplasmic reticulum. Other binding sites have either much slower binding rates or relatively low concentrations. Calcium is released at the border of the sarcomere and diffuses longitudinally. Owing to a high binding rate, calcium sites are saturated successively from the site of the release (terminal cisternae of the sarcoplasmic reticulum) toward the centre of sarcomere. The model is based on the approximation that calcium ions can diffuse in the myofilament space only when the high binding rate sites have been

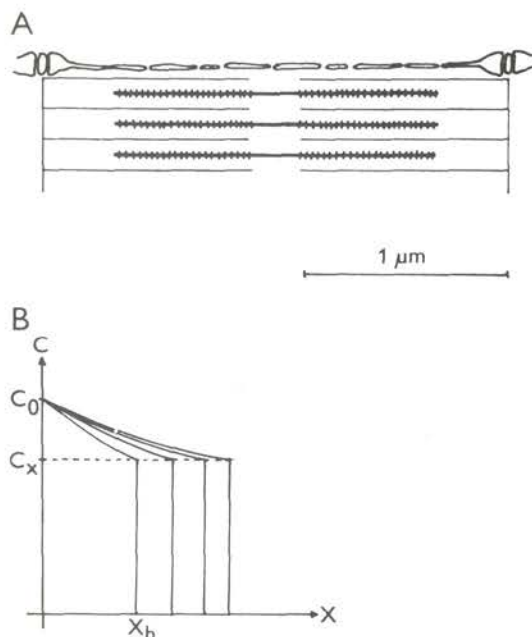


Fig. 1. Schematic drawings of a frog sarcomere (longitudinally in scale) (A) and of calcium concentration profile $c(t, x)$ with respect to the concentration of fast calcium binding sites c_x (B).

saturated. This process, schematically illustrated in Fig. 1, can be described by a one dimensional diffusion equation

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad (1)$$

where $D = 0$ for $c < c_x$, and $D = D_1 = 350 \mu m^2 s^{-1}$ for $c > c_x$. c_x is the concentration of the binding sites. The 2nd Fick's law is met at intervals where D is a constant. The condition of conserved diffusing mass is applied at the point of discontinuity of concentration c .

For the boundary condition $c(0, t) = c_0$, and for the initial condition $c(x, 0) = 0$, the solution can be found in the analytical form (Crank 1964)

$$c = c_0 + A \cdot \text{erf} \left(\frac{x}{2 \cdot (D_1 \cdot t)^{0.5}} \right) \quad \text{for } 0 < x < x_h \quad (2)$$

and

$$c = 0 \quad \text{for } x > x_h$$

where $x_h(t)$ is the coordinate of the point of discontinuity of free calcium concentration. It expresses that the frontier between saturated and free binding sites moves from the terminal cisterna ($x = 0$) and

$$x_h = k \cdot t^{0.5} \quad (3)$$

where the value of k can be obtained from

$$g(k/(2 \cdot D_1^{0.5})) = c_0/c_x - 1 \quad (4)$$

and where

$$g(z) = \pi^{0.5} \cdot z \cdot \exp(z^2) \cdot \operatorname{erf}(z) \quad (5)$$

The constant A in solution (2) can be found from

$$A \cdot \operatorname{erf}(k/(2 \cdot D_1^{0.5})) = c_x - c_0 \quad (6)$$

Only those parts of myofilaments which are in the space with saturated calcium sites can form bridges and generate force. This can be expressed as

$$dm/dt = p \cdot (k \cdot t^{0.5} - m) \quad m(0) = 0 \quad (7)$$

where p is the binding rate constant of myosin bridges, and m is the relative number of bridges formed (expressed per length of the myosin filament). The solution can be expressed in the form of the infinite series

$$m = \sum_{i=1}^{\infty} a_i \cdot s^{(i+0.5)} \quad (8)$$

with the coefficients given by the recurrent formula

$$a_i = -a_{i-1}/(2i+1); \quad a_1 = k/(3 \cdot (2p)^{0.5}) \quad (9)$$

and with $s = 2pt$.

The onset of isometric tension should be proportional to m . The coefficient of proportionality can be found from the length of myosin filaments in a half-sarcomere and from maximal tension elicited by a tetanic stimulation or a long lasting depolarization.

For frog skeletal muscle, where terminal cisternae are at the Z-lines, contraction can start when the activated region reaches the beginning of myosin filaments; this takes time τ . There equation (7) has to be modified as follows

$$dm/dt = p \cdot (k \cdot ((t + \tau)^{0.5} - \tau^{0.5}) - m); \quad t > 0; \quad m(0) = 0 \quad (10)$$

The solution can be found in the form of the Taylor series

$$m = \sum_{i=1}^{\infty} a_i \cdot t^i \quad (11)$$

with the coefficients defined by the recurrent formula

$$a_{i+1} = p/(i+1) \cdot (k \cdot \tau^{0.5} \prod_{n=1}^i (3 - 2n)/(2n \cdot \tau) - a_i) \quad a_1 = 0 \quad (12)$$

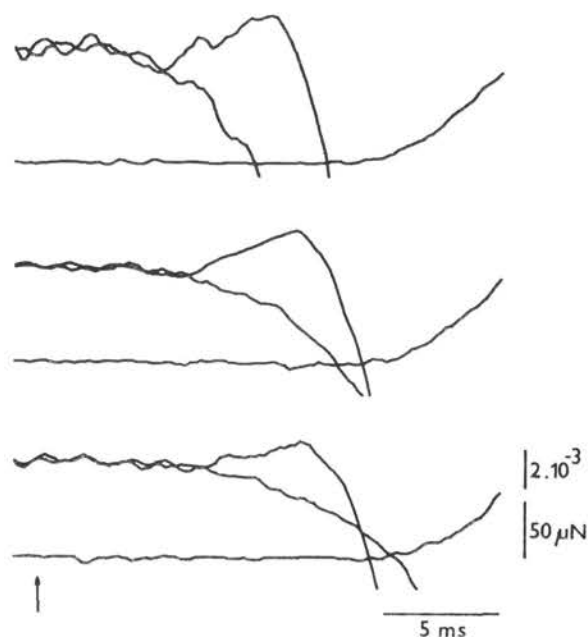


Fig. 2. Experimental records of simultaneous measurements of optical anisotropy and tension of frog muscle fibre under voltage clamp conditions. Depolarization from the resting membrane potential: 85, 75 and 65 mV. Changes in optical anisotropy were recorded with parallel and crossed polarizers.

This means that the initial phase of contraction is described by the solution (8) or (11), depending on the sarcomere morphology.

The calcium flux is given by the Fick's law

$$J = -D \cdot \partial c / \partial x \quad (13)$$

At point $x = 0$ it means the total calcium flux from the terminal cisterna. From solution (2) we get

$$J = -A \cdot (D / (\pi \cdot t))^{0.5} \quad (14)$$

and the total amount released in a half-sarcomere is

$$M(t) = \int_0^t J(\Theta) d\Theta = -2A \cdot (D \cdot t / \pi)^{0.5} \quad (15)$$

The onset of contraction can be approximated by solution (8) or (11) using the least square method, (e.g. Brown and Dennis 1972). The convergence of the

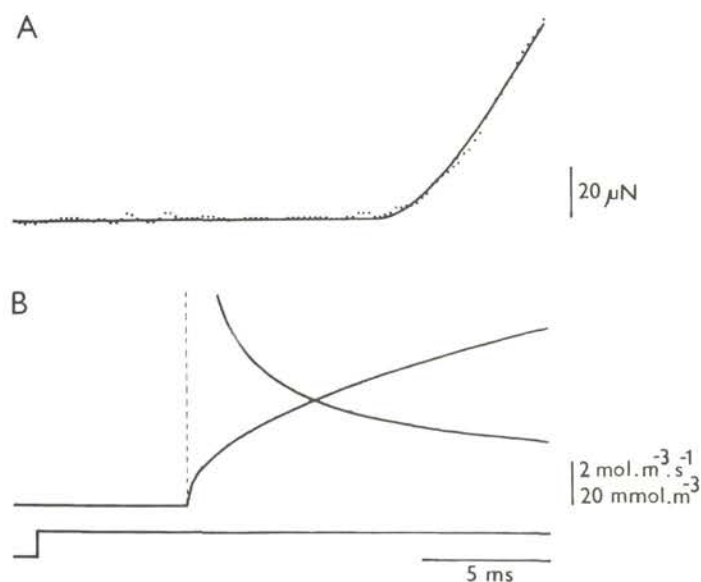


Fig. 3. (A) An example of approximation; the dotted line represents the digitalized upper tension record from Fig. 2. (B) the corresponding flux of calcium from the terminal cisterna and the total amount of calcium released. The rising phase of the calcium flux is vertical, and no assumption have been made concerning its kinetics.

series in (8) and (11) is guaranteed for $pt < 1$. This is also the condition for approximations. The approximation by this solution gives parameters which correspond to the kinetics of the bridge formation and the geometry of the sarcomere. The model can thus be tested by comparing it with the known biochemical and morphometric data.

The best experimental condition for the model testing is the activation of contraction under voltage clamp and the isometric measurement of tension at constant temperature. The data of Poledna and Lacinová (1988), with simultaneous recordings of the birefringence signal were used (Fig. 2). Frog skeletal muscle fibre activated under voltage clamp conditions (Fig. 3a) gave $p = 224 \text{ s}^{-1}$, $\tau = 7.1 \text{ ms}$ and $k = 2.7 \mu\text{m} \cdot \text{s}^{-0.5}$. The overall length of the sarcomere at an optimal stretch was $2.3 \mu\text{m}$. This means that the length of myosin filaments in a half-sarcomere was $0.7 \mu\text{m}$ (Gordon et al. 1966). τ and k determine that the calcium release started at $0.23 \mu\text{m}$ from the beginning of the myosin filaments; this exactly corresponds to the distance from the terminal cisterna. The simultaneously measured optical signal monitoring calcium release (Poledna and Lacinová 1985, 1988), started developing, in this case, 7.4 ms before the onset

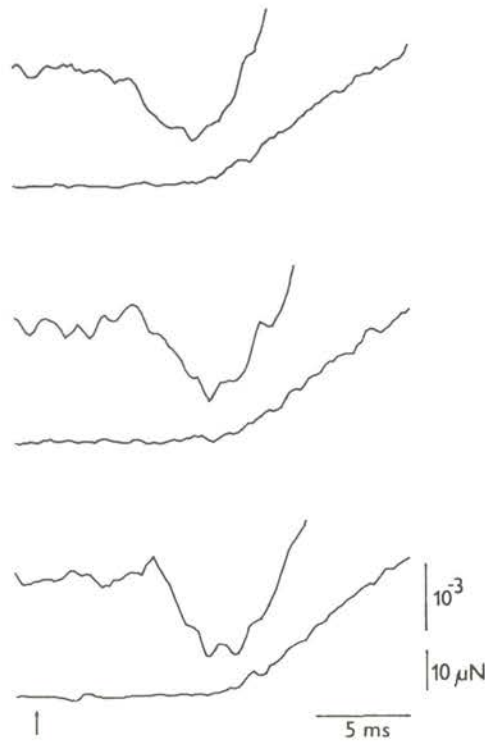


Fig. 4. Experimental records of simultaneous measurements of optical anisotropy and tension in crayfish muscle fibre under voltage clamp conditions. Depolarizations from the resting membrane potential: 85, 75 and 65 mV.

of tension. Equations (14) and (15) describe the calcium release from the terminal cisternae (Fig. 3*b*). The calcium flow, determined from the model, is qualitatively the same as experimentally measured by Melzer et al. (1987). The different rate is due to a different temperature during experiments. The value of A in the corresponding solutions was determined on the assumption that the total concentration of high rate binding sites, i.e. two specific sites on TnC and two on Ca ATPase, was 0.5 mmol/l (Baylor et al. 1983).

A similar analysis can be carried out with crayfish muscle fibres, where the terminal cisternae are located above the A-I boundary. This is also indicated by a shorter difference between the latencies of optical signals and the onsets of tension, which is less than 3 ms. Solution (8) should thus be used to approximate experimental records on Fig. 4. The obtained parameters $p = 120 \text{ s}^{-1}$ and $k = 2.29 \mu\text{m} \cdot \text{s}^{-0.5}$ are comparable to those obtained for frog muscle. It can thus

be concluded that the activation of isometric contraction under voltage clamp conditions is adequately described by the proposed model.

Discussion

The model presented herein relates the course of calcium release from the terminal cisterna to the initial part of contraction. Moreover, it approximates detailed distribution of calcium concentration in the sarcomere.

Optical measurements, which have offered most of the calcium movement data in muscle cells (Caille et al. 1984), give only spatially averaged values and cannot provide information about calcium distribution relative to the physiologically important calcium receptors. Moreover, the large number of different calcium binding sites makes mathematical modelling in the interpretation of optical measurements unavoidable (Baylor et al. 1983; Melzer et al. 1984, 1987).

The model is based on two important assumptions. The first one is that the released calcium saturates all high rate calcium binding sites along the diffusion path. Therefore, calcium ions can diffuse in the myofilament space only where all high rate binding sites have been saturated. This is expressed by the diffusion coefficient being zero until the amount of calcium ions locally exceeds the concentration of high rate binding sites; then the coefficient gets its usual value. Johnson et al. (1981), studying the binding kinetics of troponin C, found that a rapid Ca flux is able to fill the Ca-specific regulatory sites in a transient fashion, even though the equilibrium concentration of free Ca is below the amount required to occupy these sites.

The second assumption concerns the boundary condition of the constant calcium concentration at the terminal cisterna, which has allowed getting solution in an analytical form. A coupling signal activates the calcium release channels in the terminal cisternae with a rapid gating kinetics and a high unit conductance of about 100 pS (Smith et al. 1986). Therefore, calcium at the terminal cisternae can rise rapidly. Whether the concentration is held at the reached level depends on the existence of a feedback regulating the release. There is only indirect evidence for this, but the approximation of experimental data by the solution of the model is in a very good accordance with morphometric data and with independently measured calcium release. A further argument is purely biological. Cells keep very low internal concentrations of calcium ions. This is because calcium ions are an important intracellular messenger. The lower the basal concentration, the smaller amount of calcium ions is necessary to fulfill the required functions and the less energy is consumed to restore resting conditions. Hence, there must exist a feedback mechanism, which would confine the calcium release not to exceed free calcium concentration over

the regulation range. To control the calcium release, the simplest possibility is a feedback of calcium concentration at the terminal cisterna on its permeability. This process is the same as calcium dependent inactivation of calcium channels which was modelled by Poledna (1989). The analysis of the latter revealed interesting qualitative characteristics. Under stable activation conditions membrane permeability increases with an overshoot and stabilizes at a steady level. The regulated calcium concentration at the membrane monotonically reaches the steady state.

The measured onset of tension could be approximated by solution (8) or (11) only for converging corresponding series, i.e. for $pt < 1$. However, the model (7) or (10) is plausible for a longer interval, dominated by fast binding processes. The model is applicable until the calcium pump with a turnover of about 5 s^{-1} (Blasie et al. 1981) reaches the power comparable to Ca release, or until the dissociation from Ca sites is faster than the flow of free ions, and the moving frontier deteriorates. The latter also holds for low activation of Ca release, e.g. at the mechanical threshold, where this model is inapplicable.

For quantitative estimation of parameters also the calcium diffusion coefficient is of importance. Kushmerick and Podolsky (1969) compared the diffusion coefficients of ions and nonelectrolytes in aqueous solution and in muscle cell. They measured a reduction by a factor of 2 for both nonelectrolytes and ions. They concluded that the diffusion of the ions is reduced by physical rather than chemical interactions. In contrast, the diffusivity of the calcium ion, which is taken up by the sarcoplasmic reticulum, is reduced from $700 \mu\text{m}^2 \cdot \text{s}^{-1}$ to $14 \mu\text{m}^2 \cdot \text{s}^{-1}$. To take into account only physical factors, the calcium diffusion coefficient inside a muscle cell should be $350 \mu\text{m}^2 \cdot \text{s}^{-1}$. This value is close to $270 \mu\text{m}^2 \cdot \text{s}^{-1}$ determined by Zacharová and Zachar (1967) in the tubular system of crayfish muscle fibres. The method used was based on the analysis of the decoupling process in Ca-free medium (Valko et al. 1967).

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Final version accepted September 23, 1988