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Reallocation of Calcium During Activation of Skeletal Muscle Cell Contraction

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Structure of the muscle fibre

Much of the basic knowledge on muscle contraction has been obtained from experiments on isolated single muscle fibres of the frog. However, the comparative approach to different species has provided a substantial amount of information (Zachar 1971 , Huddart 1975, Ruegg 1986).

Recently, the broadening of our knowledge concerning regulatory processes of muscle contraction has moved to the molecular level. The molecular basis of contraction is the cyclic binding and dissociation of myosin heads of thick filaments to actin sites of thin filaments, generating their relative movement (Huxley 1957). This interaction is inhibited in the absence of calcium ions by the troponin-tropomyosin complex, which is a part of the thin filament. Both types of filaments are structurally arranged in a sarcomere.

Contraction is controlled by processes on the surface membrane and those occurring in intracellular membrane systems of transverse (T) tubules and the sarcoplasmic reticulum (SR). The coupling of excitation with contraction (EC) is a sequence of processes between the electrical events occurring on the surface membrane and the force generated by the contractile proteins. On the surface and the T tubule membrane, the regulation signal has the form of electrical depolarization (Hodgkin and Horowitz 1960, Zachar and Zacharová 1966). Depolarization of the plasma membrane spreads into the fibre along an internal tubular membrane system. The T tubules are branched invaginations of the surface membrane, with their internal lumen opening into the extracellular space. The tubules are in apposition with terminal cisternae of the sarcoplasmic reticulum which form specialized structures (diads or triads) and transmit the signal for calcium release. Calcium ion reallocation among muscle cell compartments on the sarcomere level, i.e. primarily between the sarcoplasmic reticulum and the myofilament space, regulates actin-myosin interaction. The transduction of the regulation signal from T tubules to the site of calcium release from the sarcoplasmic reticulum is not yet fully understood.

The main difference in EC coupling between invertebrate and vertebrate skeletal muscle follows from the type of electrogenesis in the surface membrane systems. In contrast to the sodium action potential in vertebrates, the calcium action potential may play a role in direct activation of contraction in invertebrates, e.g. by the mechanism of calcium-induced calcium release from the SR (Zacharová and

Zachar 1967, Zachar 1981). Muscle fibres of the crayfish, however, posses all basic components, which have recently been invoked as key elements of the EC coupling in vertebrate skeletal muscle (voltage sensor, T-SR junction mediators) (Zachar and Zacharová 1989, Formelová *et al.* 1990, Zacharová *et al.* 1990). The diads in muscle fibres of *Astacus fluviatilis* show a similar feet structure as do triads in vertebrate muscle (Uhrík *et al.* 1984, 1986).

Molecular mechanisms of excitation-contraction coupling

Three mechanisms have been proposed to underlie the signal transduction from membrane depolarization to Ca release from the SR in skeletal muscle: chemical mediation, a mechanical connection between tubular membrane and SR, and Ca-induced Ca release.

Inositol 1,4,5-trisphosphate (IP_3) has been proposed as a possible chemical messenger that would act as a link between these two events (Vergara *et al.* 1985, Volpe *et al.* 1985). According to this model, IP_3 would be released from the T tubule membrane following its depolarization; the released IP_3 would diffuse into the triadic space and upon reaching the sarcoplasmic reticulum, it would open calcium channels. However, there have been conflicting reports as to the ability of IP_3 to release calcium from isolated SR vesicles. Ogawa and Harafuji (1989) examined the Ca-releasing action of IP_3 on the heavy fraction of sarcoplasmic reticulum from bullfrog skeletal muscle. IP_3 concentrations of up to 55 μmol/l were ineffective. On the other hand, Hidalgo *et al.* (1989) have shown for frog skeletal muscle cells that addition of 0.5 – 1.0 μmol/l IP_3 to skinned fibres caused fast calcium release as detected from aequorin light emission. Similarly, 0.5 – 1.0 μmol/l IP_3 activated the large conductance (100 pS) calcium channel present in isolated sarcoplasmic vesicles. Both the calcium release and the activation of the calcium channel by IP_3 required a calcium concentration of 0.1 μmol/l or higher. This may support the view that IP_3 plays a role in EC coupling mechanisms in frog skeletal muscle; however, it remains to be determined how depolarization releases IP_3 from the T tubules.

Charge movement represents mobile charges inside the tubular membrane, which may serve to signalize the membrane potential to a hypothetical molecule localized in the foot of the triad, which is considered as the locus of communication between the T system and the SR membrane (Schneider and Chandler 1973, Henček *et al.* 1985). Dihydropyridine receptors are involved in EC coupling as a voltage sensor (Ríos and Brum 1987, Ríos and Pizarro 1988). Dihydropyridine receptors of skeletal muscle might not be functional Ca channels. EC coupling phenomena and calcium currents (I_{Ca}) have very similar drug sensitivities, voltage sensitivities, and time dependences of recovery. There are differences, on the other hand, which seem to indicate that the voltage sensors of EC coupling are not the pathways of I_{Ca}. All Ca agonists tested so far have had effects on EC coupling at concentrations comparable to those that affect Ca channels. However, if some Ca agonists were used, no potentiation of Ca release was observed. In general, all the agonist and antagonist dihydropyridines, the phenylalkylamines, and the benzothiazepamines have inhibitory effects on EC coupling. Voltage sensing for EC coupling and I_{Ca} gating may be carried out by different proteins (Pizarro *et al.* 1988).

The Ca release channel from rabbit muscle SR has been purified and reconstituted into lipid bilayers as a functional unit. Electron microscopy revealed the four-leaf clover structure previously described for the feet that span the T-SR junction (Lai *et al.* 1988, Hymel *et al.* 1988).

The Ca release channel in the sarcoplasmic reticulum is regulated by the endogenous ligands Ca^{2+}, Mg^{2+} and ATP. Micromolar calcium and millimolar ATP concentrations activate the channel, although the presence of both ligands is required to fully open it. In the presence of Mg^{2+} and ATP, the channel is regulated in a complex manner in that free Mg^{2+} ions inhibit channel opening, whereas the Mg.ATP complex (formed in the presence of Mg^{2+} and ATP) potentiates Ca-induced Ca release from SR vesicles. In addition to the endogenous ligands, a number of drugs and reagents able to modify proteins, such as ryanodine, doxorubicin, methylxantines, and sulfhydryl reacting agents, have been found to activate the Ca release channel from the skeletal muscle SR, similarly as does adenine. Vesicle $^{45}Ca^{2+}$ ion flux studies have suggested that caffeine, despite possessing a molecular structure related to that of adenine, exerts its effects through the calcium-activating rather than the adenine nucleotide-activating sites of the channel (Xu *et al.* 1989).

Biophysical properties of signal transduction in the T-SR junction

The nature of the coupling signal which opens the calcium channel is still controversial. Measurements of tubular voltage dependence of calcium flux into the sarcoplasm are required to find characteristics of signal transmission from the tubular membrane to terminal cisternae of the sarcoplasmic reticulum, where calcium permeability is increased. It is experimentally convenient to measure calcium concentration changes in the sarcoplasm of a muscle cell under voltage clamp conditions.

Several voltage clamp methods have been developed and used to study EC coupling (Adrian *et al.* 1969, Costantin and Taylor 1973, Costantin 1974, Dudel *et al.* 1968, Caputo and Bolanos 1979, Caputo *et al.* 1984, Heistracher and Hunt 1969). These methods have had some restrictions concerning the potential range or the choice of preparations. In the majority of calcium measurements optical methods were used (Caille *et al.* 1985).

Some voltage clamp methods (Hille and Campbell 1976, Kovács and Schneider 1978, Poledna and Lacinová 1985, 1988) allow measurements of optical changes in the activated part of the fibre.

Calcium-sensitive dyes and measurements of intrinsic optical properties have been employed to investigate the calcium release from the SR into the sarcoplasm. Changes in dye-related absorbance were used to estimate the time course of calcium release from the SR (Baylor *et al.* 1983. Melzer *et al.* 1984, Csernoch *et al.* 1990).

The birefringence signal reflects changes in optical anisotropy, i.e. in dependence of light speed on its direction relative to muscle cell geometry. This effect is a consequence of interaction of light with electron orbitals of macromolecules, and thus provides information about molecular mechanisms of physiological processes in cells. A large change in birefringence following the action potential and preceding the contraction in skeletal muscle cells was first described by Baylor and Oetliker (1975). Its origin is a change in the distribution of Ca

ATPase conformation states in the SR, which mirrors an increased Ca concentration in the sarcoplasm (Poledna and Morad 1983, Poledna and Lacinová 1988, Lacinová and Poledna 1990).

The characteristic features of birefringence and calcium transients were compared in voltage-clamped cut skeletal muscle fibres. Calcium transients were monitored by the dye antipyrylazo III. The early birefringence signal, related to excitation-contraction coupling, had a time course similar to that of calcium transients. The two signals had a superimposed onset (Kovács *et al.* 1983).

The natural indicator of calcium concentration changes in the sarcoplasm is contraction of the muscle cell. The dependence of contraction on the calcium concentration in myofilament space was determined in skinned muscle fibres under equilibrium conditions (Hellam and Podolsky 1969). The calcium concentration in the sarcomere is homogenous and there is an equilibrium between free and bound calcium. During the activation of contraction, processes of calcium distribution are far from equilibrium. The sarcomere has several types of binding sites for calcium with different rate constants for binding and dissociation. Therefore, this equilibrium relation is not relevant, and it is necessary to estimate the dependence of contraction on calcium release from the dynamics of calcium redistribution in the sarcomere. Several approaches to model these processes have been published (Cannell and Allen 1984, Poledna 1987, 1989b,c, Hollý and Poledna 1989).

Calcium ions are released from the terminal cisternae. This event is controlled by tubular membrane voltage. In the frog muscle cell, T tubules are located at the Z-line level. Calcium ions bind to specific regulatory sites of troponin C. Due to the high binding rate constant, the calcium flux is able to temporarily fill them, even though the equilibrium concentration is below the amount required to occupy these sites (Johnson *et al.* 1981). This means that during calcium release specific binding sites saturate and form a region, which expands from the Z-line towards the centre of the sarcomere. Only in this region can actin and myosin filaments interact and generate force.

Calcium movements in the sarcomere are described under these conditions by the diffusion equation

$$\partial c/\partial t = D.\partial^2 c/\partial x^2 \quad c(0,t) = c_0 \quad c(x,0) = 0 \tag{1}$$

The diffusion constant, D, is nonzero only in the region of saturated specific sites (Poledna 1989b).

The solution of Eq. 1 implies (Crank 1964)

$$x_h(t) = kt^{0.5} \tag{2}$$

where x_h is the coordinate of the border line between saturated and free binding sites.

The force generated is proportional to the number of bridges formed by myosin heads in the region of saturated regulatory sites. Their number is described by

$$dm/dt = p\{k[(t + \gamma)^{0.5} - \gamma^{0.5}] - m\} \tag{3}$$

where γ is the time needed by the saturated region to reach the A-band, where the thick and thin filaments overlap.

Based on this model, we can approximate experimental records of contraction under voltage clamp conditions and determine parameter A of calcium flux from terminal cisternae (Fig. 1)

$$J = -A[D/(\pi t)]^{0.5} \tag{4}$$

The calcium flux, J, was derived from Fick's first law of diffusion.

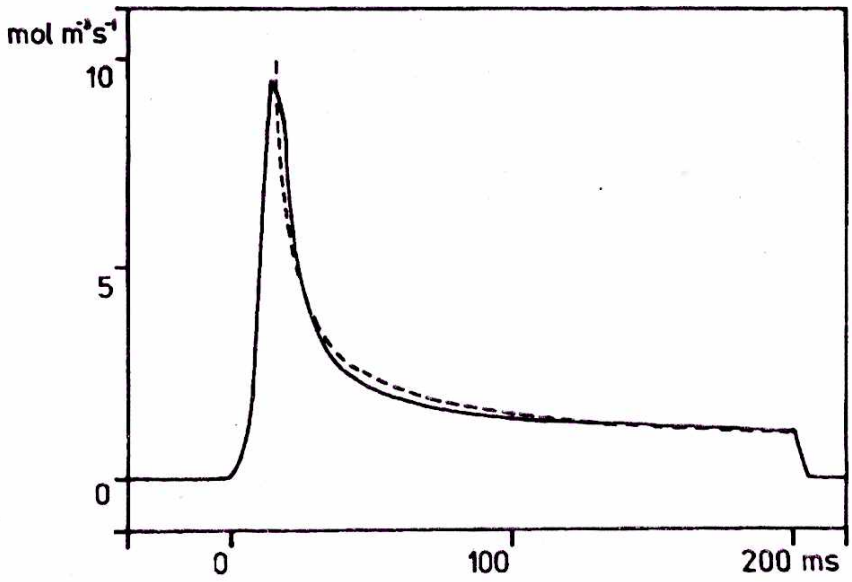

Fig. 1
The calcium release from the terminal cisternae, as determined from the model (Eq. 4) and experimental data (Poledna 1989b) (broken line), is qualitatively the same as measured by Melzer *et al.* (1987) (solid line). Temperature differences were accounted for with setting $Q_{10} = 2$.

The calcium release for contraction in skeletal muscle cells is the largest source of intracellular calcium concentration changes. Therefore, it is natural to suppose the existence of feedback inhibition of this very process (Poledna 1987, Schneider and Simon 1988, Poledna 1989a). The boundary condition of model (1) is then the constant calcium concentration at the terminal cisternae. It follows from the solution with this boundary condition that the width of the region with saturated regulatory sites is proportional to the square root of the activation time (cf. Eq. 2). This conclusion was tested experimentally.

Contractile responses to rectangular depolarizing pulses of different amplitudes and durations were measured. In accordance with Eq. 2, the maximal amplitude of contraction, which is approximately proportional to the width of the region of saturated calcium sites, depends on the square root of the pulse duration (Fig. 2). This supports the assumption concerning the steady calcium concentration at the terminal cisterna under voltage clamp conditions. Simultaneously, it confirms the existence of feedback calcium release inhibition by calcium ions at the terminal cisternae.

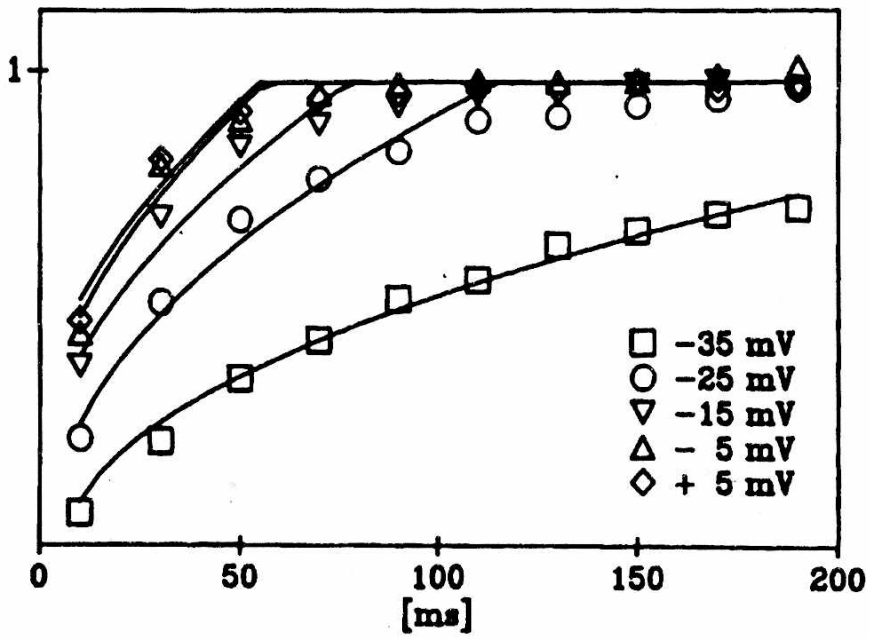

Fig. 2
Contractile responses to rectangular depolarizing pulses measured under voltage clamp conditions (Poledna and Lacinová 1988). The maximal amplitude of contraction has been plotted against the depolarizing pulse width. Experimental values were approximated by parabolas (Eq. 2) for different pulse amplitudes.

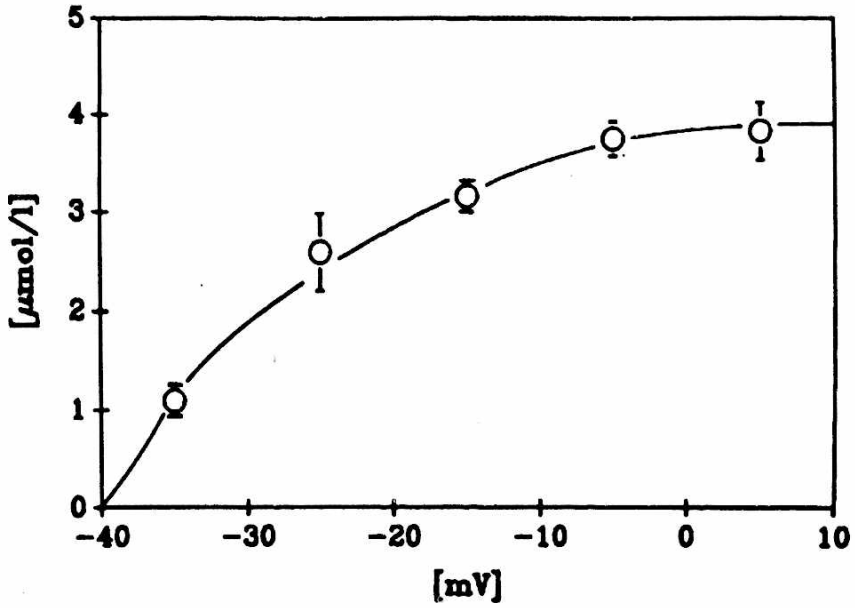

Fig. 3
The dependence of calcium at the terminal cisterna plotted against the tubular membrane potential. Values were determined from the model; the parameters of the parabolas are shown in Fig. 2.

Moreover, based on the model we can determine the voltage dependence of the steady state calcium concentration at the terminal cisternae from the time and voltage dependencies of the contraction amplitude (Fig. 3).

The proposed model is based on the assumption that the saturated region of troponin C regulatory sites spreads from the Z-line towards the centre of the sarcomere. At the end of activation the calcium release ceases. However, the calcium continues to be taken up and redistributed among the binding sites. The redistribution can be expressed as a fictive diffusion of the occupied binding sites, and is described by the diffusion equation (Poledna 1989c)

$$\partial \mathbf{c}/\partial t = \mathbf{D}.\partial^2\mathbf{c}/\partial x^2 - \alpha\mathbf{c} \tag{5}$$

where **c** is the concentration of the occupied binding sites, **D** is the fictive diffusion constant of the occupied sites, and α is the rate of calcium uptake by the sarcoplasmic reticulum, and/or its binding to parvalbumin. The initial and boundary conditions are

$$\mathbf{c}(x,0) = 1 \text{ for } 0<x<\beta \text{ and } \mathbf{c}(x,0) = 0 \text{ for } \beta<x<1 \tag{6}$$

$$\partial \mathbf{c}/\partial x = 0 \text{ at the points } x = 0 \text{ and } x = 1 \tag{7}$$

where β gives the relative width of the saturated part of the sarcomere.

The solution of the equation (5) has the form

$$\mathbf{c} = \exp(-\alpha t)[\beta + \sum_r 2(-1)^r \exp(-\mathbf{D}(r\pi)^2 t)\sin(r\pi(\beta - 1))\cos(r\pi x)/(r\pi)] \tag{8}$$

We have obtained the distribution of the occupied troponin C binding sites in the sarcomere during relaxation. The inhibition of troponin-tropomyosin complex is removed when the both calcium specific sites are occupied; therefore, tension should be proportional to the integral through half-sarcomere of the square of the occupied sites concentration

$$T(t) = K\int_0^1 \mathbf{c}^2(x,t)dx =$$

$$= K[\beta^2\exp(-2\alpha t) + 2\sum_r(\sin(r\pi(\beta - 1))\exp(-\alpha t - \mathbf{D}t(r\pi)^2)/(r\pi))^2] \tag{9}$$

From Eq. 9 it follows that for long depolarizing pulses, which saturate all regulatory sites, i.e. $\beta = 1$ and only the first term is nonzero, the relaxation has monoexponential time course corresponding to the calcium uptake only. For short pulses, Eq. 9 expresses the contribution of the redistribution of the occupied sites. This conclusion was confirmed experimentally. For short pulses, the relaxation is at

least biexponential (Fig. 4). The slower time constant for relaxation at short pulses is the same as the time constant for long pulses.

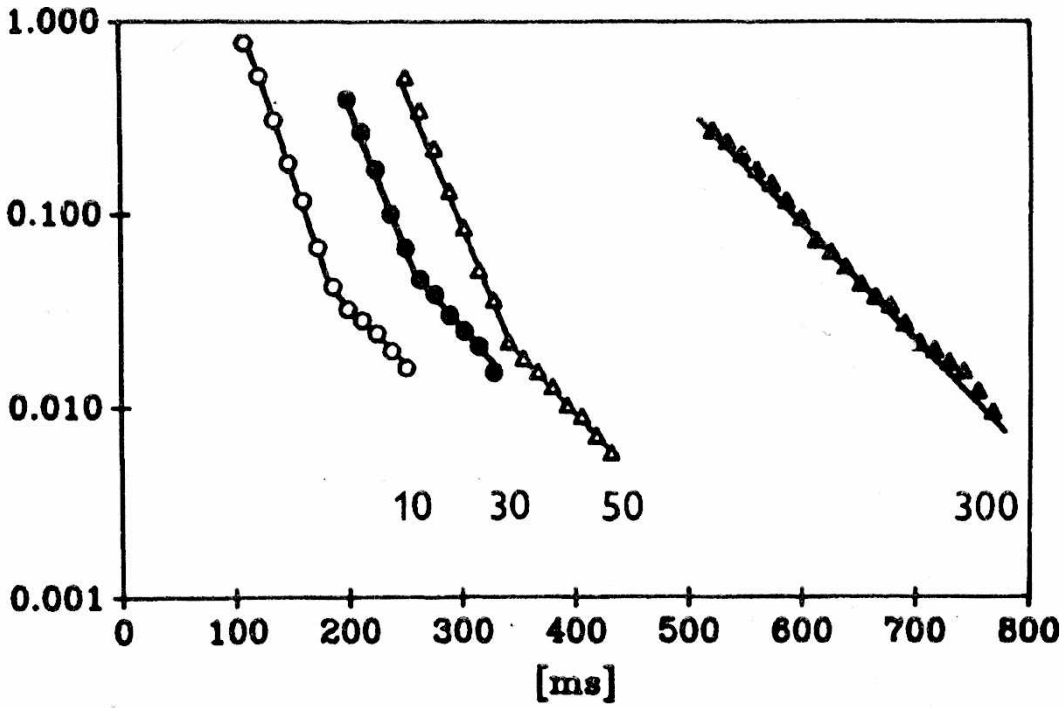

Fig. 4
Semilogarithmic plots of the relaxation phase of contraction for different durations of the depolarizing pulse (10, 30, 50, 300 ms). Values on the abscissa are relative units of the maximal amplitude. The solid line represents the approximation by the model (Eq. 9).

The presented hypothesis, expressed by the model, includes the essential mechanisms which determine the course of activation and relaxation of contraction. Confining this to the onset of contraction, where the calcium uptake can be neglected, or to relaxation, where calcium release is already absent, the model supplies an explicit solution applicable to experimental records. It makes it possible to find otherwise nonmeasurable variables, such as the time course of calcium release and the time and spatial distribution of calcium in the sarcomere. This is important for the analysis of regulation processes in the skeletal muscle cell.

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