Calcium model for mammalian skeletal muscle

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Abstract—A model is presented describing quantitatively the events between excitation and force development in skeletal muscle. It consists of a calcium mediated activation model (c.m.a.m.) in series with a force generator model (f.g.m.). The c.m.a.m. was based on intracellular processes such as cisternal Ca-release, Ca-troponin interaction and Ca-uptake by SR. Ca-troponin complex concentration is an output of the c.m.a.m. and input of the f.g.m., the latter being a simplified actin-myosin cross-bridge model. Simulated and measured forces were compared for the rat slow (soleus) muscle. With the present structure and parameter values there is reasonable agreement between simulated and measured forces for single- and double-pulse responses. The parameters of the f.g.m. mainly determine the rising phase of the twitch since the Ca-release is brief and the binding of Ca to troponin is fast. In relaxation the Ca-troponin interaction and the Ca transporting ATPase parameters are also important. The behaviour of the muscle after a second action potential depends strongly on the level of Ca occupancy for both the troponin and Ca transporting ATPase.

Keywords—Calcium activation, Excitation-contraction coupling, Force development, Muscle model, Rat skeletal muscle

1 Introduction

IN recent years much information has been gained as to the processes involved in excitation-contraction (EC-) coupling of skeletal muscles. Sarcoplasmic reticulum is a key factor in EC-coupling (see FUCHS, 1974) and calcium distribution over a number of intracellular compartments also has an essential function. Excitation results in changed inter-compartmental calcium flows (CHANDLER *et al.*, 1976b; MILEDI *et al.*, 1977; POTTER *et al.*, 1978; FROELICH and TAYLOR, 1975; WINEGRAD, 1970) the nature of which has been described in part quantitatively.

Only when the individual processes, as calcium release from sarcoplasmic reticulum (SR), calcium-troponin interaction and calcium uptake by SR, are combined, can the effect of a single process on the overall behaviour be analysed. This can be done only with help of a simulation model in which the individual processes can be recognised.

Fig. 1 Calcium mediated activation (c.m.a.-) model describing flows between and storage in the calcium compartments. Input is the action potential, output is calcium-troponin complex concentration. The latter is input for the force-generation (f.g.-) model

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In this paper a calcium model is quantitatively described. It enables the simulation of calcium flows between the compartments and the calcium (complex) concentrations within them by using the relevant data of the individual processes.

Simulated forces generated by the model (Fig. 1) for single and repetitive stimulations are compared with experimental results obtained from rat skeletal muscle.

2 Calcium mediated activation model (c.m.a.m.)

2.1 Outline of calcium pathways

The c.m.a.m. describes the changes of the calcium (-complex) concentration in the intracellular compartments. Briefly, what happens is together with the calcium flows indicated in Fig. 2. During rest the calcium is stored for the greater part in the terminal cisternae of the sarcoplasmic reticulum (SR). As a result of an action potential over the T-tubules calcium flows into the sarcoplasm (flow ϕ_1), where it can bind to troponin (ϕ_2). A resulting calcium-troponin

calcium mediated activation model

Fig. 2 Block diagram of the calcium compartments and calcium flows. Flows (ϕ s) are described in the text

complex enables force generation. Dissociation of the calcium-troponin complex is forced by low sarcoplasmic calcium concentration realised by a calcium carrier pump in the longitudinal SR-membrane. Carriers remove calcium from sarcoplasm (ϕ_3) and transport it into the longitudinal SR against a calcium gradient (ϕ_4). During rest and after an action potential calcium leaks from the SR into the sarcoplasm due to the calcium gradient (ϕ_5). Inside the SR-system the calcium flows back to the cisternae by passive diffusion (ϕ_6). Owing to their significant calcium binding capacity, troponin and calcium carriers are considered as separate compartments.

The calcium concentration is assumed to be uniform inside each compartment, implying that all intracompartamental diffusion processes are virtually instantaneous.

2.2 Calcium release from SR (ϕ_1)

The mechanism of the calcium release from the terminal cisternae is not yet clear. For skeletal muscle, the depolarisation of the tubular membrane probably initiates the enhancement of the calcium permeability of the terminal cisternal membrane (ENDO, 1977).

The sarcolemmal action potential also propagates into and along the T-tubules (GONZALEZ-SERRATOS, 1971). The time course of the tubular membrane depolarisation is not known; detailed information about propagation velocity of the action potential and eventual damping is lacking (ADRIAN and PEACHEY, 1973). Consequently, these aspects are not incorporated in the c.m.a.-model. The depolarisation of the whole tubular membrane was taken as identical to sarcolemmal depolarisation. The impact of this assumption on the simulations is discussed in Section 6. The course of the sarcolemmal action potential has been derived from HANSON (1974) for rat soleus muscle fibres. The resting membrane potential is -73.7 mV; the amplitude 93.8 mV. The calcium release flow is calculated from

$$\phi_1(t) = n_{ch} \cdot A_T \cdot N^2(t) \cdot \phi_{ch} \cdot \frac{[Ca(t)]_c - [Ca(t)]_s}{[Ca]_{c,rest} - [Ca]_{s,rest}} \quad (1)$$

where

- n_{ch} the maximum number of open calcium channels per unit cisternal membrane surface,
- A_T the cisternal membrane surface concerned in release,
- $N^2(t)$ the fraction open calcium channels,
- ϕ_{ch} the calcium capacity per (open) channel,
- $[Ca]_c$ the calcium ion concentration in cisternae and
- $[Ca]_s$ the calcium ion concentration in sarcoplasm.

The component elements of eqn. 1 will now be introduced consecutively. Calcium channels have been claimed to occur in the cisternal membrane (see ENDO,

1977). The number of calcium channels per surface unit was equated to the number of 'feet' between the tubular and SR-membrane per unit surface in frog muscle (FRANZINI-ARMSTRONG, 1970).

The surface of the SR-membrane that contains the calcium channels was taken the same as the surface of the T-system.

Calcium channels may occur in two states in the model: open and closed. We assumed that the fraction of open channels depends on membrane potential and time, since $N(t)$ behaves like a Hodgkin-Huxley variable

$$\frac{dN(t)}{dt} = \alpha\{1 - N(t)\} - \beta \cdot N(t) \quad (2)$$

where

$$\alpha = \frac{\alpha_{max}(E - \bar{V})/k}{1 - \exp(\bar{V} - E)/k} \quad (3)$$

and

$$\beta = \frac{\beta_{max}(\bar{V} - E)/k}{1 - \exp(E - \bar{V})/k} \quad (4)$$

where

α_{max} , β_{max} , $\bar{V}$ and k are constants and E is the transmembrane potential.

The behaviour of the calcium channels is not known. The value of ϕ_{ch} , the flow per channel in the rest situation, and which was taken as a constant, was obtained from the assumption that about $0.1 \mu\text{mol Ca}$ per ml sarcoplasm is released during a single twitch (BAYLOR *et al.*, 1979a).

For a given $N^2(t)$ function the product of n_{ch} , A_T and ϕ_{ch} determines the calcium release in the twitch (the influence of the last term in eqn. 1 is quite small). So the release of a certain amount of calcium can also be realised by another combination of values of n_{ch} and ϕ_{ch} .

The calcium flow through each open channel was assumed to depend on the calcium concentration gradient over the membrane. The last factor of eqn. 1 equals 1 in the rest situation. It is always less than 1 after release of calcium from the cisternae into the sarcoplasm and thereby reduces the calcium flow.

2.3 Calcium binding to troponin (ϕ_2)

The troponin-C molecule of skeletal muscle has two specific calcium binding sites and two competitive $\text{Ca}^{2+} - \text{Mg}^{2+}$ binding sites (POTTER and GERGELY, 1975; HAIKCH *et al.*, 1979).

We assumed that there is no interdependency between the binding to specific calcium and $\text{Ca}^{2+} - \text{Mg}^{2+}$ sites, so that the troponin molecules can be thought to be split into two sets: troponin with two specific calcium sites and troponin with two $\text{Ca}^{2+} - \text{Mg}^{2+}$ sites. The amount of both 'sorts' of troponin is then equal to the total amount of troponin.

2.5 Calcium leakage from longitudinal SR into sarcoplasm (ϕ_5)

At rest and during prolonged muscle activity the calcium concentration inside the longitudinal SR is much higher than in the sarcoplasm. This results in a flow from SR to sarcoplasm (WEBER *et al.*, 1966). Since the nature of this flow is unknown, it is described in the model as a passive diffusion leakage flow:

$$\phi_5(t) = D_1([Ca(t)]_L - [Ca(t)]_S) \quad (11)$$

where

D_1 is a diffusion constant.

2.6 Calcium backflow from longitudinal SR to terminal cisternae (ϕ_6)

The longitudinal calcium flow within the SR to the terminal cisternae can be studied only in intact muscle fibres. Quantitative data of WINEGRAD (1970) (frog muscle) indicate that it is controlled by the concentration gradient:

$$\phi_6(t) = D_2([Ca(t)]_L - [Ca(t)]_C) \quad (12)$$

where

D_2 is a diffusion constant and
 Ca_C free calcium ions in terminal cisternae.

The greater part of the calcium in the cisternae is bound to proteins in a fast equilibrium (MARTONOSI, 1971). In our model only 1% is present in free ionised form. An instantaneous relation between free and bound calcium is used:

$$[Ca(t)]_C = 0.01 [Ca(t)]_{C, total} \quad (13)$$

where $Ca_{C, total}$ is the sum of free and bound calcium in the terminal cisternae.

2.7 Additional equations

In the above Sections the intercompartmental flows were expressed in terms of the underlying processes. To complete this model additional equations are needed to relate compartmental calcium concentrations to the flows. Differential equations to describe the calcium concentrations in the compartments involved follow from the differences between the flows in and out of these compartments. Also the system was supposed to be closed so the quantities of calcium, troponin and ATPase are constant.

The resting values of the compartmental concentrations were derived by putting all time derivatives zero. These values have been used as the starting values for the integration procedure.

3 Force generation model

Our force generation model is derived from the cross-bridge model of JULIAN *et al.* (1974). Fig. 3

indicates schematically the processes determining the number of force generating cross-bridges. The presence of binding places on actin for coupling with free myosin heads (AS in Fig. 3) depends on the troponin state. When both calcium specific binding sites of one troponin (resulting in $Ca_2 Tn_s$) have been occupied, 7 actin binding places AS are recruited (PERRY, 1979). A change in $[AS]$ depends on its relations illustrated in Fig. 3 and on its production by $Ca_2 Tn_s$:

$$\frac{d[AS(t)]}{dt} = k_a[A_\beta(t)] - k_a[AS(t)][D(t)] + 7 \frac{d[Ca_2 Tn_s(t)]}{dt} \quad (14)$$

Fig. 3 Diagram of force-generation model with k_a , k_d , k_x and k_β reaction rate constants.

AS = active sites; available binding places on actin for free myosin heads

D = free myosin heads

A_α = non-force generating cross-bridges

A_β = force generating cross-bridges

The symbols are defined in the legend of Fig. 3. Eqn. 14 implies that the concentration of $Ca_2 Tn_s$ does not depend on the state of the myosin head (D, A_α or A_β) and a change in $[Ca_2 Tn_s]$ only influences $[AS]$. Muscle force depends on the number of parallel muscle fibres whose effects summate linearly. The force per muscle fibre depends on the number of force-generating cross-bridges per half sarcomere (HUXLEY, 1974). If the force exerted by all force generating cross-bridges was assumed to be uniformly distributed, then:

$$F(t) = F_{br} C[A_\beta(t)] \quad (15)$$

where

F_{br} is the mean cross-bridge force and
 C is a constant.

Table 1. Values of the most critical parameters

Parameters	Explanation	Sim. value	Dim.	Lit.† value	References	Animal species
ϕ_1 ϕ_{ch}	calcium capacity per open channel	27.5×10^{-13}	$\frac{\mu\text{mol}}{\text{s}}$	5×10^{-13}	CHANDLER <i>et al.</i> (1976b)	frog
ϕ_2 $k_{1,s}$ $k_{2,s}$	binding } rate constant of calcium for specific, low affinity troponin sites decoupling }	0.8×10^5 80	$\frac{\text{ml}}{\mu\text{mol s}}$	10^5 20–35		
$k_{1,r}$ $k_{2,r}$	binding } rate constant of calcium for Ca^{2+} – Mg^{2+} , high affinity troponin sites decoupling }	1.65×10^4 1.3	$\frac{\text{ml}}{\mu\text{mol s}}$	10^5 0.1–0.5	POTTER <i>et al.</i> (1978), POTTER (1979)	rabbit
ϕ_3 E_{tot}	total amount of calcium carriers in V_{eq}	2.8	$\frac{\mu\text{mol}}{\text{ml}}$	0.24–10.5	MARTONOSI (1973)–OGAWA (1970), KANAZAWA <i>et al.</i> (1971)	rabbit
k_3	rate constant calcium binding	8×10^4	$\frac{\text{ml}}{\mu\text{mol s}}$	$4\text{--}13 \times 10^4$ †	OGAWA (1970)–FROELICH and TAYLOR (1975)	rabbit
k_4 k_5	rate constant calcium decoupling rate constant calcium decoupling	0.4 1300	$\frac{\text{s}^{-1}}{\text{s}^{-1}}$	2×10^{-4} –80† 40–80	OGAWA (1970)–SUMIDA <i>et al.</i> (1978) MARTONOSI <i>et al.</i> (1974)–SUMIDA <i>et al.</i> (1978)	rabbit rabbit
$F.G.$ k_a k_x k_β k_d	rate constants force generation model	158 190 155 40	$\frac{\text{ml}}{\mu\text{mol s}}$ $\frac{\text{s}^{-1}}{\text{s}^{-1}}$ $\frac{\text{s}^{-1}}{\text{s}^{-1}}$ $\frac{\text{s}^{-1}}{\text{s}^{-1}}$	— — — —		

† The literature values may have been calculated or put in other dimensions; sometimes adapted to the high temperature with $Q_{10} \sim 3$ (when the Q_{10} was not mentioned).

‡ As indicated in the section on numerical aspects, these values are listed here only in order to indicate the great variance in parameters values, rather directly related to experiments.

Table 2. Values of less critical parameters.

Parameter	Explanation	Sim. value	Dim.	Lit. value	References	Animal species
V_S	volume sarcoplasm	600×10^{-4}	ml	600×10^{-4}	STONNINGTON and ENGEL (1973), LUFF and ATWOOD (1971), EISENBERG and KUDA (1976)	rat, mouse, Guinea pig
V_L	volume long. SR	17.1×10^{-4}	ml	17×10^{-4}	"	
V_C	volume term. cisternae	8.4×10^{-4}	ml	8×10^{-4}	"	
V_{eq}	volume long. SR membrane + a layer at both sides of 15 Å	13×10^{-4}	ml	2×10^{-4}	MARTONOSI (1971), STONNINGTON and ENGEL (1973), EISENBERG and KUDA (1976)	
G	amount of calcium in the muscle surface T-tubuli	0.3	μmol	-11×10^{-4}	MARTONOSI (1971)	
A_T		138.0	cm^2	84-138	EISENBERG and KUDA (1976)-LUFF and ATWOOD (1971)	Guinea pig, mouse
n_{th}		6.5×10^{10}	cm^2	6.5×10^{10}	FRANZINI-ARMSTRONG (1970), CHANDLER (1976a, b)	frog
α_{max}	see equations 1-4	1560	s^{-1}	1560	CHANDLER (1976a, b)	
β_{max}		1560	s^{-1}	1560		
k		8.7	mV	8.7		
$\bar{V}$		-32.0	mV	-44		
Tn_{total}	$Tn + CaTn + Ca_2Tn$	0.14	$\frac{\mu\text{mol}}{\text{ml}}$	0.14	BENDALL (1969), WALKER and SCHRODT (1969)	frog, rat
D_1	diffusion constant (ϕ_s)	17×10^{-5}	$\frac{\text{ml}}{\text{s}}$	—		
D_2	diffusion constant (ϕ_n)	28×10^{-3}	$\frac{\text{ml}}{\text{s}}$	0.28×10^{-3}	WINEGRAD (1970)	frog
MH_{tot}	free myosin-heads + cross-bridges	0.177	$\frac{\mu\text{mol}}{\text{ml}}$	0.177	BENDALL (1969), WALKER and SCHRODT (1969)	frog, rat

4 Numerical aspects

In this study model responses, calculated with the help of processes described in the literature, and actual muscle performance after electrical stimulation were compared quantitatively. Therefore, the literature dealing with the subcellular mechanism of EC-coupling was searched and the information obtained was incorporated, after any necessary transformation or scaling, into the model. The force output of the model was then compared with the results of suitably designed measurements.

In the course of doing this a number of problems arose. As already mentioned, virtually all the component processes have been described and parameter values published in the literature. In most cases, however, the conditions of the measurements differ from those in the muscle for which the model was intended to be valid. For instance, the temperature could have been more than 30°C lower, a different sort of animal may have been used, or concentrations may not be comparable with those in actual muscle conditions. This resulted—at least for some parameters—in a large degree of uncertainty (see Tables 1 and 2). How this problem was further dealt with will be described below after introducing the experimental procedure.

Isometric force developed by the slow m. soleus of albino rats (Nembutal anesthesia) was measured *in vivo* at 37°C. Supramaximal stimulation pulses with a duration of 50 μ s were applied to the nerve, while the muscle was adjusted to the optimum twitch length. Detailed information about experimental set-up and results has been described before (WALLINGA-DE JONGE *et al.*, 1980). Twitches, double-pulse responses with various interval times, tetani and prolonged series of twitches were measured. Characteristics as indicated in Fig. 4 were derived from twitch and double pulse responses and compared with those derived from the simulated patterns.

Fig. 4 Definition of characteristics of force patterns for twitch and double pulse response
Contraction time = time interval between onset of force generation and time of maximum force
Half relaxation time = time interval between moment of maximum force and time at which force amplitude

Actual model parameters were found as follows. In the first instance the values for the f.g.m. were fully unknown and had to be found by trial and error in such a way that a twitch was simulated accurately (least summed squares of differences between simulated and measured characteristics). The mean force per cross-bridge (F_{br} in eqn. 15) was derived from measured tetanic force (WALLINGA-DE JONGE, 1980) taking into account the organisation of sarcomeres and myofilaments, and data from the literature (SZENT-GYÖRGYI, 1975; GRAY and GONDA, 1978). The two sets of values were in good agreement and indicated 6 pN to be a reasonable approximation.

The parameter values of the c.m.a.m. which are not accurately known (especially the nine mentioned in Table 1) were adjusted so as to minimise the deviation between simulated and experimental force patterns. In most cases an optimal fit could be obtained with parameter values in accordance with the literature (Tables 1 and 2), but note the uncertainty range mentioned above.

BAYLOR *et al.* (1979a) calculated on the basis of their arsenazo III-measurements in frog muscle fibres that a single action potential releases about 0.1 μ mol calcium per ml sarcoplasm. In our model an action potential arriving in the resting state releases 0.14 μ mol calcium/ml. This amount is sufficient for the generation of an adequate twitch. This indicates the greater amount estimated by ENDO (1977), about 0.4 μ mol/ml, is not required.

The greatest differences occur in the referenced values of the parameters of the calcium transport from sarcoplasm to the longitudinal SR. The great ranges given in Table 1 for k_3 ($1:3 \cdot 10^4$) and k_4 ($1:4 \cdot 10^5$) make them virtually unknown. Moreover, the *in vivo* calcium transport situation does not allow direct comparison with the literature values for the reaction constants defined.

Realisation of the model was further complicated by

decreased to a half
Maximum positive slope = max of derivative of force in the rising phase
Maximum negative slope = min of derivative of force, in the descending phase
Slopes are indicated with an angle-sign

the fact that it represents a fairly large and stiff set of differential equations. This necessitated widely different integration step lengths in the time. The special c.s.m.p. (continuous simulation modeling program) variable step integration procedure for stiff differential equation systems (with large differences in time constants) was used. A twitch simulation thus takes approximately 1 s of c.p.u.-time.

5 Force simulation results

The parameter set ultimately chosen produced the simulated twitch shown in Fig. 5a from a single action potential. The resemblance between simulated and measured twitch patterns is good, although the form of the descending phases differ slightly. This is reflected by the difference in values of the maximum negative slope in the simulation and measurement, while the values of the other characteristics agree. Although reasonable twitch force patterns were obtained with a number of different parameter sets, only two of these showed acceptable behaviour with respect to double

pulse responses. They differed with respect to only two parameter values of the force generation model (k_s and k_p).

The simulated and measured force of a double pulse response are plotted in Fig. 5b. In contrast to the twitch the simulated relaxation is faster than the measured one. The results at different interval times for the characteristics indicated in Fig. 4 are depicted in Fig. 6. Note that the normalised amplitude and maximum positive slope agree satisfactorily for simulated and measured patterns in the tested interval time range (5 to 200 ms). The contraction time of the simulated double pulse responses however is always too small. It is apparent from Fig. 6d that the maximum negative slope decreases with increasing interval time. This decrease is less pronounced for the simulations. If the characteristic values of simulated and measured twitch agree, the normalised plots of Fig. 6 can be interpreted directly in a quantitative way. This holds for all characteristic values except for the maximum negative slope, the value of which for the simulated twitch is too small. At the smallest interval

Fig. 5 Comparison of simulated (1) and measured (2) force patterns for twitch (a), double pulse response with 60 ms interval (b), tetanus with 20 stimuli, 10 ms intervals (c), and prolonged series of twitches with 200 ms interval (d). In (a)–(c) the force is plotted as function of the time, in (d) the force is normalised with respect to the force amplitude of the first twitch, and plotted against the rank order of the twitches

times the difference in the absolute values of the maximum negative slope of simulated and measured double pulse responses is reduced with respect to that of a twitch.

Deviations of the model are appreciable in the tetanus situation. The ascending phase of the simulated tetanus is too fast, while the descending phase is too slow (Fig. 5c). The most conspicuous deviation occurs after stimulation has finished: the simulated force stays high, while the measured force decreases gradually. This appears to be associated with the value chosen for the total amount of calcium (see discussion). The effect disappears if this amount is

reduced.

Prolonged series of twitches were also studied because they offer the possibility of analysing the measure to which the system is returned to the resting state after a twitch. That this is not entirely the case is found experimentally since the amplitude of twitches changes during such a series. The plots of the amplitude of twitches, with an interval time of 200 ms, show that during the onset the model performs reasonably well (Fig. 5d). In the simulations the twitch amplitude stays constant from the 7th twitch upwards, in the experiment it decreases gradually from about the 10th twitch (WALLINGA-DE JONGE, 1980).

Fig. 6 Comparison of simulated (1) and measured (2) characteristic values of double pulse responses for different interval times (5, 10, 15, 20, 30, 40, 60, 80, 100 and 200 ms). Ordinate values are normalised to the corresponding values for a single twitch (indicated for clarity by the broken line)

— interval time, ms

Fig. 7 Some examples of sensitivity of the model to parameter values. *a-e* for twitch, *f* for double pulse response with 60 ms interval time. In all plots force versus time.

- (a) same amount of calcium released for the three curves, but duration of calcium release resp. 0.2, 1.0 and 2.2 ms
 (b) further increasing durations 1: 2.0 ms, 2: 6.0 ms, 3: 10.0 ms
 (c) calcium capacity per channel (ϕ_{ch}) changed 1:

- 11%, 2: optimised value, 3: +15%
 (d) changed interaction of calcium with troponin ($k_{1,s}$ and $k_{2,s}$ changed comparably so that $k_{1,s}/k_{2,s}$ is constant) 1: –50%, 2: optimised value, 3: +50%
 (e) changed amount of calcium carrier (E) 1: –14%, 2: optimised value, 3: +14%
 (f) changed release of calcium from carrier (k_s) 1: –38%, 2: optimised value, 3: +38%

6 Influence of parameter values on force development

Since the literature values of the parameters occasionally show appreciable uncertainty (see Tables 1 and 2), it is of interest to test the model sensitivity for a number of parameters. The most prominent results with respect to c.m.a.-model parameters have been plotted in Fig. 7. A general aspect found is that the changes in contraction and half relaxation time are always similar with respect to direction, although they sometimes differ in extent. This fact implies a considerable degree of dependency between some parameters at least and causes difficulties in simultaneously realising correct contraction and half relaxation times. Here either the contraction time was too short or the relaxation was too slow.

The duration of the calcium release, which is directly related to the (unknown) form of the tabular action potential, is varied from 0.2 to 2.2 ms (Fig. 7a), conserving the amount of calcium released: the three twitches are indistinguishable. When the same amount is released during times > 2.2 ms, the twitch is slower and reaches a lower amplitude (Fig. 7b). Thus, in the case of a short duration release, only the released amount is important rather than the course of the release.

In reality the total amount of calcium released per action potential is uncertain (see Section 4 on numerical aspects). One way of adapting the released amount is with the help of the capacity of the calcium channels ϕ_{ch} (1). A change of ϕ_{ch} influences both amplitude and form of the twitch, at least in the range shown (Fig. 7c).

Because the duration of the T-tubule depolarisation is expected to be less than 10 ms, the main action of the action potential is controlling the amount of calcium released. Therefore changes in action potential will have effects comparable with changes in ϕ_{ch} , displayed in Fig. 7c.

There is a strong competition between the calcium binding to troponin and the calcium transporting ATPase. The effect of a simultaneous change in $k_{1,s}$ and $k_{2,s}$ (the reaction rate constants for calcium binding to and decoupling from the specific sites of troponin respectively) without change in the affinity constant $k_{1,s}/k_{2,s}$ is shown in Fig. 7d. This change markedly influences the form of the twitches. An enhancement introduces an increase of the twitch amplitude and a shortening of both contraction and half relaxation time.

Model performance depends fairly strongly on the amount of calcium transport ATPase with the chosen parameter set. An adaptation of the ATPase amount changes the twitch amplitude and particularly the relaxation of the twitches (Fig. 7e). The change in the twitch form, caused by variation of the reaction rate for calcium binding to the ATPase (k_3), are not shown. An enlargement of k_3 reduces the amplitude, and shortens the half relaxation time more than the contraction time. Finally, the effect of a change in reaction rate constant k_5 (the decoupling of calcium from the

ATPase at the lumen side of the SR-membrane) does not influence the twitch noticeably, but it is an important parameter in the quotient of double pulse amplitude/twitch amplitude (Fig. 7f). If k_5 increases, the resting state will be reached sooner and there will be relatively less effect of the second stimulus.

When each of the four reaction rate constants in the f.g.m. is varied, the time course of the twitch changes. In conclusion, all the simulations described in this

Fig. 8 Processes from action potential to force generation. In all cases a single action potential occurs, but its starting moment is different [in (a) at 0.5 ms, in (b) at 1.5 ms and in (c) at 0.5 ms].

- (a) 1 = membrane potential (mV), 2 = ϕ_1 (calcium release flow in sarcoplasm, $\mu\text{mol s}^{-1}$)
- (b) 1 = $[Ca^{2+}]_s$, $\mu\text{mol ml}^{-1}$, 2 = $[Ca_2 T n_s]$, $\mu\text{mol ml}^{-1}$, 3 = force (N)
- (c) 1 = $[A_\alpha]$, $\mu\text{mol ml}^{-1}$, representing the non-force generating cross-bridges, 2 = $[A_\beta]$, $\mu\text{mol ml}^{-1}$, representing the force generating bridges

Section do not indicate that one of the processes is rate limiting.

7 Simulation results of intermediate processes

One of the possibilities with the c.m.a. model is the calculation of the concentrations of calcium in the various compartments during different stimulation patterns.

An action potential, used as model input, is shown in Fig. 8a together with the resulting calcium release flow ϕ_1 starting after the crossing of a transition voltage (constant $\bar{V}$ in eqns. 3 and 4). Its duration is slightly increased with respect to that of the action potential (note this is less than 2 ms). The release causes the rise of the sarcoplasmic calcium concentration (Fig. 8b). The shape of this concentration curve is comparable with the ϕ_1 curve. The resting state sarcoplasmic calcium concentration equals 10^{-5} $\mu\text{mol/ml}$ [referenced to the range from 0.5×10^{-5} (SZENT-GYÖRGYI, 1975) to 2×10^{-5} $\mu\text{mol/ml}$ (FORD and PODOLSKY, 1972) in frog muscle fibres]. The maximum concentration reaches 1.8×10^{-2} $\mu\text{mol/ml}$ after one action potential. The prolonged presence of a concentration six times higher than this is able to introduce full activation (CHANDLER *et al.*, 1976b). The rise in sarcoplasmic calcium concentration is followed by a very fast increase in $[Ca_2Tn_s]$. This curve reaches its peak value at the end of the $[Ca^{2+}]_s$ peak and declines slowly thereafter (Fig. 8b). The Ca_2Tn_s is followed by an increase in the number of cross-bridges, those generating no force (A_x) rising the first (Fig. 8c). Note that the time scales in Figs. 8b and c

differ by a factor of 15, indicating that the reaction rate constants in the cross-bridge processes are essential in the slow pattern of the force. The simulated results were worse in the condition where all the cross-bridges contributed to the force. Two reactions with a reaction rate constant relatively low with respect to the calcium binding to troponin and to calcium transporting ATPase are essential. The time course of $[A_\beta]$ is identical with that of muscle force (see eqn. 15).

The competition between the calcium binding to troponin and the calcium transporting ATPase is indicated by the curves in Fig. 9a. The cross-hatched area is almost equivalent to the amount of calcium that directly is bound to the ATPase. This calcium (about one third of the total amount released) does not contribute to the force generation. The binding of calcium on the ATPase at the sarcoplasmic side of the SR-membrane is fast in contrast to the splitting off at the luminal side of the membrane causing the time course of the calcium flows to and from the ATPase to be very different (Fig. 9b). In a twitch the flow ϕ_3 will contribute essentially to relaxation which in a tetanus is only driven by ϕ_4 since the occupancy of the ATPase with calcium is maximum at the moment the stimulation stops.

There is still an increased flow within the SR to the cisternae after the mechanical relaxation of the muscle is complete. Comparison of Fig. 9c and 8c illustrates this aspect up to 120 ms. The resting state is reached again only after a time well beyond 1 s after the start of twitch.

Unfortunately, the experimental data on calcium flows and concentrations are very scarce for

Fig. 9 Calcium flows simulated by the model.

- (a) 1 = ϕ_1 , release flow, 2 = ϕ_2 , flow to troponin. The shaded area represents something like the capacity of carriers and sarcoplasm
- (b) 1 = ϕ_3 , flow representing the removal of calcium from sarcoplasm by calcium transporting ATPase, 2 = ϕ_4 , flow of calcium from the carrier into the longitudinal SR. Note the different scales
- (c) 1 = ϕ_5 , leakage flow from longitudinal SR into sarcoplasm, 2 = ϕ_6 , backflow from longitudinal SR to the terminal cisternae

mammalian muscle fibres. Data for $[Ca^{2+}]_s$ are more often described for frog muscle fibres (MILEDI *et al.*, 1977; BLINKS *et al.*, 1978) than for mammalian muscle fibres (EUSEBI *et al.*, 1980). In frog skeletal muscle fibres the light signal with aequorin reaches its crest value about 10 ms after the onset of the action potential (30°C; Fig. 3 of BLINKS *et al.*, 1978), but this probably reflects more the kinetics of the aequorin reaction than the calcium transient in sarcoplasm (BLINKS *et al.*, 1978). In our model simulations the action potential is very brief and causes a sharp peak in sarcoplasmic calcium concentration (Fig. 8a), its highest value being reached about 1 ms after the onset of the action potential (37°C).

8 Discussion

The force generated by skeletal muscle depends mainly on the nature of the muscle fibres of which it is composed, on the stimulation pattern and on the degree of elongation of the muscle. The model presented was developed to study whether the isometric force of a muscle during a number of stimulation patterns could be understood in terms of the processes known to play a role in the excitation-contraction coupling and in force generation. For such an approach it is necessary to realise a model that is based upon physiologically recognisable component processes. Presently available models for skeletal muscle, in which activation processes are implemented, are not sufficiently extensive for this purpose (ASHLEY and MOISESCU, 1972; AKAZAWA *et al.*, 1976; HATZE, 1977; RADU *et al.*, 1974) or are based on obsolete data (TAYLOR, 1969). Such extensive models are also lacking for cardiac muscle, although a related approach such as ours is sometimes taken (BASSINGTHWAIGHTE and REUTER, 1972; KAUFMANN *et al.*, 1974; TSATURYAN and IZAKOV, 1978; MARKHASIN and MIL'SHTEIN, 1979; VAN DEN BROEK, 1979).

Before evaluating the model, the main assumptions and simplifications inherent in the model processes will be discussed in some detail. In contrast to heart muscle fibres, the calcium content of skeletal muscle fibres is independent of the stimulation pattern (KIRBY *et al.*, 1975). Isolated skeletal muscle fibres are able to generate force, even in a calcium free medium (BLINKS *et al.*, 1978). So the calcium flow through the sarcolemma has been neglected.

It is generally accepted that the depolarisation of the tubular membrane is the main cause for calcium release in skeletal muscle fibres (ENDO, 1977). An action potential is propagated along the tubular membranes with a velocity of about 8 cm s^{-1} in frog muscle fibres at 20°C (GONZALEZ-SERRATOS, 1971). In soleus muscle fibres at 37°C this should introduce a delay $< 0.25 \text{ ms}$ between superficial and medial tubular membrane depolarisation and consequently between the calcium release in the corresponding myofibrils. It ensures an adequate transversal

synchronisation within the fibre, while such a delay will not change the twitch form noticeably (Fig. 7a).

The depolarisation of the tubular membrane determines the number of open calcium channels in the cisternal membranes. This is described by a formalism that might be compatible with the membrane charge movement mechanism (CHANDLER *et al.*, 1976a and b). A relation between the number of open calcium channels in the cisternal membranes and the amount of charge movement has been proposed by several authors (CHANDLER, 1976b; CAPUTO and FERNANDEZ DE BOLAÑOS, 1979; SHLEVIN, 1979), but has not been proved (ADRIAN, 1978). The continuity of membrane layers of T-tubules and cisternae (SOMLYO, 1979) may be an important link in the control of opening calcium channels in the SR membrane by depolarisation of the tubular membrane.

In the model $0.14 \mu\text{mol}$ calcium release per millilitre of sarcoplasm is sufficient to reach normal force amplitudes; this amount agrees with estimations from recent experiments (BAYLOR *et al.*, 1979a).

While contraction is triggered by the release of calcium from the cisternae, the relaxation is driven by the calcium uptake by longitudinal SR. The relaxation phase depends strongly on the calcium transport from sarcoplasm to longitudinal SR. Parameter values of the calcium flows involved in this transport are uncertain to a great extent (Table 1). So it is not surprising that there is no agreement on the question as to whether the transport velocity is sufficient for adequate relaxation. WEBER (1971) and FORD and PODOLSKY (1972) expect it will be under *in vivo* conditions. In our model the transport appears to be sufficient for the realisation of relaxation, but due to the simplicity of the structure of the component that modelled the calcium transport into the SR no general conclusions can be drawn.

After the uptake in the longitudinal SR, the calcium is transported back to terminal cisternae. In a tetanus the repeated release of calcium perceivably diminishes the amount of calcium stored in the cisternae. Were it not replenished the tetanus force would collapse at a certain moment. The diffusion rate constant for the backflow given by WINEGRAD (1970) is not sufficient to overcome such a collapse in our model. With a value of about 100 times that of Winegrad corrected for temperature the plateau phase can hold for at least 200 ms. The calcium backflow has no importance for the twitch and double pulse response, but it will influence both the tetanus and the staircase.

The calcium storage in the cisternae may be considerable since, for the main part, it is present in bound form (MARTONOSI, 1971). The calcium is probably bound to proteins with high calcium binding capacity (MACLENNAN and WONG, 1971). The properties of the interaction between free and bound calcium and the concentration of calcium binding protein as used in our model are described in more detail elsewhere (HEIJINK, 1980). During the resting state the main part of calcium is present in the

cisternae. The total amount of calcium in the muscle fibres has been estimated to be $0.3 \mu\text{mol}$ [equal to 0.2 times the estimated weight of SR membrane protein in mg, MARTONOSI (1971)]. If this amount is reduced, the twitch and double pulse response can be maintained as they were, while the prolonged plateau of the tetanus disappears (HEIJINK, 1980).

The model makes it possible to study the effect of other muscle parameters in the excitation-contraction coupling. For example, examine whether a change from the slow to the fast muscle type parameters is sufficient to realise an adequate fast muscle model (e.g. BRIGGS *et al.*, 1977).

9 Conclusions

Present day knowledge enables the realisation of a model containing quantitatively essential processes of excitation-contraction coupling and force generation. Such a model is necessarily specific for one particular type of muscle. The force patterns generated by the model as developed for the m. soleus of the rat after one or two action potentials agree satisfactorily with measured performance of that muscle and in their dependency upon the time intervals used.

The amount of calcium transporting ATPase in the SR membrane is so considerable that its buffer action is appreciable. In the model the ATPase competes strongly with troponin in the binding of the released calcium. The behaviour of the muscle after the second action potential depends strongly on the degree of occupancy of both the troponin and the ATPase with calcium.

A literature search for parameters needed to render the model operational reveals that many data required are incompletely defined or partly conflicting. A model as presented in this study can be used as a guide for further study of the processes involved.

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References

- ADRIAN, R. H. and PEACHEY, L. D. (1973) Reconstruction of the action potential of frog sartorius muscle. *J. Physiol.*, **235**, 103–131.
- ADRIAN, R. H. (1978) Charge movement in the membrane of striated muscle. *Ann. Rev. Biophys. Bioeng.*, **7**, 85–112.
- AKAZAWA, K., YAMAMOTO, M., FUJII, K. and MASHIMA, H. (1976) A mechanochemical model for the steady and transient contractions of the skeletal muscle. *Jap. J. Physiol.*, **26**, 9–28.
- ASHLEY, C. C. and MOISESCU, D. G. (1972) Model for the action of calcium in muscle. *Nature New Biol.*, **237**, 208–211.
- BASSINGTHWAIGHTE, J. B. and REUTER, H. (1972) Calcium movements and excitation-contraction coupling in cardiac cells. In *Electrical phenomena in the heart*, DE MELLO, W. C. (Ed.) Academic Press, New York.
- BAYLOR, S. M., CHANDLER, W. K. and MARSHALL, M. W. (1979a) Arsenazo III signals in singly dissected frog twitch fibres. *J. Physiol.*, **287**, 23–24P.
- BAYLOR, S. M., CHANDLER, W. K. and MARSHALL, M. W. (1979b) Arsenazo III signals in frog muscle. *Biophys. J.*, **25**, 141a.
- BENDALL, J. R. (1969) *Muscles, molecules and movement*, Heineman, London.
- BLINKS, J. R., RÜDEL, R. and TAYLOR, S. R. (1978) Calcium transients in isolated amphibian skeletal muscle fibres: detection with aequorin. *J. Physiol.*, **277**, 291–323.
- BRIGGS, F. N., POLAND, J. L. and SOLARO, R. J. (1977) Relative capabilities of sarcoplasmic reticulum in fast and slow mammalian skeletal muscles. *J. Physiol.*, **266**, 587–594.
- BROEK, J. H. J. M. VAN DEN (1979) A model study of left ventricular function. Thesis State University of Utrecht, The Netherlands.
- CAPUTO, P. and FERNANDEZ DE BOLAÑOS, P. (1979) Membrane potential, contractile activation and relaxation rates in voltage clamped short muscle fibres of the frog. *J. Physiol.*, **289**, 175–189.
- CHANDLER, W. K., RAKOWSKI, R. F. and SCHNEIDER, M. F. (1976a) A non-linear voltage dependent charge movement in frog skeletal muscle. *J. Physiol.*, **254**, 245–283.
- CHANDLER, W. K., RAKOWSKI, R. F. and SCHNEIDER, M. F. (1976b) Effects of glycerol treatment and maintained depolarization on charge movement in skeletal muscle. *J. Physiol.*, **254**, 285–316.
- EISENBERG, B. R. and KUDA, A. M. (1976) Discrimination between fiber populations in mammalian skeletal muscle by using ultrastructural parameters. *J. Ultrastruct. Res.*, **54**, 76–88.
- ENDO, M. (1977) Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.*, **57**, 71–108.
- EUSEBI, F., MILEDI, R. and TAKAHASHI, T. (1980) Calcium transients in mammalian muscles. *Nature*, **284**, 560–561.
- FORD, L. E. and PODOLSKY, R. J. (1972) Calcium uptake and force development by skinned muscle fibres in EGTA buffered solutions. *J. Physiol.*, **223**, 1–19.
- FRANZINI-ARMSTRONG, C. (1970) Studies of the triad: I. Structure of the junction in frog twitch fibres. *J. Cell Biol.*, **47**, 488–499.
- FROELICH, J. P. and TAYLOR, E. W. (1975) Transient state kinetic studies of sarcoplasmic reticulum adenosine triphosphatase. *J. Biol. Chem.*, **250**, 2013–2021.
- FUCHS, F. (1974) Striated muscle. *Ann. Rev. Physiol.*, **36**, 461–502.
- GONZALEZ-SERRATOS, H. (1971) Inward spread of activation in vertebrate muscle fibres. *J. Physiol.*, **212**, 777–799.
- GRAY, B. F. and GONDA, I. (1978) The sliding filament model of muscle contraction II. The energetic and dynamical predictions of a quantum mechanical transducer model. *J. Theor. Biol.*, **69**, 187–230.
- HAIECH, J., DERANCOURT, J., PECHÈRE, J.-F. and DEMAILLE, J. G. (1979) Magnesium and calcium binding to parvalbumins: evidence for differences between parvalbumins and an explanation of their relaxing function. *Biochemistry*, **18**, 2752–2758.
- HANSON, J. (1974) The effects of repetitive stimulation on the action potential and the twitch of rat muscle. *Acta Physiol. Scand.*, **90**, 387–400.
- HATZE, H. (1977) A myocybernetic control model of skeletal

- muscle. *Biol. Cybernetics*, **25**, 103–119.
- HEIJINK, R. J. (1980) A contribution to the development and parameter estimation of a calcium mediated activation model for rat skeletal muscle. Research report nr. 219, Bio-information Group, Twente University of Technology, The Netherlands.
- HUXLEY, A. F. (1974) Muscular contraction. *J. Physiol.*, **243**, 1–43.
- JOHNSON, J. D., CHARLTON, S. C. and POTTER, J. D. (1979) A fluorescence stopped flow analysis of Ca^{2+} exchange with troponin C. *J. Biol. Chem.*, **254**, 3497–3502.
- JULIAN, F. J., SOLLINS, K. R. and SOLLINS, M. R. (1974) A model for the transient and steady-state mechanical behavior of contracting muscle. *Biophys. J.*, **14**, 546–562.
- KANAZAWA, T., YAMADA, S., YAMAMOTO, T. and TONOMURA, Y. (1971) Reaction mechanism of the Ca^{2+} -dependent ATPase of sarcoplasmic reticulum from skeletal muscle. V. Vectorial requirements for calcium and magnesium ions of three partial reactions of ATPase: formation and decomposition of a phosphorylated intermediate and ATP-formation from ADP and the intermediate. *J. Biochem.*, **70**, 95–123.
- KAUFMANN, R., BAYER, R., FÜRNISS, T., KRAUSE, H. and TRITTHART, H. (1974) Calcium-movement controlling cardiac contractility II. Analog computation of cardiac excitation-contraction coupling on the basis of calcium kinetics in a multi-compartment model. *J. Mol. Cell. Cardiol.*, **6**, 543–559.
- KIRBY, A. C., LINDLEY, B. D. and PICKEN, J. R. (1975) Calcium content and exchange in frog skeletal muscle. *J. Physiol.*, **253**, 37–52.
- LUFF, A. R. and ATWOOD, H. L. (1971) Developmental changes in the sarcoplasmic reticulum and transverse tubular system in skeletal muscle of the mouse. *J. Gen. Physiol.*, **57**, 243–244.
- MACLENNAN, D. H. and WONG, P. T. S. (1971) Isolation of a calcium-sequestering protein from sarcoplasmic reticulum. *Proc. Nat. Acad. Sci., USA*, **68**, 1231–1235.
- MARKHASIN, V. S. and MILL'SHEIN, G. N. (1979) Modelling the influence of rhythm on the force of contraction of heart muscle. *Biophysics*, **23**, 688–696.
- MARTONOSI, A. (1971) The structure and function of sarcoplasmic reticulum membranes. In *Biomembranes*, MANSON, L. (Ed.), Vol. I, 191–256, Plenum, New York.
- MARTONOSI, A. (1973) Biochemical and clinical aspects of sarcoplasmic reticulum function. In *Topics in membranes and transport*, BRONNER, F. and KLEINZELLER, A. (Eds.), **3**, 83–197, Academic Press, New York.
- MARTONOSI, A., LAGWINSKA, E. and OLIVER, M. (1974) Elementary procedure in the hydrolysis of ATP by sarcoplasmic reticulum membranes. *Ann. N.Y. Ac. Sci.*, **227**, 549–567.
- MILEDI, R., PARKER, I. and SCHALOW, G. (1977) Measurements of calcium transients in frog muscle by the use of arsenazo III. *Proc. R. Soc. Lond. B.*, **198**, 201–210.
- OGAWA, Y. (1970) Some properties of fragmented frog sarcoplasmic reticulum with particular reference to its response to caffeine. *J. Biochem.*, **67**, 667–683.
- PERRY, S. V. (1979) The regulation of contractile activity in muscle. *Biochem. Soc. Trans.*, **7**, 593–617.
- POTTER, J. D. (1979) Personal communication.
- POTTER, J. D. and GERGELY, J. (1975) The calcium and magnesium binding sites on troponin and their role in the regulation of myofibrillar adenosine triphosphatase. *J. Biol. Chem.*, **250**, 4628–4633.
- POTTER, J. D., JOHNSON, J. D. and ROBERTSON, S. P. (1978) Studies on the regulation of skeletal and cardiac muscle contraction. 6th International Biophysics Congress, Kyoto, p. 235.
- RADU, H., DA RONCH, A. and CALDESI-VALERI, V. (1977) Calcium and myotonia: a mathematical model. *Bull. Math. Biol.*, **39**, 577–585.
- SHLEVIN, H. H. (1979) Effects of external calcium concentration and pH on charge movement in frog skeletal muscle. *J. Physiol.*, **288**, 129–158.
- SOMLYO, A. V. (1979) Bridging structures spanning the junctional gap at the triad of skeletal muscle. *J. Cell Biol.*, **80**, 743–750.
- STONNINGTON, H. H. and ENGEL, A. G. (1973) Normal and denervated muscle. A morphometric study of fine structure. *Neurology*, **23**, 714–724.
- SUMIDA, M., WANG, T., MANDEL, F., FROELICH, J. P. and SCHWARTZ, A. (1978) Transient kinetics of Ca^{2+} transport of sarcoplasmic reticulum. A comparison of cardiac and skeletal muscle. *J. Biol. Chem.*, **253**, 8772–8777.
- SZENT-GYÖRGYI, A. G. (1975) Calcium regulation of muscle contraction. *Biophys. J.*, **15**, 707–723.
- TADA, M., YAMAMOTO, T. and TONOMURA, Y. (1978) Molecular mechanism of active calcium transport by sarcoplasmic reticulum. *Physiol. Rev.*, **58**, 1–79.
- TAYLOR, C. P. S. (1969) Isometric muscle contraction and the active state: an analog computer study. *Biophys. J.*, **9**, 759–780.
- TSATURYAN, A. K. and IZAKOV, V. YA. (1978) Mathematical model of the coupling of excitation with contraction in the heart muscle. *Biophysics*, **23**, 904–910.
- WALKER, S. M. and SCHRODT, G. R. (1969) Filament lengths and distribution of staining material in the I-bands of rat skeletal muscle. *Am. J. Phys. Med.*, **48**, 178–192.
- WALLINGA-DE JONGE, W. (1980) Force development in rat skeletal muscle-measurement and modeling. Thesis, Twente University of Technology, The Netherlands.
- WALLINGA-DE JONGE, W., BOOM, H. B. K., BOON, K. L., GRIEP, P. A. M. and LAMMERÉE, G. C. (1980) The force development of fast and slow skeletal muscle at different muscle lengths. *Am. J. Physiol.*, **239** (Cell Physiol. 8), C98–C104.
- WEBER, A. (1971) Regulatory mechanisms of the calcium transport system of fragmented rabbit sarcoplasmic reticulum. I. The effect of accumulated calcium on transport and adenosine triphosphate hydrolysis. *J. Gen. Physiol.*, **57**, 50–63.
- WEBER, A., HERZ, R. and REISS, I. (1966) Study of the kinetics of calcium transport by isolated fragmented sarcoplasmic reticulum. *Biochem. Z.*, **345**, 329–369.
- WINEGRAD, S. (1970) The intracellular site of calcium activation of contraction in frog skeletal muscle. *J. Gen. Physiol.*, **55**, 77–88.