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Calcium transients in frog slow muscle fibres

MANY muscles in the frog contain slow as well as twitch muscle fibres, which differ in their innervation, electrical and contractile properties and fine structure[1]. Slow fibres give graded slow contractions with nerve stimulation, and can maintain a prolonged contracture when depolarised. There is evidence that contractile activation in slow fibres is mediated by a rise in myoplasmic calcium concentration[2], but it is not clear whether this calcium originates from the sarcoplasmic reticulum, as in twitch fibres, or enters the fibre from the external solution[1,3–5]. We have used the calcium indicator dye arsenazo III (refs 6–9) to follow changes in intracellular free calcium concentration occurring during depolarisation of slow fibres, and find that the membrane potential dependence of these calcium transients in slow fibres is very similar to that observed in twitch fibres[9]. The time courses of the calcium transients in slow fibres are, however, very much slower than in twitch fibres[9,10], and may be a major factor in determining the time courses of tension development and relaxation.

Experiments were performed on the pyriformis muscle of *Rana temporaria*[11], using a bathing solution of composition (mM); NaCl, 120; KCl, 2; $CaCl_2$, 12; *p*H, 7. Intracellular calcium changes with arsenazo III were measured as previously described for twitch fibres[8,9]. Briefly, by cutting away the lower part, the muscle was reduced to a thin sheet of fibres which was stretched until contraction was practically abolished. Fibres were impaled with two electrodes, about 150 μm apart, for voltage recording and dye injection. Slow fibres were identified by their inability to generate action potentials and their slow electronic time constant when tested with current pulses applied through the dye pipette. The membrane potential was held at −100 mV by passing a hyperpolarising current through the dye pipette, and this served also to slowly inject arsenazo III into the fibre. Changes in light absorption of the dye were monitored by focusing light from a 50-W bulb on to a spot of about 80 μm diameter between the pipettes, and measuring the transmitted light at wavelengths of 532 and 602 nm using two photomultipliers and interference filters. The light absorbance of arsenazo III changes in opposite directions at these wavelengths in the presence of calcium, and by subtracting the signals from the two photomultipliers a record is obtained which gives a quantitative measure of changes in free calcium concentration, but is little affected by movement artefacts[8,9].

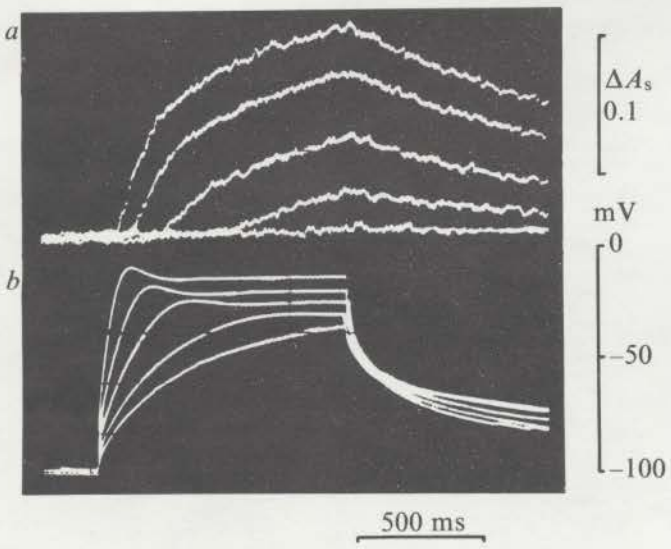

Fig. 1 Light absorbance records from a fibre injected with arsenazo III, showing responses to depolarising pulses of different amplitudes. *a*, Light absorbance changes at the wavelength pair 532–602 nm. *b*, Membrane potential. The light absorbance traces were recorded through a low-pass filter of time constant 10 ms, and the calibration (ΔA_s) gives the change in absorbance as a fraction of the total absorbance of the injected dye at a wavelength of 532 nm. An upward deflection indicates an increase in free calcium concentration, and ΔA_s of 0.1 is equivalent to an increase in free calcium of 2 μM (ref. 9). Five superimposed sweeps are shown. Stimuli were constant current pulses of 1 s duration and varying amplitudes. Temperature 7 °C.

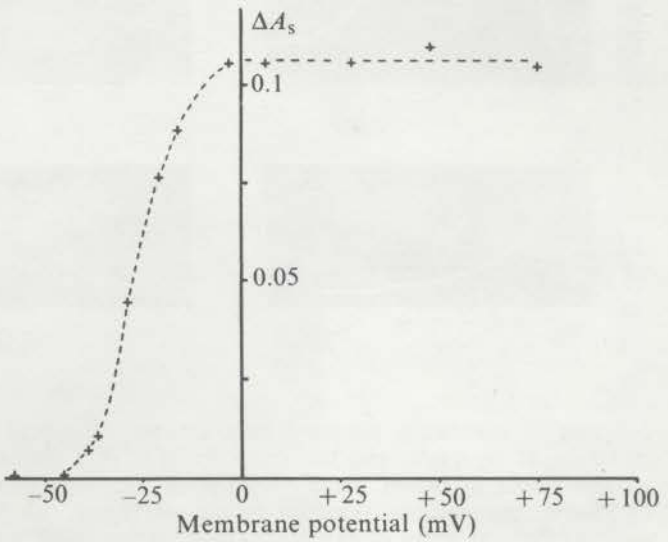

Fig. 2 Relationship between calcium response and membrane potential recorded in one fibre using depolarising pulses of 100 ms duration. Abscissa: membrane potential during pulse. Ordinate peak change in standardised light absorbance (ΔA_s) at the wavelength pair 532–602 nm. The fibre was voltage clamped using the dye pipette as a current-passing electrode, and the membrane potential was held at −90 mV between pulses. Temperature, 7 °C.

Changes in intracellular free calcium concentration produced by depolarising pulses are shown in Fig. 1. The size of the calcium transient is a graded function of membrane potential, and the rates of increase and decay of calcium concentration are much slower than in twitch fibres[9,10]. The decay time constant measured in six fibres varied between 0.6 and 4 s, and the maximum rate of increase in free calcium concentration in 10 fibres was 0.045 μM ms^{-1} ±0.025 (mean ±s.d.), when depolarised to a potential above that required to obtain a maximal response (compare Fig. 2). Corresponding values for twitch fibres are 50 ms and 0.95 μM ms^{-1} (ref. 9). The rise in calcium concentration during depolarisation shows two phases: an initial rapid increase, followed by a transition to a much slower rate of rise. This is probably a property of the calcium release mechanism, and is unlikely to be due to any nonlinearity in the recording system as the transition occurs at different calcium concentrations for different sized pulses, and the rate of calcium uptake is probably too slow to account for the transition.

To examine the relationship between membrane potential and calcium release the membrane potential was clamped at the measuring spot, and the peak size of the calcium transient measured for different amplitudes of 100 ms depolarising pulses (Fig. 2). The calcium transient is a sigmoid

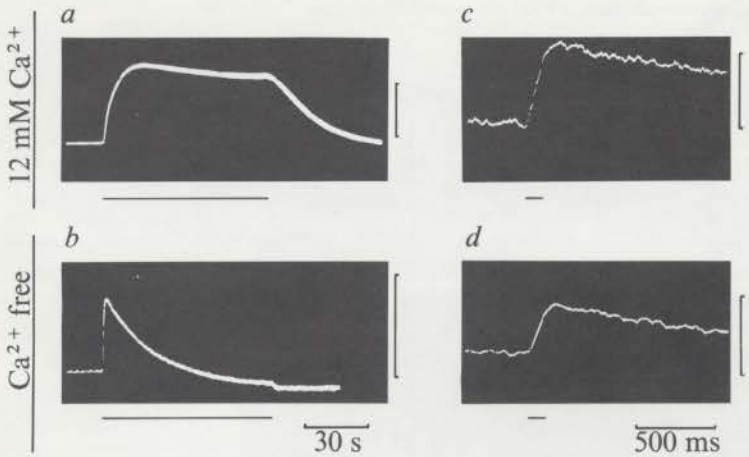

Fig. 3 Effects of removal of external calcium on the calcium responses to prolonged (*a* and *b*) and short (*c* and *d*) depolarising pulses. In each record the fibre was depolarised from −100 mV to +20 mV during the times indicated by the bars, and all records were obtained from different fibres. Ringer solution containing 12 mM $CaCl_2$ (plus 120 mM NaCl and 2 mM KCl) was used in *a* and *c*, while in *b* and *d* the solution was (mM); NaCl, 120; KCl, 2; $MgCl_2$, 5; EGTA, 1; *p*H 7. Traces show changes in light absorbance at the wavelength pair 532–602 nm, and the calibration bars are $\Delta A_s = 0.5$ for *a* and *b*, and $\Delta A_s = 0.1$ for *c* and *d*. In *b* a small shift of the baseline occurred due to movement of the fibre. Temperature, 7.5 °C.

function of membrane potential over a range of approximately −45 to 0 mV, and the relationship is very similar to that found in twitch fibres[9]. With large depolarisations (+75 mV) the calcium response is not depressed, which suggests that the entry of external calcium is not important in the generation of the calcium transient, since the driving force for calcium entry would be reduced at this potential[9,12].

The role of external calcium was further studied by buffering the calcium concentration in the bathing solution at $<10^{-7}$ M with 1 mM EGTA and 5 mM $MgCl_2$. We found no changes in the calcium transients produced by 100-ms depolarising pulses in this solution: mean response sizes produced by supramaximal depolarising pulses in 12 mM calcium and calcium-free solution were respectively 2.0 μM ±1.1 ($n = 9$) and 1.4 μM ±0.4 ($n = 4$), while the relationship between membrane potential and calcium release and uptake remained the same (Fig. 3*c*,*d*). During a prolonged depolarisation, however, the calcium response is considerably affected by removal of calcium from the bathing solution, and the response becomes transient, rather than maintained (Fig. 3*a*, *b*). This effect is well known from studies of contractile tension[13], and might result from either an action of external calcium on the activating sites in the T-tubule membrane, or from the absence of a calcium influx which is required to maintain the intracellular calcium level. To test the importance of calcium influx during maintained contractures in 12 mM calcium, we gave a step depolarisation to +80 mV during prolonged depolarisations to +20 mV. This additional depolarisation should have reduced any calcium influx, but all experiments showed a small rise in intracellular calcium concentration, rather than a fall.

These experiments are in agreement with other work[3] in demonstrating than an influx of external calcium is not directly involved in contractile activation in slow fibres, and the striking similarity in the relationships between membrane potential and calcium response for slow and twitch fibres supports the view that activation in slow fibres occurs by the same mechanisms as in twitch fibres[4,5,13]. External calcium is, however, important in the maintenance of prolonged contractures, possibly by affecting the rate of inactivation of the activating sites within the T tubules[13,14]. The slow rates of increase and decay of intracellular calcium in slow fibres may arise simply as a consequence of the relative scarcity of sarcoplasmic reticulum and triads[15–17], and are probably major factors in determining the rates of contraction and relaxation in slow muscle. Our values for the rate of decay of the calcium transient are in good agreement with measurements of tension relaxation[1,5,18], but the slow shortening velocity of the myofibrils[2] may also be an important factor in determining the rate of tension development.

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R. Miledi
I. Parker
G. Schalow*

Department of Biophysics,
University College London,
Gower Street, London WC1, UK

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*Present address: I Physiologisches Institut der Universität des Saarlandes, 665 Homburg/Saar, FRG.

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