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Birefringence signals and calcium transients in skeletal muscle

TWITCHES in skeletal muscle are preceded and accompanied by changes in optical properties of the muscle fibres[1–11]. Measurements of birefringence give large signals which can be separated into two main components[6–8]: the first begins on the falling phase of the action potential, and reaches a peak at the onset of tension development; this is followed by a late signal which is thought to be associated with development of tension by the contractile proteins[2,3,6]. Since the early signal precedes tension development, it could provide an important tool for studying intervening steps in excitation–contraction coupling. This signal has recently been attributed to potential changes in the sarcoplasmic reticulum (SR) membrane, associated with Ca^{2+} release into the sarcoplasm[6,10]. We have simultaneously recorded birefringence signals and changes in intracellular Ca^{2+} concentration, using arsenazo III, in frog muscle fibres. The results show that the onset of the early birefringence signal coincides with the rise in sarcoplasmic Ca^{2+} concentration, and that both can be abolished by injecting EGTA to chelate the sarcoplasmic Ca^{2+}. This suggests that the early birefringence signal arises from some process dependent on the rise in sarcoplasmic Ca^{2+}, and is not caused by events in the SR associated with the calcium release mechanism.

Experiments were performed on the cutaneous pectoris muscle of *Rana temporaria*, using a bathing solution of the following composition (in mmol l^{-1}): NaCl, 120; KCl, 2; $CaCl_2$, 2; phosphate buffer (*p*H 7.2), 3. Intracellular Ca^{2+} changes were measured with arsenazo III as previously described[12,13]. Briefly, the muscle was stretched sufficiently to block visible contraction, and a superficial fibre was impaled with two microelectrodes about 150 μm apart, for voltage recording and dye injection. Arsenazo III was injected by ionophoresis. The dye pipette also served to initiate action potentials by passing 1-ms depolarising current pulses or, in some experiments, to voltage-clamp a region of the fibre. White light was focused on a spot of 80 μm diameter between the pipettes, and the light transmitted through the fibre was diverted on to three photomultipliers. Calcium-dependent changes in absorption of arsenazo III were recorded by subtracting electrically the measurements of transmitted light at wavelengths of 532 and 602 nm, obtained by using two photomultipliers and interference filters. Birefringence changes were recorded with the third photomultiplier and two crossed polarisers, once placed in front of the light source and the other in front of the photomultiplier. The plane of polarisation was at 45° to the longitudinal axis of the muscle fibres[4,8].

Preliminary experiments indicated that intracellular injection of arsenazo III did not affect the time course of the birefringence signals recorded following action potentials. The birefringence records show two components; an early transient decrease in light intensity, followed by a signal with a similar time course to the movement artefact detected when one polariser was removed. This late component could appear as either an increase or a decrease in light intensity, depending critically on the positioning of the light spot. In a few fibres, the applied stretch sufficiently reduced the late signal to allow the early signal to be recorded with minimal distortion (Fig. 1*a*), but generally the early signal was obscured after its peak (Fig. 1*b* and *c*). At 10 °C the early signal takes off within 2 ms of the foot of the action potential, and reaches a peak within 6 ms. The onset of the birefringence signal coincides with the onset of the calcium response as detected by the arsenazo III, and the latencies of both are affected to the same extent by changes in temperature (Fig. 1*b* and *c*).

Intracellular injection of EGTA provides a means of exploring the relationship between the events underlying these optical changes, since it reduces the increase in sarcoplasmic Ca^{2+} by binding the Ca^{2+} ions released by the SR (ref. 14). A micropipette containing 0.2 M EGTA (buffered to *p*H 7 with KOH) was inserted with its tip in the centre of the measuring light spot. A depolarising backing current (10 nA) was applied to the pipette while the control records were taken. To minimise movement artefacts resulting from contraction of distant regions of the fibre, action potentials were blocked with tetrodotoxin (10^{-6} g ml^{-1}); tetraethylammonium bromide (30 mM) was added to the medium and the membrane was voltage-clamped in the recording area. The fibre was stimulated by giving 5- or 10-ms depolarising pulses and EGTA was injected by applying a small hyperpolarising current through the microelectrode. The results are shown in Fig. 2. Blockade of the

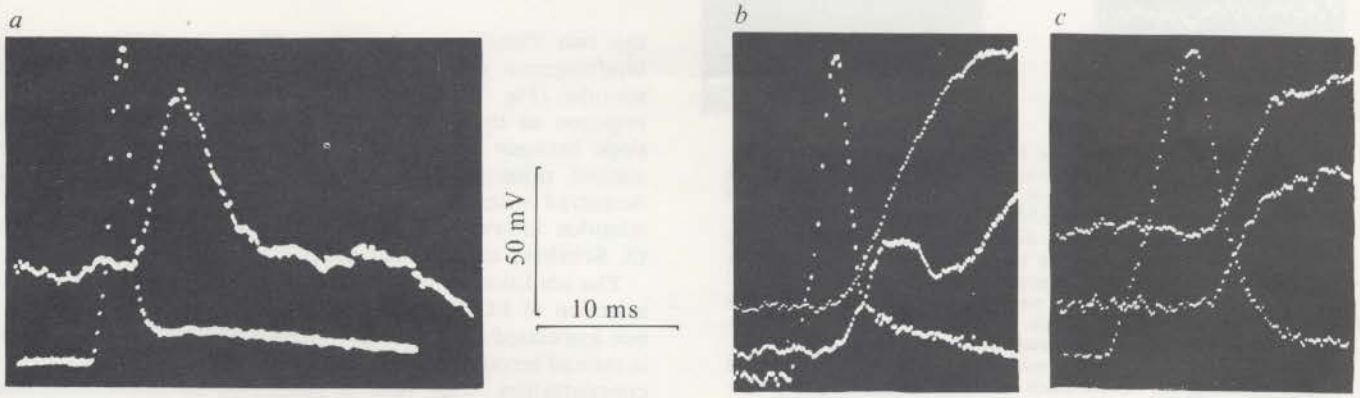

Fig. 1 Changes in birefringence and light absorption of arsenazo III following action potentials. Traces are signal averages of 32, 16 and 24 sweeps in *a*, *b* and *c*, respectively. In all panels, the bottom trace is the membrane potential; birefringence changes are shown in the upper trace in *a* and in the middle traces in *b* and *c*. Changes in sarcoplasmic Ca^{2+} concentration detected with arsenazo III are shown in the top traces in *b* and *c*. Both optical records were low-pass filtered with a time constant of 1 ms, and are expressed as the change in light transmitted through the fibre, divided by the resting transmission. Fractional changes in light transmission; 10^{-2} for the arsenazo III trace, and 2.5×10^{-3} for the birefringence. An upward deflection in both optical traces indicates a decrease in light intensity. Temperature: *a* and *b*, 10 °C; *c*, 4 °C.

rise in free sarcoplasmic Ca^{2+} was accompanied by inhibition of both the early and the late birefringence signals (Fig. 2*a*); the intensity of the block could be graded by progressive increase in the amount of EGTA injected (Fig. 2*b*). In one fibre, a partial recovery of the birefringence and calcium signals was observed when recording was continued for about 30 min after injection. The most direct interpretation of these results is that the birefringence signals were abolished as a consequence of the reduction in size of the Ca^{2+} transient due to Ca^{2+} binding to the EGTA (ref. 14), but the possibility cannot be entirely ruled out that the EGTA additionally interfered in some way with the Ca^{2+} release mechanism.

Experiments with paired stimuli provide further evidence against the early birefringence signal being associated with the calcium release mechanism. When the interval between

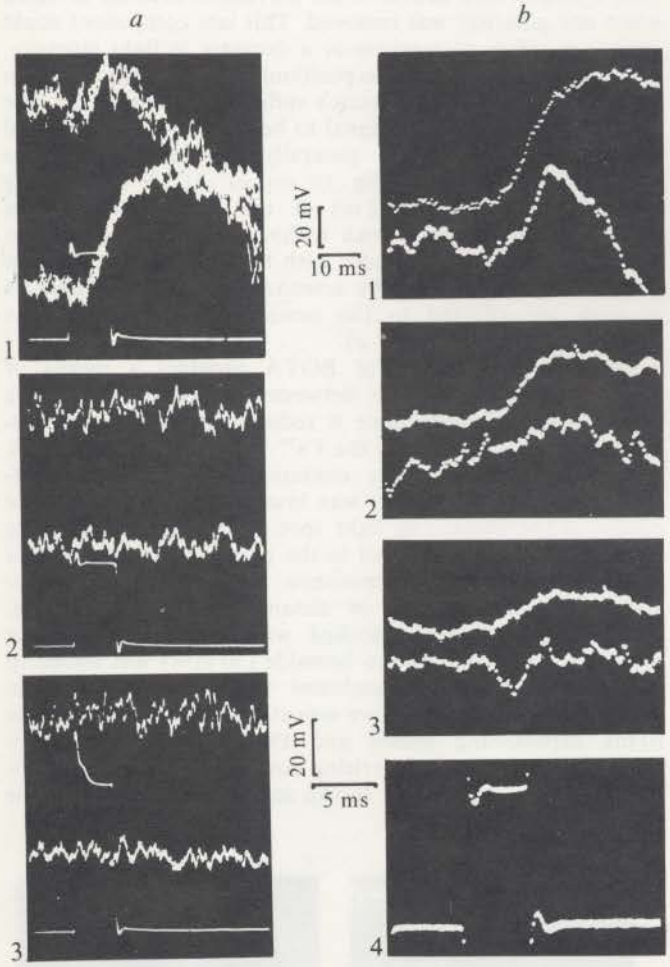

Fig. 2 Effect of EGTA on the birefringence signals and Ca^{2+} transients. The fibres were stimulated with 10-ms depolarising pulses in *a* and 5-ms pulses in *b*; tetrodotoxin (10^{-6} g ml^{-1}) and tetraethylammonium (30 mM) were present in the medium. *a* and *b* are records from two different fibres. *a*, Bottom trace is membrane potential, middle trace is arsenazo III responses and top trace shows birefringence changes. *a*1 shows control responses (three superposed sweeps); *a*2 and *a*3 show the blockade of the optical signals following injection of EGTA for 8 min with an average current of 50 nA. Resting potential: 85 mV. *b*, Changes in dye absorption (upper trace in *b*1–*b*3) and in birefringence (lower trace in *b*1–*b*3) induced by depolarising pulses (shown in *b*4). Records are averages of 48 sweeps. EGTA was applied for 2 min at 40 nA between *b*1 and *b*2, and for 2 min at 60 nA between *b*2 and *b*3. Upper calibration bars apply to fibre *a*: horizontal, 10 ms; vertical, 20 mV, 2×10^{-3} (arsenazo III response) and 4×10^{-3} (birefringence changes). Lower calibration bars refer to fibre *b*: horizontal, 5 ms; vertical, 20 mV, 4×10^{-3} (arsenazo III) and 2×10^{-3} (birefringence). Temperature: *a*, 7 °C; *b*, 6 °C.

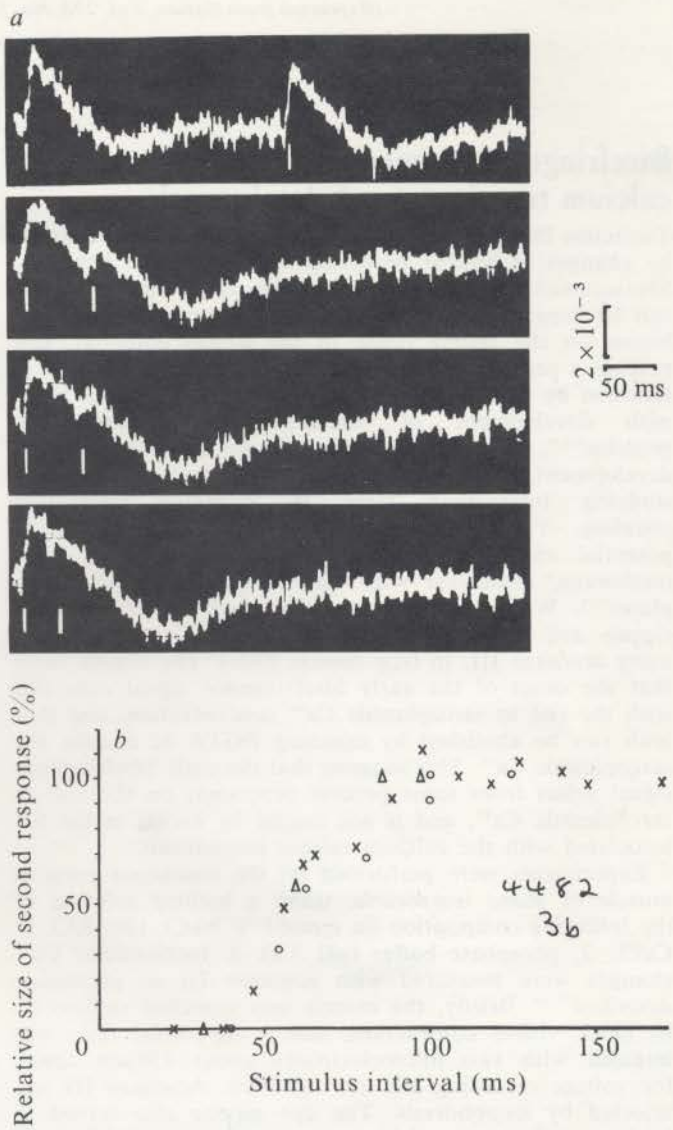

Fig. 3 Birefringence response to action potentials. *a*, Single sweeps showing four runs at stimulus intervals of 250, 65, 55 and 35 ms; vertical bars under the birefringence traces indicate the peak time of the action potentials; temperature, 4 °C. *b*, Relative size of the birefringence signal elicited by the second pulse as a function of stimulus interval. Data from three different fibres; temperature, 4 °C.

the two stimuli was less than 40 ms (at 4 °C), no early birefringence change was detected following the second stimulus (Fig. 3*a*). The curve relating the size of the second response to the stimulus interval (Fig. 3*b*) shows a steep slope between 50 and 90 ms, with recovery to 50% of the control value in about 60 ms. In contrast, Ca^{2+} transients measured with arsenazo III summate almost linearly with stimulus intervals as close as 20 ms (R. Miledi, I.P. and G. Schalow, unpublished data).

The abolition of the birefringence signals by intracellular injection of EGTA strongly suggests that the early signal is not associated with the Ca^{2+} release process by the SR, but is instead secondary to the increase in free sarcoplasmic Ca^{2+} concentration. This view is supported by the coincidence in time courses of the Ca^{2+} transient and the early birefringence signal, and by the experiment with the paired stimuli. The simplest interpretation of the paired stimulus experiment is that the early birefringence signal arises from a process which is fully saturated by the rise in sarcoplasmic Ca^{2+} concentration resulting from a single action potential.

A possible mechanism for this early birefringence signal is a conformational change in the thin filament caused by the Ca^{2+} binding to troponin[3,10]. Latency relaxation and elongation have recently been attributed to a lengthening of the thin filament resulting from Ca^{2+} binding, and the time course of these phenomena parallels that of the early optical changes[1,3].

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