

## TEMPERATURE DEPENDENCE OF CALCIUM TRANSIENTS EVOKED BY ACTION POTENTIALS AND VOLTAGE CLAMP PULSES IN FROG TWITCH MUSCLE FIBRES

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**Abstract:** Intracellular calcium transients, monitored with arsenazo III, were recorded from frog skeletal muscle fibres at different temperatures. The peak size of the arsenazo signal elicited by action potentials did not change appreciably with temperature, whilst that elicited by a fixed depolarization in voltage clamped fibres increased with increasing temperature, with a  $Q_{10}$  of about 1.6. Although the peak free calcium change during a normal twitch appears to change little with temperature, the duration of the calcium transients is longer at lower temperatures. This effect may, at least partly, account for the increased twitch tension at low temperatures.

**Key words:** muscle contraction; calcium transients; arsenazo III

### INTRODUCTION

The contraction of skeletal muscle fibres is controlled by changes in the intracellular level of free calcium ions (Costantin, 1975; Ebashi, 1976; Endo, 1977; Grinnell & Brazier, 1981), and the transient increases in free calcium resulting from action potentials can be monitored using various techniques (Miledi, Parker & Schalow, 1977; Miledi, Parker & Zhu, 1982, 1983a; Blinkes, Rudel & Taylor, 1978; Baylor, Chandler & Marshall, 1982; Eusebi, Miledi & Takahashi, 1983). The calcium indicator dye arsenazo III gives a signal which is roughly linearly proportional to the intracellular free calcium concentration change (Miledi et al. 1982; Miledi, Parker & Schalow, 1980; Scarpa, 1979; Gorman & Thomas, 1978; Thomas, 1982). We have used it here to examine the temperature dependence of the intracellular calcium transient elicited in frog skeletal muscle fibres by action potentials and by fixed depolarizing pulses applied in voltage clamped fibres. A preliminary account has appeared (Miledi, Parker & Zhu, 1983b).

### METHODS

Experiments were made on the isolated cutaneous pectoris muscle of *Rana temporaria*. Techniques for recording calcium transients with arsenazo III and for voltage clamping

fibres were as previously described (Miledi et al. 1982, 1983a). Amplitudes of the arsenazo light absorbance signals are expressed as  $\Delta A/A_{570}$ , where  $\Delta A$  is the absorbance change at the wavelength pair 650–700 nm produced by stimulation, and  $A_{570}$  is the resting absorbance of the injected dye at 570 nm (see Miledi et al. 1982 for details). This expression compensates for differences in fibre diameter and amounts of dye injected, and gives a roughly linear measure of free calcium for concentrations which are small compared to the dissociation constant of arsenazo III (Miledi et al. 1982). Action potentials were elicited by applying brief depolarizing current pulses through the dye injection pipette, which was positioned just outside of the measuring light spot. To study the calcium transients evoked by fixed depolarising pulses, fibres were voltage clamped using a conventional two-point clamp. The measuring light spot was positioned between the voltage and current electrodes, which were inserted about 100  $\mu\text{m}$  apart. Tetrodotoxin ( $5 \times 10^{-7}$  g ml $^{-1}$ ) and tetraethylammonium bromide (20 mM) were added to the bathing solution in these experiments.

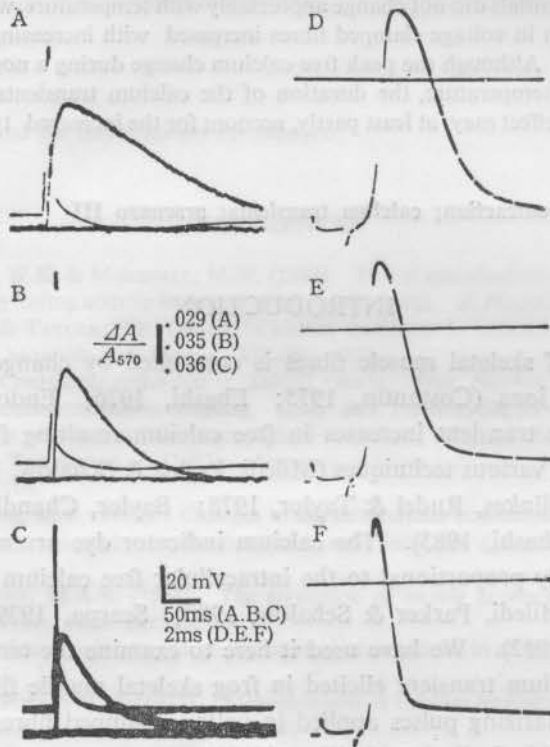


Fig. 1 Arsenazo absorbance signals evoked by action potentials at different temperatures. A–C, action potentials (upper traces) and arsenazo signals (lower traces) recorded at temperatures of 6°C (A), 10°C (B) and 20°C (C). Arsenazo signals show the differential light absorbance change at 650–700 nm, expressed as  $\Delta A/A_{570}$ , and were low pass filtered at 1 KHZ. Baseline absorbance records without stimulation are superimposed on the traces. The records were obtained from three different fibres, and hence the  $\Delta A/A_{570}$  calibration differs slightly for each record. D–F, action potentials recorded on a faster sweep, at the corresponding temperatures to (A–C). These records were obtained from different fibres to the traces in (A–C), and are included only for illustration. Lines indicate the 0 mV potential reference.

to block the action potential and improve the clamp performance. Peltier elements in the base of the chamber were used to control the temperature, which was monitored using a small thermocouple (Comark Ltd) placed close to the muscle. Each measurement at a new temperature was made on a different fibre.

## RESULTS

### Calcium transients evoked by the action potential

We previously found that the peak size of the arsenazo signal to an action potential showed very little variation with temperature, although the decay phase of the response became slower at low temperatures (Miledi et al. 1982). These features are illustrated in Fig. 1 A-C, and Fig. 3 shows an Arrhenius plot of the temperature dependence of the mean peak size. Over the range 6–25°C, the arsenazo signal was almost unchanged in size.

However, the duration of the action potential became much longer at low temperatures

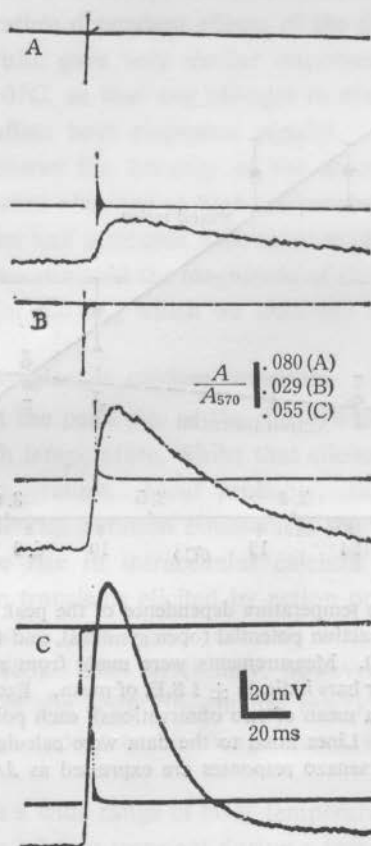


Fig. 2 Arsenazo signals recorded in the same way as Fig. 1, but using voltage clamp pulse (to 0 mV for 5 ms) as the stimulus. Records were obtained at temperatures of; (A), 3.5°C; (B), 10°C; (C), 29°C. The three traces in each frame are, from top to bottom, 0 mV potential level, membrane potential, and arsenazo absorbance signal.

(Fig. 1 D-F), and this factor will affect the size of the arsenazo signal, since depolarizations of longer duration are known to elicit larger arsenazo responses (Miledi et al. 1977, 1983a). We therefore examined the arsenazo responses elicited at different temperatures by a voltage clamped depolarization of fixed amplitude and duration.

### Calcium transients evoked by a fixed depolarization

The pulse chosen to elicit arsenazo signals was of 5 ms duration to 0 mV, from a holding potential of -75 mV. This gave a response similar in size and time course to that evoked by an action potential at a temperature of 10°C. In contrast to the action potential response, changes in temperature had a large effect on the arsenazo signal elicited by the pulse (Fig. 2). For example, the fibres illustrated in Fig. 2 gave values of  $\Delta A/A_{570}$  of 0.074, 0.09 and 0.317 at temperatures of, respectively, 3.5, 10 and 29°C. Figure 3 shows an Arrhenius plot of the mean peak response sizes elicited by the fixed pulse. The data can be fitted adequately by a straight line, the slope of which corresponds to a  $Q_{10}$  of 1.6 between 10 and 20°C.

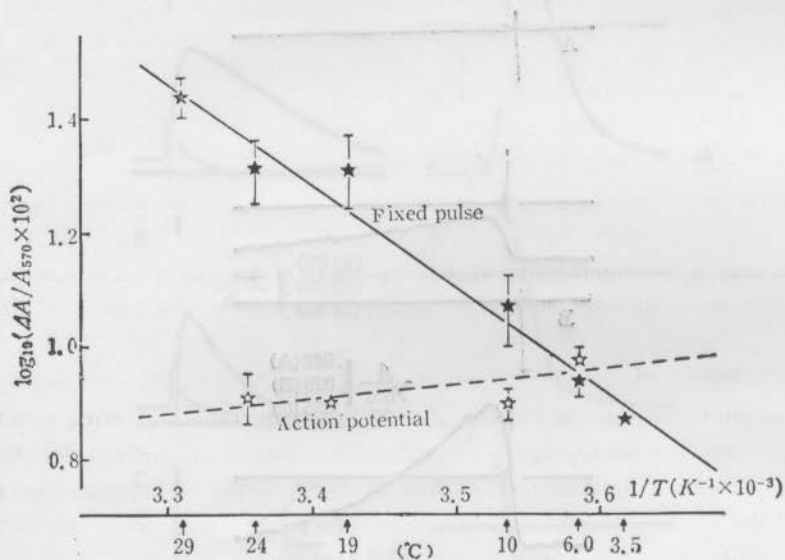


Fig. 3 Arrhenius plot of the temperature dependence of the peak size of the arsenazo response elicited by the action potential (open symbols), and the fixed voltage clamped pulse (filled symbols). Measurements were made from records such as those in Figures 1 & 2. Error bars indicate  $\pm 1$  S.E. of mean. Except for the point without error bars (which is a mean of two observations), each point is a mean value from at least three fibres. Lines fitted to the data were calculated by linear regression. Amplitudes of the arsenazo responses are expressed as  $\Delta A/A_{570}$  (see Methods).

## DISCUSSION

### Possible errors in arsenazo recording

A number of factors complicate the interpretation of arsenazo signals in terms of free-

calcium levels. We have previously discussed some of them (Miledi et al. 1982, 1983a) but for the present experiments where signals elicited at different temperatures were compared, two main points must be considered: (1) Does the sensitivity of arsenazo III for calcium vary with temperature? (2) Is the dye response sufficiently fast to register the rapid peak of the calcium transient at high temperatures without distortion? Regarding (1), we monitored the absorbance of a solution containing  $50 \mu\text{M}$  arsenazo III and about  $15 \mu\text{M}$  calcium at a wavelength of 650 nm, and found no changes over the temperature range from 10 to  $50^\circ\text{C}$ . This indicates that the dissociation constant of the dye for calcium would have been constant over the temperature range explored in the muscle. The situation regarding (2) is less clear. The arsenazo signal may be slower than the true time course of the calcium transient (Baylor & Quinta-Ferreira, 1983), but it is not yet known whether the kinetics of the dye reaction would cause the peak amplitude of the signal to vary with temperature.

Perhaps the best argument is that our experiments included an internal control, which demonstrates that the differences observed between the temperature dependence of the responses elicited by action potentials and clamped depolarizations arose from properties of the muscle, and not from temperature dependent effects of the dye. This is because the action potential and the clamped pulse gave very similar responses, in both amplitude and time course, at a temperature of  $10^\circ\text{C}$ , so that any changes in arsenazo properties with temperature would be expected to affect both responses equally.

A different problem concerns the linearity of the arsenazo signal. With the voltage clamp pulse, large responses were obtained at high temperatures, which approached the level where the dye would have been half saturated with calcium (Miledi et al. 1982). These signals, therefore, may have underestimated the magnitude of the true free calcium transient, and as a consequence the value for the  $Q_{10}$  which we obtained may also be an underestimate.

### Temperature dependence of myoplasmic calcium transients

The major finding is that the peak size of the calcium transient during a normal twitch remains roughly constant with temperature, whilst that elicited by a fixed depolarizing pulse increases with increasing temperature. Most probably, this is because the temperature dependence of the action potential duration counteracts the temperature dependence of the processes responsible for the rise in intracellular calcium.

Measurements of calcium transients elicited by action potentials using the photoprotein aequorin show that the aequorin light signal increases with increasing temperature (Blinks et al. 1978; Eusebi et al. 1983). This effect may, however, be largely accounted for by the temperature dependence of the aequorin-calcium reaction (Blinks et al. 1978; Eusebi et al. 1983), so these findings appear to be in agreement with our results using arsenazo III.

Since the frog experiences a wide range of body temperatures, it might seem a useful property for the amplitude of the calcium transient during a twitch to remain constant with temperature. However, the duration of the calcium transient is fairly brief compared to the time required for full activation of the contractile filaments (Blinks et al. 1978), so that the duration of the transient will also affect the twitch tension. In this respect, the time integral of the calcium transient should give a better indication of the degree of contractile activation

than the peak magnitude. Since the calcium transient consists of a rapid rise, followed by a slower exponential decay (Miledi et al. 1982), the integral is approximately given by the product of the peak magnitude and the decay time constant. The decay time constant increases with decreasing temperature, with a  $Q_{10}$  of about 2.4 (Miledi et al. 1982), so that the calcium activation of the contractile filaments during twitches is expected to be greater at low temperatures. It is well known that the twitch tension of frog skeletal muscle increases at low temperatures (Hill, 1951), and the lengthening of duration of the calcium transient without any change in peak size is probably a major factor contributing to this effect.

At present, we do not know which stage(s) in the excitation-contraction coupling mechanism are responsible for the temperature dependence seen with fixed depolarizations. It would not be surprising if the calcium release from the sarcoplasmic reticulum were temperature dependent, but it is also possible that the release may be constant, and that variations in peak free calcium arise from temperature dependent changes in the calcium buffering systems in the myoplasm.

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