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Transition temperature of excitation–contraction coupling in frog twitch muscle fibres

THE mechanism by which depolarisation of the muscle fibre membrane leads to release of stored Ca^{2+} ions from the sarcoplasmic reticulum, although crucial, is perhaps the least understood stage in excitation–contraction coupling[1,2]. We report here our investigation of the temperature dependence of this mechanism, using intracellular injection of arsenazo III (refs 3, 4), a Ca^{2+} indicator dye with a fast response time[5]. We have found that the latency between depolarisation of the fibre and the onset of the rise in intracellular free Ca^{2+} is proportional to the reciprocal temperature, but that on an Arrhenius plot this relationship shows a change in slope at a temperature which depends upon the Ca^{2+} concentration in the bathing solution.

Experiments were carried out on the cutaneous pectoris muscle of *Rana temporaria*, stretched to a striation spacing of about 3.6 μm to reduce contraction. The bathing solution contained (in mM): NaCl, 120; KCl, 2; 0, 1.8 or 12 $CaCl_2$. In the 0 Ca^{2+} solution, 1 mM EGTA and 5 mM $MgCl_2$ were added; *p*H was adjusted to 7.2. The temperature of the solution was controlled by Peltier elements in the base of the chamber, and

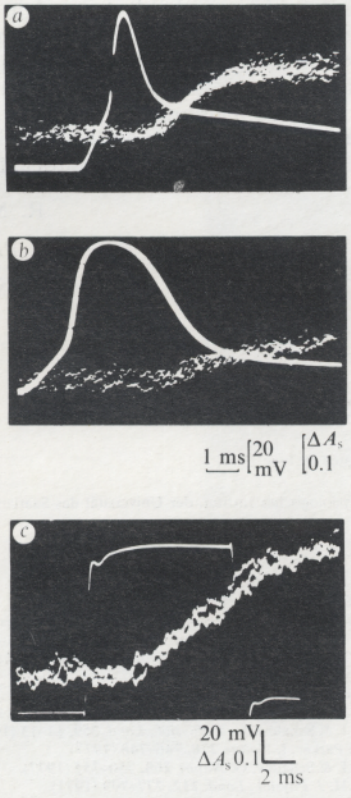

Fig. 1 Delay of the arsenazo III light response following stimulation by action potentials (*a*, *b*), and a voltage-clamped depolarising pulse (*c*). In each record the upper (noisy) trace shows light transmission changes from the arsenazo III-loaded fibre, at the wavelength pair 532–602 nm, and the calibration bars give the standardised change in light absorption, ΔA_s (see ref. 6). The lower traces show the membrane potential. Each record is a superposition of three to four sweeps. The records were obtained from different fibres, at the temperatures indicated. Ringer's solution contained 1.8 mM Ca^{2+} in all cases, and in *c* 2×10^{-6} tetrodotoxin and 30 mM tetraethyl ammonium bromide were also added to the solution. In *a* and *b* the fibres were stimulated by passing depolarising current pulses of 1 ms duration through the dye pipette. In *c* the fibre was voltage clamped, and stimulated by a 10-ms duration depolarising command pulse. Temperature: *a*, 29 °C; *b*, *c*, 7 °C.

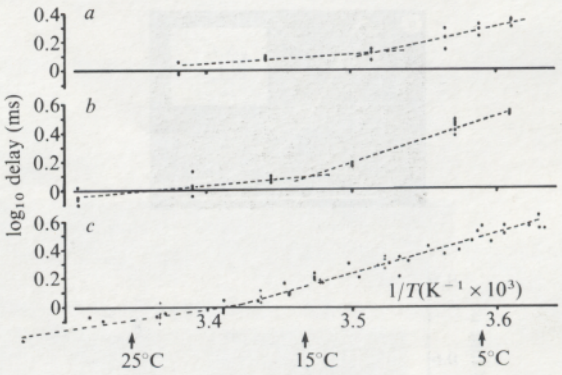

Fig. 2 Arrhenius plots of the log delay of the Ca^{2+} response against T^{-1}. The delay was measured by eliciting action potentials by a 1-ms depolarising pulse applied through the dye pipette, and measuring the latency between the foot of the action potential (about −40 mV) and the onset of the Ca^{2+} response (see Fig. 1). The three graphs show data from muscles bathed in Ringer's solution containing: *a*, 0 mM Ca^{2+} + 1 mM EGTA and 5 mM $MgCl_2$; *b*, 1.8 mM Ca^{2+}; *c*, 12 mM Ca^{2+}. A different muscle was used for each solution, and results from two muscles (●, and +) are shown in *c*. Each datum point was obtained from a different fibre, and is a mean of nine measurements. Readings were generally obtained from three or four fibres while holding the muscle at a given temperature, and different temperatures were tested in random order. Optical recording time constant was 0.5 ms. The lines drawn through the data were fitted by eye.

was measured by a thermistor close to the muscle. Fibres were penetrated with two micropipettes, for voltage recording and dye injection. The dye pipette also served as a current-passing electrode for initiating action potentials, or for voltage clamping. Intracellular Ca^{2+} changes were recorded as previously described[3,6,7]. Briefly, arsenazo III was injected into a fibre by ionophoresis, and light transmission through the fibre was measured at wavelengths of 532 and 602 nm from a spot of 80 μm diameter. Electrical subtraction of these two signals gave a record sensitive to Ca^{2+}-dependent changes in absorption of the dye, but in which movement artefacts were reduced.

When a fibre was depolarised to potentials above 0 mV, there was a definite latency between the rise in membrane potential and the onset of the Ca^{2+} response (Fig. 1 and refs 6, 8). This delay increased with decreasing temperature and was similar when the response was elicited by a depolarising pulse after blocking the action potential mechanism with tetrodotoxin, rather than by an action potential. For example, in 12 mM Ca^{2+} Ringer's solution, mean values of the delay-following action potentials and depolarising pulses were, respectively, 1.5 ± 0.2 ms (8) and 1.45 ± 0.42 ms (6) at a temperature of 15–16 °C, and 2.6 ± 0.8 ms (7) and 3.0 ± 0.6 ms (6) at 3–4 °C [mean, s.d. (number of fibres)]. Figure 2 shows the temperature dependence of the delay following an action potential, measured in muscle fibres bathed in Ringer's solutions containing various Ca^{2+} concentrations. The latency increased exponentially with the inverse of the absolute temperature (K) in all conditions studied, but at each Ca^{2+} concentration the slope of the relationship changed at a particular temperature. This transition temperature increased with increasing external Ca^{2+} concentration, and was 11 °C in 0 mM Ca^{2+}, 15 °C in 1.8 mM Ca^{2+} and 19.5 °C in 12 mM Ca^{2+}. This effect of Ca^{2+} concentration is probably specific to Ca^{2+} ions and not simply due to a change in divalent cation concentration, as the 0 Ca^{2+} solution contained 5 mM Mg^{2+}.

Another parameter of the excitation–contraction coupling system which displayed a similar temperature dependence to the initial latency, was the time during which a depolarised fibre could maintain an elevated Ca^{2+} level. Figure 3*a* shows a typical light absorbance record from a voltage-clamped fibre which was

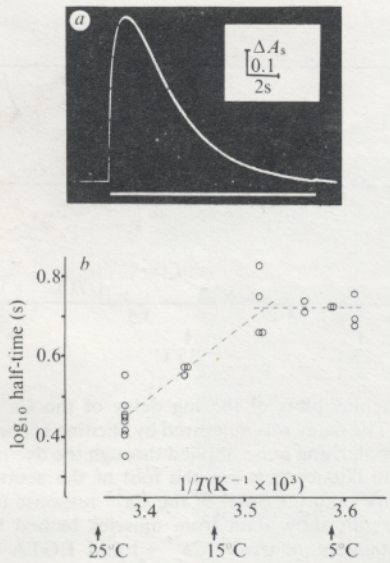

Fig. 3 *a*, Optical record showing the Ca^{2+}-induced change in differential light absorbance at 532–602 mn of an arsenazo III-loaded fibre, in response to a prolonged depolarisation. The fibre was voltage clamped to a potential of −80 mV at rest, and depolarised to +20 mV for the duration indicated by the bar. The vertical calibration, ΔA_s, gives the standardised change in light absorbance[3,6]. Ringer's solution contained 12 mM Ca^{2+}; temperature 5 °C; recording time constant 33 ms. *b*, Arrhenius plot of the log half-duration of the Ca^{2+} response during prolonged depolarisation. The half-duration was measured as the time for which the light absorbance record exceeded a value of one-half of the peak value. Each point is a single measurement from a different fibre from one muscle. Same experiment as Figs 2*a* and 3*a*.

depolarised from −80 mV to +20 mV for 10 s. An initial large Ca^{2+} response was recorded which declined to the baseline over the duration of the pulse. Figure 3*b* shows an Arrhenius plot of the temperature dependence of the half-duration of this response, measured in a muscle bathed in 0 mM Ca^{2+} Ringer's solution. At temperatures below 11 °C the response half-duration remained constant at about 5.2 s, but at higher temperatures it decreased exponentially with the inverse of the absolute temperature. This transition temperature corresponds closely with that measured for the latency of the arsenazo signal during twitches on 0 mM Ca^{2+} solution. Technical difficulties prevented us from obtaining temperature dependence data at other Ca^{2+} concentrations, but at a temperature of 22.5 °C the response half-duration was slightly dependent on external Ca^{2+} concentration. Mean half-durations were 0 mM Ca^{2+}, 2.8 s (s.e.m. 0.14); 1.8 mM Ca^{2+}, 3.22 s (0.08); 12 mM Ca^{2+}, 3.8 s (0.12). This contrasts with frog slow muscle fibres, in which the duration of the maintained Ca^{2+} response is more strongly dependent on external Ca^{2+} concentration[9]. The peak sizes of the responses were not significantly affected by changes in temperature, suggesting that the shorter duration of the maintained Ca^{2+} response at high temperatures was not the result of exhaustion of Ca^{2+} reserves due to a higher rate of release. This agrees with the view that the amount of Ca^{2+} released during a prolonged depolarisation is controlled by a time-dependent inactivation of the release process[21,22].

Possible sources of the latency of the Ca^{2+} response include (1) response time of the dye, and diffusion time of Ca^{2+} within the myoplasm; (2) propagation time of the action potential inwards along the T-system; (3) a delay in the coupling mechanism between T-tubule depolarisation and Ca^{2+} release from the sarcoplasmic reticulum. The response time of arsenazo III is reported as faster than 400 μs at 21 °C (ref. 5) and the complex temperature dependence of the response latency makes it unlikely that the factors in (1) can account for the delay. Active propagation along the T-tubules has a velocity of about 7 cm s^{-1} at 7 °C in frog twitch fibres[10], and would therefore introduce a delay of about 1 ms for conduction to the centre of a 100-μm fibre. However, the initial rise in the Ca^{2+} response probably results from activation of the sarcoplasmic reticulum at the periphery of the fibre, so that inward conduction time would not be an important factor in determining the minimal delay. A large part of the observed latency may therefore be attributed to the coupling mechanism.

The two component Arrhenius plots shown in Figs 1 and 2 resemble recent results on the elementary properties of drug receptor channels in muscle cells[11–15]. Possible causes of these temperature transitions include a membrane lipid phase transition[16–18], a conformational change in some protein[19] associated with the excitation–contraction coupling process, or different steps of a reaction sequence in the coupling process, being rate limiting in different parts of the temperature range[20]. Whatever the explanation, the influence of external Ca^{2+} concentration on the transition temperature of the latency in the rise of internal Ca^{2+} during a twitch, suggests that a delay in excitation–contraction coupling is introduced by a structure which is influenced by changes in Ca^{2+} concentration in the bathing medium, and is therefore probably located in the T-tubule membrane. The close similarity of transition temperature, and dependence on extracellular Ca^{2+}, of the duration of maintained Ca^{2+} response further suggests that this is also regulated by a structure in the T-tubule membrane[21,22].

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