KAWASAKI MED. J. VOL. 13 : NO. 2, 69-73 (1987)

Effects of Temperature on Arsenazo III Ca^{2+}-transients in *Xenopus* Skeletal Muscle Fibers

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Accepted for Publication on March 12, 1987

ABSTRACT. Arsenazo III (AZ), a Ca^{2+} indicator dye, was injected iontophoretically into *Xenopus* skeletal muscle fibers. AZ Ca^{2+}-transients (AZ signals) following action potentials were measured by a pair of photomultipliers at the wavelength of 651-721 nm. As the temperature was lowered, the latency of the AZ signal (T_1), the time to peak (T_2) and the decay time constant (T_3) were prolonged. The values of T_1, T_2 and T_3 were about 1.5, 4 and 25 msec at 20-21°C and about 5, 10 and 80 msec at 7°C. An Arrhenius plot for T_2 and T_3 indicated that the values of Q_{10} for T_2 and for T_3 were 1.7 and 2.2. It was suggested that, at low temperatures, the dissociation of Ca from Ca-binding proteins appears to be the rate limiting factor of the falling phase of the AZ signal.

Key words : E-C coupling — arsenazo III — Ca^{2+}-transients — temperature — Q_{10}

The muscle contraction is initiated by the Ca ions released from the sarcoplasmic reticulum (SR) and the relaxation takes place as the result of sequesteration of Ca ions into SR. The time course of the transient increase in Ca ions concentration have been measured with the metallochromic dyes such as arsenazo III (AZ)[1] and antipyrylazo III[2] and Ca^{2+}-sensitive protein, aequorin.[3] The time course of the intracellular Ca^{2+} concentration thus measured indicates the processes of Ca^{2+} release from SR, binding of Ca^{2+} to troponin and parvalbumin, and Ca^{2+} re-uptake into SR and so on. All of the processes are dependent on temperatures. The present work is carried out to investigate in detail the temperature dependence of the Ca^{2+}-transients and to explain the intracellular Ca^{2+} movement.

METHODS

The materials and the experimental procedures were already described.[4] The temperature of the Ringer solution was changed by circulating temperature-regulated water through the sides and the bottom of the muscle chamber. The range of the temperatures investigated was 7-29°C. Usually, the measurements were started at high temperature and then they were done during lowering temperatures. The AZ signals were detected by a pair of photomultipliers

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(Hamamatsu Photonics, R 1463) as the difference of transmitted light intensity at 651–721 nm. In this paper, the AZ signal was expressed in terms of the absorbance changes ΔA, calculated from the equations (1) and (2).

$$A = \log \frac{I_0}{I} \tag{1}$$

$$\Delta A = -\frac{1}{\ln 10} \cdot \frac{\Delta I}{I} \fallingdotseq -0.43 \cdot \frac{\Delta I}{I} \tag{2}$$

where A is the absorbance of AZ and I_0 and I are the transmitted light intensities of the fiber before and after injection of AZ, respectively. ΔI is the change in the transmitted light intensity of the AZ signal.

RESULTS

For the investigation on the effect of temperature on Ca^{2+}-transients it was necessary to leave the muscle preparation in the solution of newly attained temperature for at least 5 min. Therefore, it took about an hour to obtain the records at different temperatures. The problem was that the injected AZ diffused away and the AZ concentration might change during a course of the measurements. According to Baylor *et al.*,[5] the AZ concentration affect not only the amplitude but also the falling phase of the AZ signal. To avoid the errors due to a progressive decrease of the AZ concentration, the AZ-electrode was left inside the muscle fiber and AZ was additionally injected prior to measurement at the new temperature. The amount of additional injection of

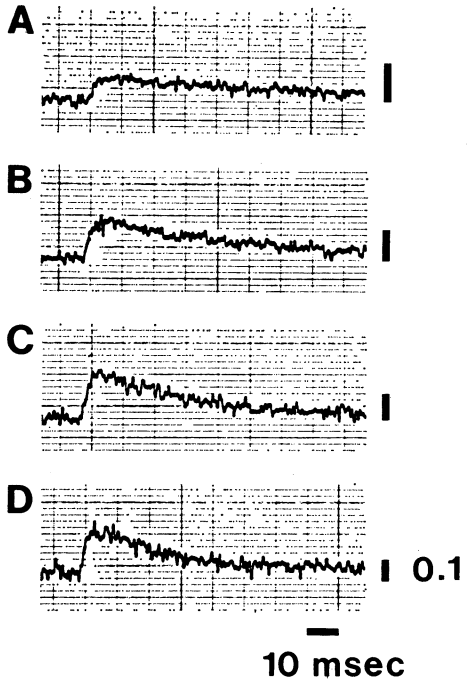

Fig. 1. AZ signals evoked by intracellular stimulation at various temperatures. A, B, C and D show the response at the temperatures of 9.1, 11.1, 14.0 and 20.5°C. Four signals at 25 sec intervals are averaged. Calibration of the signal is indicated by $\Delta A/A$.

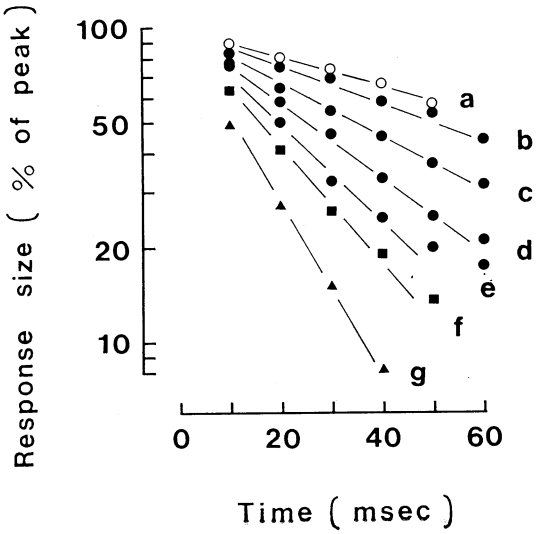

Fig. 2. Effects of the temperature on falling phase of the AZ signals. a, b, c, d, e, f and g indicate 7.0, 9.1, 11.1, 14.0, 20.5, 25.4 and 29.2°C. The peak amplitude of each signal was taken as 100% and the time taken from the peak of the signal. Different symbols indicate the different fibers.

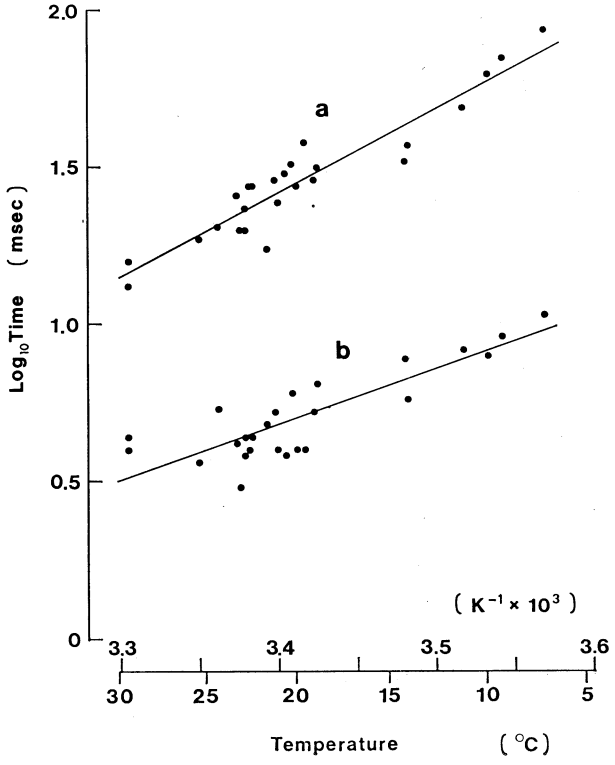

Fig. 3. Temperature dependence of decay time constant (a) and time to peak (b) of the AZ signal in 18 fibers. Arrhenius plot of these two parameters is shown.

AZ was adjusted by monitoring whether the transmitted light intensity (I in eq. (1)) might be equal in every measurement. The AZ concentration, however, was not kept exactly constant, so it was not reasonable to compare the peak amplitude of the AZ signal at one temperature with that at another temperature. One advantage was that the concentration dependence was excluded if the value of $\Delta A/A$ was taken, although ΔA was dependent on AZ concentration. In this paper, the AZ signal was expressed as $\Delta A/A$ and only the time parameters were analysed.

Fig. 1 shows the AZ signals when the temperature of Ringer solution was changed from 20.5°C to 9.1°C. There were about 10 min intervals between each of the measurements. As the temperature was lowered, the latency of the AZ signal (T_1), the time from the onset of the signal to the peak (T_2) and the decay time constant (T_3) were prolonged. The values of T_1, T_2 and T_3 were 5, 10, 80 msec at 7°C in comparison with 1.5, 4, 25 msec at 20–21°C. Fig. 2 shows the effect of the temperature on the falling phase of the AZ signal. Prolongation of the falling phase became marked with lowering the temperature. In Fig. 3, an Arrhenius plot was shown on T_2 and T_3 measured from different fibers over the temperatures of 7–29°C. The values of Q_{10} for T_2 and T_3 were calculated to be 1.7 and 2.2 from the slope of the straight line drawn by the method of least squares.

DISCUSSION

Effect of the temperature on the rising and falling phase of the AZ signals was investigated over the temperature of 7–29°C. The falling phase of the AZ signal was prolonged by lowering the temperature with Q_{10} of 2.2 for the time constant of the falling phase. Miledi *et al.*[6] investigated the effect of the temperature and obtained Q_{10} of 2.4, which was slightly larger than our result. Eusebi *et al.*[7] also reported Q_{10} of 2.3 for the falling phase of the aequorin signal. The value of Q_{10} for Ca^{2+} uptake in isolated fragmented SR was indicated around 2.5 to 3 by Weber *et al.*[8] or 3.1 by Ogawa.[9] This value of Q_{10} was a little different from that of the falling phase of the AZ signal and other mechanisms may be considered in the AZ signal. Ca ions once released from SR are bound to troponin, which leads muscle contraction. At the same time, Ca^{2+} are buffered by another Ca-binding protein, parvalbumin, and are sequestered by SR. At the low temperatures, not only Ca-uptake into SR but also the dissociation of Ca^{2+} from Ca-binding proteins is slowed altogether. If the dissociation was more dependent on temperature than Ca-uptake, the free Ca^{2+} concentration will decrease more rapidly than expected from Q_{10} value of Ca-uptake. It may be said that the falling phase of the AZ signal is mainly determined by Ca-uptake into SR at room temperatures of above 20°C, but at low temperatures below 10°C, the dissociation of Ca^{2+} from Ca-binding proteins appears to be the rate limiting factor of the falling phase of the AZ signal. The roles of parvalbumin in the falling phase of the AZ signal is probable because the association rate of Ca to parvalbumin is a little less temperature dependent than Ca-uptake into SR.[10]

In regard to the rising phase of the AZ signal, Q_{10} for T_2 is 1.7 and smaller than that for the falling phase. The rising phase is decided by the processes

of Ca-releasing from SR and of Ca-binding to AZ and Ca-binding proteins. It is assumed that the Ca-release from SR is the rate limiting factor of the rising phase of the AZ signal because of the small Q_{10} for the rising phase.

Acknowledgment

This work was supported in part by the Research Project Grant No. 60-507 from Kawasaki Medical School.

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