CALCIUM TRANSIENTS IN SINGLE MUSCLE FIBERS

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The kinetics of *in vivo* calcium release and reabsorption in skeletal muscle are of interest because calcium ions are known to provide an essential link between membrane depolarization and contraction (4). We have been able to follow the time course of the intracellular release and reabsorption of calcium during the contraction of single muscle fibers.

Our method involves the intracellular injection of the calcium-sensitive bioluminescent protein, aequorin, into the giant single muscle fibers of the acorn barnacle, *Balanus nubilus* (2). Aequorin is derived from the medusa, *Aequorea aequorea*, abundant in the environs of Friday Harbor, Washington during the summer months. The extraction, purification, and properties of aequorin have already been described by Shimomura, Johnson, and Saiga (5). As a sensitive *in vivo* calcium indicator, the bioluminescent protein has the following advantages over such well known indicators as murexide (3). First, aequorin is a highly specific calcium indicator and is not affected by other physiologically occurring ions. The reaction of aequorin with calcium is brief; following the emission of light the protein is apparently inactivated and the calcium ion released. Aequorin is sufficiently soluble so that only small volumes of a concentrated solution need be injected. The rate of light emission from a given amount of aequorin is a monotonic, although not necessarily linear function of the calcium ion concentration. The protein is apparently sensitive to all levels of calcium, but the practical lower limit as

measured by Shimomura, Johnson, and Saiga (5) was 10^{-6} - 10^{-7} M. Aequorin has an estimated molecular weight of ca. 35,000, and is apparently small enough to diffuse throughout the sarcoplasm and yet too large to diffuse out of the fiber across the sarcolemma. Finally, the presence of this foreign protein appears to be well tolerated by the Balanus fiber.

Several thousand Aequorea were collected and the protein extracted and purified using a modification of the procedure described by Shimomura et al. (5). Once purified, the protein was dissolved in 300mM KCl and 0.3 - 0.5ul. of this concentrated solution was injected along almost the full length of the cannulated Balanus fiber. The injection process produced little detectable fiber damage. Preliminary studies showed that the fiber could be seen by the dark adapted eye to glow on stimulation. In order to record this light emission, the fiber was placed in a chamber, provided with a window through which the emitted light could be monitored by an RCA 6342A end-window photomultiplier. Fresh saline circulated throughout the chamber during the course of the experiment and the fiber was stimulated by passing current between two full fiber-length silver electrodes. A period of thirty minutes was allowed between the injection and the stimulation of the fiber, to allow the aequorin to diffuse throughout the sarcoplasm. The fiber was always cannulated at the shell end (1) so that it was held in position in the chamber by the cannula mounting. The tendon or scutal end of the fiber was attached by an inextensible chain to the anode peg of an RCA 5734 mechanoelectric transducer to monitor tension responses. The output from the photomultiplier was D. C. coupled to an amplifier and the resulting signal, together with the tension response, stored in a Technical Measurement Corporation, Computer for Average Transients (CAT) # 400B. The memory contents of the CAT could be displayed on a small associated cathode ray screen which was photographed directly.

The results shown in Fig. 1 illustrate the relationship between the calcium-mediated light emission and the onset of tension in a single Balanus

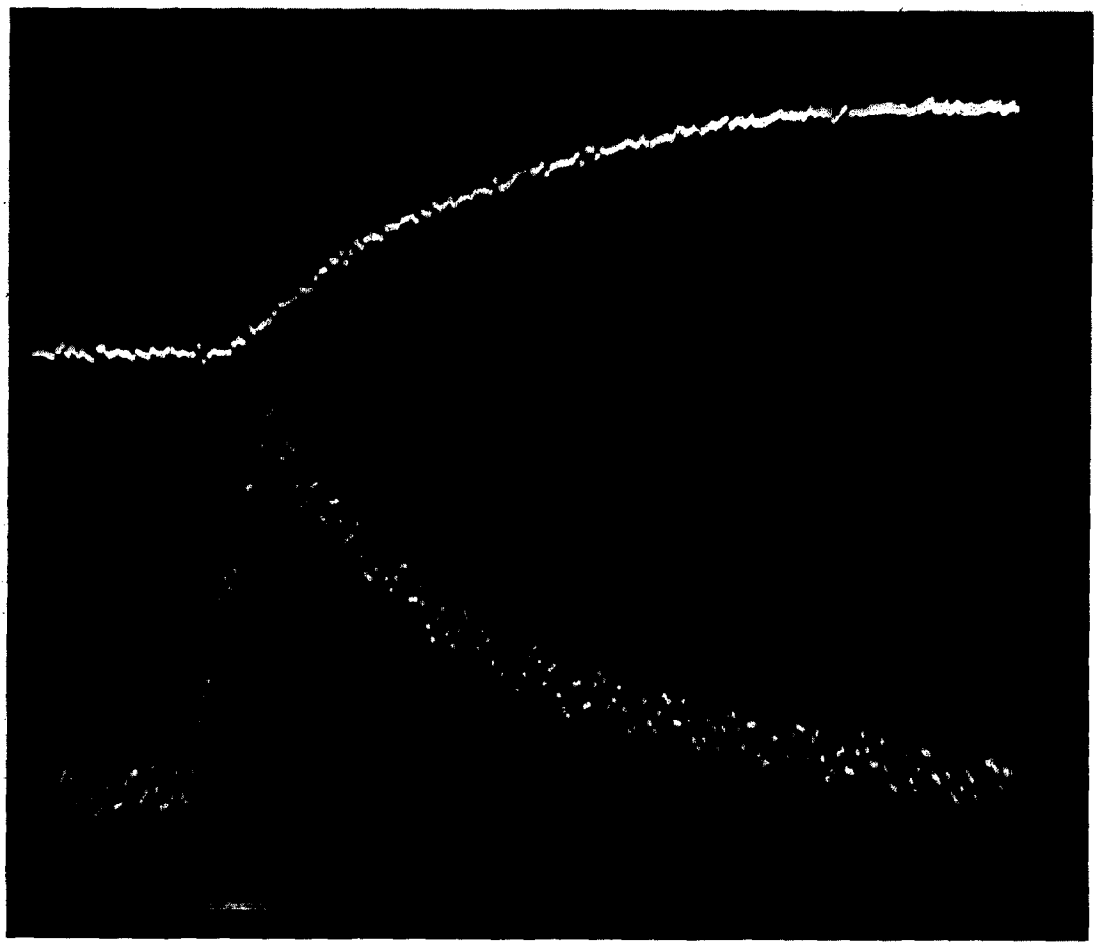

Fig. 1. Single stimulus pulse: Light emission (lower trace); tension (upper trace). Stimulus duration (indicated by horizontal bar) 20 msec, traces the result of 5 additions into the CAT. Full sweep 250 msec, temp: 14°- 16°C.

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fiber injected with aequorin. The fiber was stimulated after a 40 msec delay with a square wave pulse of 20 msec duration. As can be seen, the onset of light emission occurs almost simultaneously with the stimulus, the delay being 1 msec or less. Similarly, light emission begins to fall immediately on the cessation of the stimulus. In contrast to this, tension development, which does not begin until about 5 msec after the beginning of stimulation, continues to rise throughout the record, approaching its maximum when light emission has virtually ceased. Moreover, the peak calcium concentration occurs at a time when tension is no greater than 10% of its maximum value,

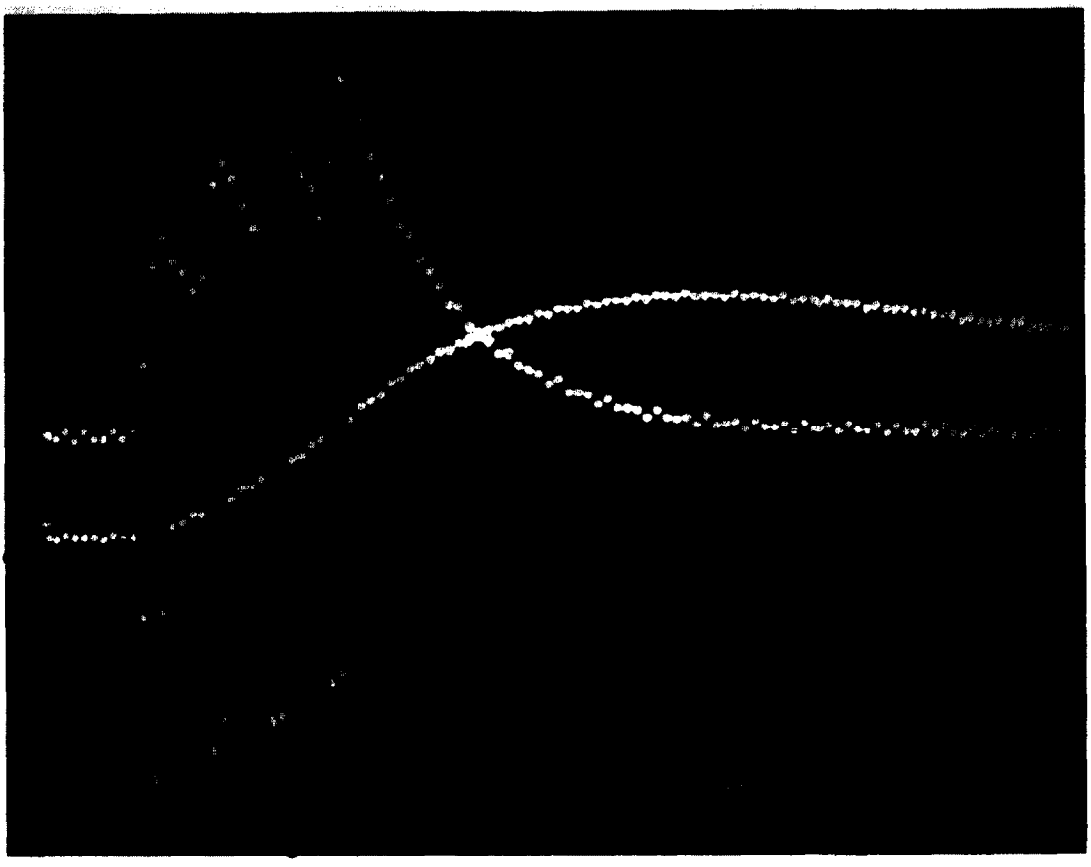

Fig. 2. Brief tetanus: Light emission (upper trace); tension and stimulus artefacts (lower trace). Each stimulus pulse 20 msec duration, traces the result of 6 additions into the CAT. Calibration bar: 200 msec, temp: 14°- 16°C.

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hence there appears to be no simple relationship between the ionized calcium concentration and tension.

In Fig. 2, the results indicate the relationship between the calcium-mediated light output and tension during a brief tetanus. Associated with each of the 20 msec stimuli, there is a rapid rise and subsequent fall of light output, despite a smooth rise of tension. The light output falls rapidly after the final stimulus and is almost back to the baseline at peak tension. Each additional stimulus produces a slightly greater peak light output than the preceeding one, thus giving a 'staircase' effect. As in the

case of the single stimulus record (Fig. 1), the peak in the calcium-mediated light output occurs before peak tension. The peak in the light output occurring some 220 msec, peak tension occurring some 600 msec, after the beginning of the first stimulus pulse. At this CAT sweep, tension and light output appear to rise simultaneously.

During the course of these experiments, we have observed that, following a single injection of aequorin, many hundred such light emitting responses may be elicited over a period of several hours. Hence the injected aequorin must reside in an area of low ionized calcium, the most likely position being the sarcoplasm proper (1).

The results illustrated above indicate that in these gradedly-responsive fibers, the rising phase of the calcium transient is closely coupled to the duration of the stimulus; calcium being released so long as the stimulus and resulting depolarization are maintained.

The peak of the calcium transient appears to coincide with the maximum rate of rise of tension. This observation suggests at least a limited relationship between the first derivative of tension and the calcium ion concentration during the rising phase of tension.

The falling phase of the calcium transient is roughly concomitant with the fall in the rate of tension increase during contraction. This fall in ionized calcium must reflect the binding of calcium to various components in the muscle fiber.

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