CALCIUM RELEASE AND REABSORPTION IN THE SARTORIUS MUSCLE OF THE TOAD

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Since the early experiments of Heilbrunn and Wiercinski (1), it has been well established that changes in the intracellular calcium concentration must play a crucial role in muscular contraction (2), but a direct demonstration of such changes in intact, contracting muscle has been lacking thus far. In 1963, Ohnishi and Ebashi (3) showed that it is possible to monitor in vitro the uptake of Ca^{++} by particulate fractions derived from the sarcoplasmic reticulum. To this end, they measured the changes in light absorption that occur when Ca^{++} complexes with murexide, a well-known calcium indicator. We have used the same approach to study the kinetics of Ca^{++} changes in intact muscles after in vivo incorporation of small amounts of murexide.

Toads (Bufo marinus) were injected intraperitoneally with 50 mg of murexide (ammonium purpurate) twice daily for at least two days before sacrifice. In some cases, the indicator was dissolved in a small quantity (0.5-1.0 ml) of dimethyl sulfoxide to facilitate permeation through the muscle membrane. The animals were decapitated and the hind legs perfused with Ringer's solution via the descending aorta. After careful excision, a sartorius muscle was stretched to approximately 120 per cent of body length over a

gently curved surface of nylon netting. Stimulation was by supramaximal shocks of 1 msec duration delivered at 2 pairs of bar electrodes, one above and one below the optical window through the muscle. A monochromatic light beam (5 mμ band width) was used for transillumination of the middle half of the muscle. The light that traversed the muscle impinged on an end-on photomultiplier. After suitable amplification, the resulting current was bucked out and the output of the amplifier was AC-coupled to 0.1 cps to the input of a Nuclear Chicago #7100 computer for average transients.

Twitches or short tetani were induced at 30 second intervals under illumination at either the peak (470 mμ) or at the trough (540 mμ) of the difference spectrum of Ca^{++}-murexide vs murexide. The optical changes accompanying 5 or 10 contractions were averaged on the computer. In order to cancel the light scattering effects, the optical signals at two adjacent wave lengths were subtracted. For the 470 mμ peak 440 and 505 mμ were employed as reference wave lengths; for the 540 mμ trough 505 and 580 mμ. The effects of the same number of contractions under 440 or 505 mμ illumination were averaged with opposite polarity at the input of the computer and thus, were subtracted from the above accumulated average. This was followed by the same number of contractions with 505 or 580 mμ illumination, and finally, another series was measured again at 470 or 540 mμ with the original polarity. With well mounted muscles, not exposed to murexide, this regimen cancels light scattering effects during the contraction cycle, with the occasional exception of the last part of the relaxation phase. In such cases, fatigue was usually noticeable in the mechanical performance of the muscle, with consequent changes in the shape of the relaxation phase. The lack of a similar effect on the light scattering changes during the latent period and the contraction phase is probably related to the

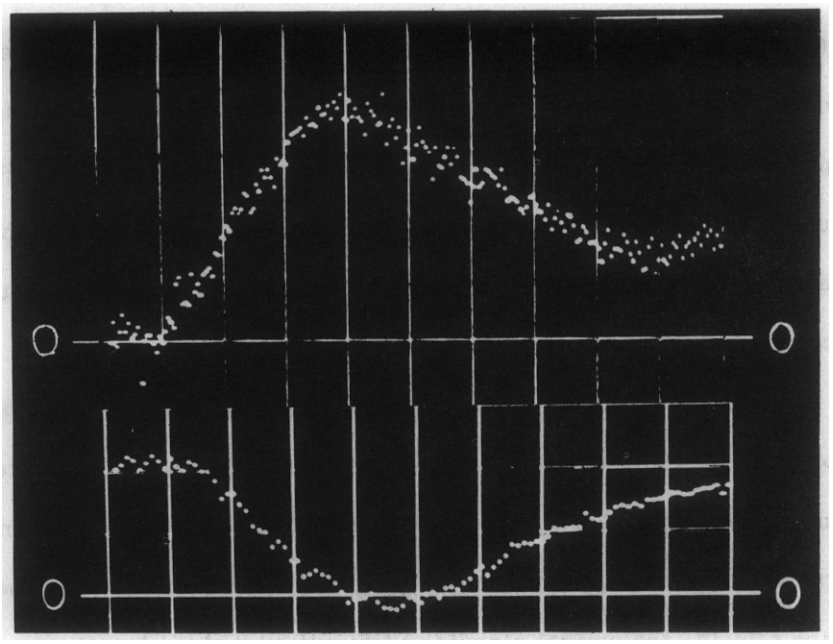

Fig. 1 Kinetics of the Ca-murexide complex: effects of 4 series of 10 contractions. Top: 540 mμ (minus 505 and 580 mμ); Bottom: 470 mμ (minus 440 and 505 mμ). Increase in transmission is recorded in the upward direction. The vertical lines provide a time scale at every 25 msec. The stimulus was delayed 25 msec after the start of the sweeps. Results on two preparations from different toads. Temp: 9-10°C.

fact that fatigue produces considerably less change in the time relations of the early phases of the contraction cycle.

In Fig. 1, results are given which are obtained at 10°C with a sartorius muscle from a murexide treated toad. The decrease in light trans-

mission at 470 and the increase at 540 mμ - each as compared with two neighboring wave-lengths - coincide with the expected spectral changes for the formation of Ca-murexide. The Ca^{++} concentration is seen to increase within a few msec after the stimulus, to reach a peak at 75-80 msec afterwards and

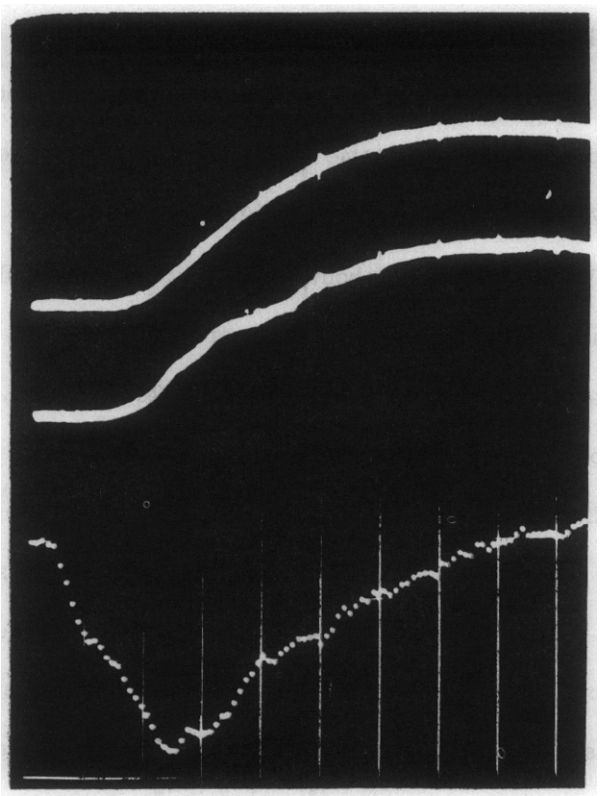

Fig. 2 Time relations between tension development (upper trace), light scattering (middle trace) and the kinetics of the Ca-murexide complex (470 mμ). Time scale: 25 msec per division. Stimulus delayed 12 msec after the start of the sweeps. Temp: 12°C. Otherwise as Fig. 1.

to decrease again to half the peak value at 150 msec. Some variability occurred between preparations especially in the time of occurrence of the maximum. This can only partially be ascribed to the temperature which fluctuated between 9 and 12°C between experiments. The peak has been observed in practically all the preparations from toad treated with murexide + dimethyl sulfoxide and in about half of those injected with murexide alone.

In the next traces (Fig. 2) the time relations are explored between the tension, the light scattering changes and the absorption effects specific to the 470 mμ region. The top two records were photographed from an oscilloscope display with the same sweeping speed (25 msec/division) as the display of the memory contents of the computer. The Ca^{++} signal of Fig. 2 reaches its maximum about 55 msec after the stimulus. At this time the tension has reached about 20 per cent of its peak value. The trace has returned to half its maximum value at 110-120 msec and at the moment of peak tension attainment virtually no free Ca^{++} remains.

The effect of a number of stimuli delivered at a tetanic rate was also explored; one result is presented in Fig. 3. Here it can be seen that the level of Ca^{++} increases stepwise when four stimuli are presented at a rate of 20 cps. Four discontinuities can be distringuished in the rise in Ca^{++} concentrations. Starting with the first peak at 75 msec after the first stimulus, small shoulders can be distinguished at approximately 50 msec intervals; i.e. equal to the spacing of the stimuli. This occurs notwithstanding the fact that the tension rises smoothly throughout the entire period, and reaches its maximum at 275-300 msec; i.e. much after the moment of the last maximum of sarcoplasmic Ca^{++}.

From the measurements at 470 and 540 mμ with and without murexide treatment, it has been established that the observed responses are directly

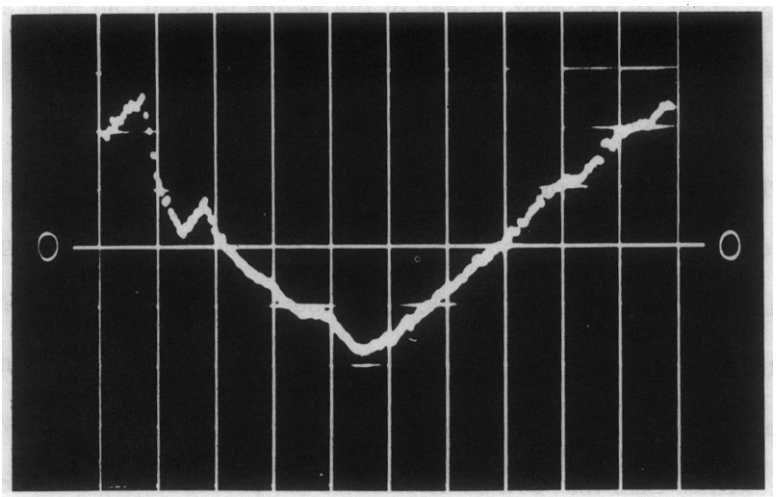

Fig. 3 Response to 4 stimuli at 20 cps. Time scale: 50 msec. No delay between onset of sweep and the stimulus. Temp: 12°C. Otherwise as Fig. 1b.

dependent on the murexide treatment. Furthermore the direction of the transmission effect is also in agreement with the formation and subsequent dissolution of a Ca-murexide complex. Since the endoplasmic reticulum probably contains a very large internal Ca^{++} concentration, changes in its level would hardly reflect on the total concentration and, if noticeable, would generate the opposite signal. Neither should any murexide adsorbed to the external surface of the muscle membrane show much of a change in Ca^{++} concentration. It is, therefore, concluded that the sarcoplasm proper is by far the most likely location of Ca-murexide formation in response to a stimulus.

Study of a number of experiments as depicted in Fig. 2 has made it clear that the time relations between the contraction and the Ca^{++} kinetics

are generally representative as they are shown in that figure. The exact occurrence of the peak showed some variability but always occurred at a time when about 25 per cent or less of the twitch tension had been achieved. Some effect of the temperature was apparent, but, as yet, no further analysis has been performed.

The exact onset of the first increase in Ca^{++} varies but little between experiments from perhaps 1 or 2 msec to a maximum of about 5. In general the initial rate is highest (c.f. Fig. 2) although a more gradual onset is noted at times (c.f. Fig. 1, top). If it be considered that excitation requires the inward spread of a disturbance originated at the membrane, a few msec is the maximum time that can be allotted to the process. An electrical process appears to be the most logical choice for the process which leads to the liberation of Ca^{++} in the sarcoplasm. This notion is in good agreement with current speculations and hypotheses (c.f. Sandow 4).

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