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External Calcium and Contractility in Single Giant Muscle Fibres

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Isometric tension and intracellular calcium movements detected using the calcium sensitive photoprotein aequorin have been studied on single striated fibres. The decrease of tension and light response upon deprivation of external calcium would support the notion that calcium ions are involved in the transmission of the electrical potential change from the tubular system to the sarcoplasmic reticulum, by occupying sites on the external (extracellular) surface of tubular system in direct correspondence with the terminal sacs of the sarcoplasmic reticulum.

Introduction. The state of activity of the contractile system in muscle fibres appears closely related to the myoplasmic calcium concentration, $[Ca^{2+}]_m$, (I,2) whose level is the result of a series of events which are initiated with the muscle action potential in the motor end plate (3). This electrical change spreads along the surface of the fibre, as well as towards its interior along the transverse tubular system (T-system) (4). The sarcoplasmic reticulum (SR) is then triggered by the electrical wavefront, in a way that is not yet entirely known, to release calcium which in its turn initiates contraction (5).

However, in certain preparations such as vertebrate cardiac muscle (6) and some invertebrate muscle (7) the level of contraction seems related to the external calcium concentration, $[Ca^{2+}]_o$. Whether this calcium acts upon entering the membrane of the fibre during depolarization, thereby modifying the $[Ca^{2+}]_m$, or is involved in some step of the cycle that leads to the release of calcium from the SR (8), or in some other way, has not yet been completely clarified. This work is therefore concerned with the relation between $[Ca^{2+}]_o$ and tension development on invertebrate muscle.

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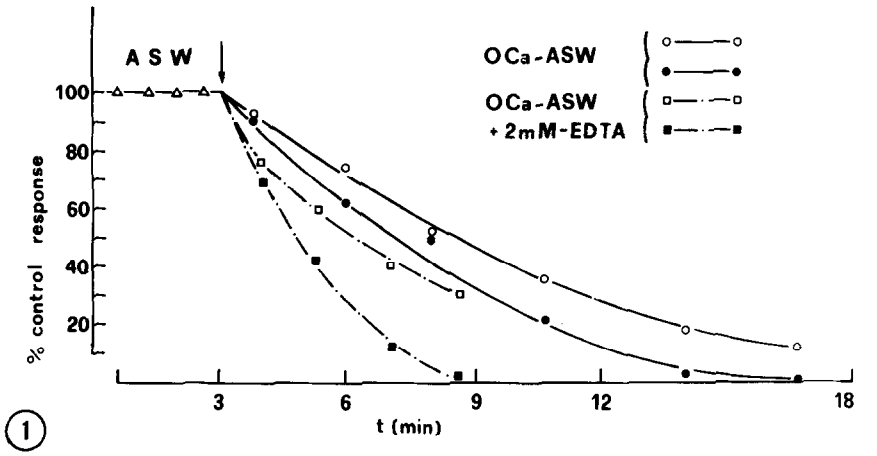
Materials and Methods. Single striated muscle fibres, usually I-2 mm in diameter and 3-4 cm in length from Balanus nubilus were used. The experimental apparatus, general procedure, and light recording were similar to those described by Ashley and Ridgway (2). The fibres were cannulated with a glass capillary (ca. 400 μm Ø) and resting potentials were determined with a KCl (0.5 M) probe (I00-I20 μm Ø). The mean values, ranging between 45-55 mV, were not corrected for liquid junction potential. The replaced salines did not significantly alter the resting potential of the fibre over the time course of the experiment. Tension in the range 0.5-I.0 $kgcm^{-2}$, (I0-20% Tmax), was recorded by connecting the tendon to the shaft of an RCA-5734 mechano-electric transducer by means of a lever system. The stimulating-recording electrode consisted of a glass capillary double-coiled platinum wires. 0.3 μl of the photoprotein aequorin (9) were injected axially with a microsyringe giving a final internal concentration of I-2 μM. Light emission was detected by an RCA 6342 photomultiplier whose output was coupled to the RCO. The artificial sea water (ASW) used contained (mM): NaCl 5I0.4, KCl I2.9, $MgCl_2$ 23.6, $CaCl_2$ II.8, $NaHCO_3$ 2.6 and 2mM-TES; pH was 7.25.

Results and Discussion. A decrease in $[Ca^{2+}]_o$ by replacing part or all of the $CaCl_2$ in ASW with an osmotically equivalent amount of NaCl resulted in a proportional decrease of isometric tension and light in response to a test stimulus, while the membrane potential time course was unaffected. As shown in Fig.I, responsiveness of the fibres was not maintained for longer than I5 min, the majority failing within I0-I5 min (I3 out of I7). When the 0Ca-ASW bathing solution contained 2 mM-EDTA the fibres became refractive in a shorter time (Fig.I). Fibres were kept no longer than 30 min in 0Ca-ASW since prolonged exposure causes irreversible damage (I0). As a control non aequorin-injected fibres were bathed for 20 min in 0Ca-ASW and subsequently tested with salines at different calcium concentration. Fig.2 shows such an experiment where, on reapplication of normal saline (I2Ca-ASW), the tension response recovered to at least 90% within 20 min. For $[Ca^{2+}]_o$ of 9, 6 and 3 mM the tension recovered to 50%, 20% and I0% respectively of the initial response. Contractile failure of striated frog muscle upon deprivation of external calcium has been reported and attributed to the parallel progressive depolarization of the fibre (II,I2). Caputo (I3), on frog single muscle fibre, observed a transient increase of the twitch response during a short exposure to 0Ca-Ringer, which he interpreted as a result of membrane depolarization and alteration of the action potential time course. Since depolarization of the fibre which occurs upon calcium withdrawal was found to be I, 2, 3, and 3 mV in four fibres, which is only ca. I/4 of the figure on skeletal muscle fibres (I3,I4), and no modification of the



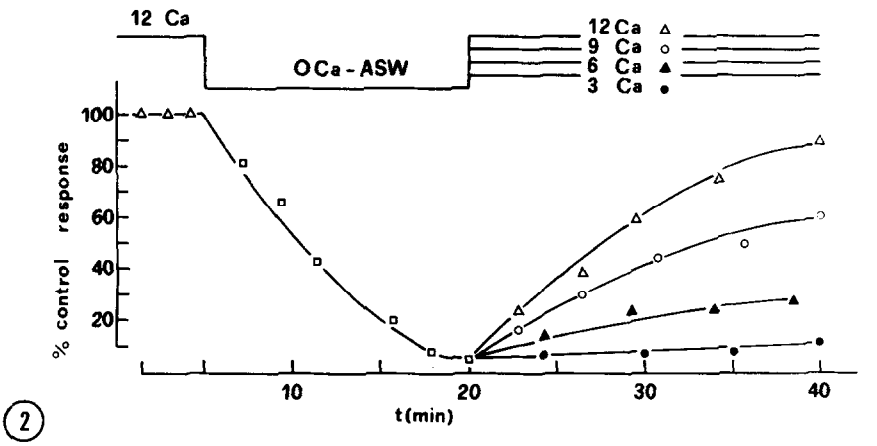


Figure 1. Tension (filled symbols) and light (open symbols) vs. time in response to constant electrical stimulation upon replacement (at the arrow) of ASW with either OCa-ASW (circles) or OCa-ASW + 2 mM-EDTA (squares) on an aequorin-injected single fibre. Tension and light were expressed as percentage of the value obtained at the beginning of the experiment in ASW.

Figure 2. Tension response to constant electrical stimulation as a function of time of recovery in different $[Ca^{+2}]_o$, after 15 min treatment of the fibre with OCa-ASW. Tension was expressed as percentage of control value.

membrane response time course was observed, another explanation for our results is needed.

The decline of responsiveness in OCa-ASW might be thought to depend on calcium ions diffusing down their concentration gradient i.e. from the SR into the myoplasm and hence to the cleft system and external solution. This would lead to a smaller light response accompanied by a decrease in tension as $[Ca^{2+}]_{SR}$ and $[Ca^{2+}]_m$ decline (Fig.I). Two experiments have been performed to test this line of reasoning. If fibres lose calcium and the final net decrease occurs on the SR and myoplasmic free calcium fraction,

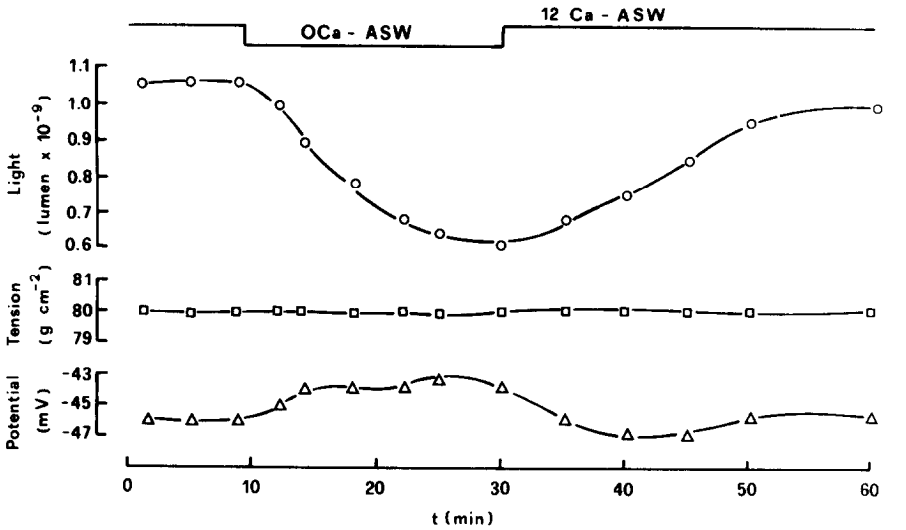


Figure 3. Light (triangles), tension (squares), and transmembrane potential (circles) time courses during OCa-ASW application and recovery recorded at rest.

resting light and, consequently, resting tension should decrease in the same way. Light, tension and membrane potential from a single muscle fibre were recorded at rest, as shown in Fig.3. After 20 min exposure to OCa-ASW, while resting light decreased by ca. 40%, tension remained constant (generally, it was not possible to detect any change in tension higher than 0.25% of the resting tension (I5), or 0.005% of P_o). The depolarization in this fibre was average. One possible explanation that could account for this result is that a decrease of I5% in $[Ca^{2+}]_m$ (as resting light in Fig.3 would suggest for a relation: light = $[Ca^{2+}]_m^{2.5}$ (I6)) would not induce the muscle to relax. It has in fact been shown that an increase of the same amount in $[Ca^{2+}]_m$ does not bring the fibre to the mechanical threshold (5).

In the second experiment caffeine was used, because of its properties as a calcium releasing agent (I7,I8), to assess the degree of depletion of the SR as a result of free calcium media soaking. Table I shows tension output and its rate of rise (time to reach plateau tension) in ASW and I2 min OCa-ASW bathed fibres in response to 5 mM-caffeine solution. These results which show differences of little significance for both parameters considered are in line with the observation that only a net loss of calcium equal to 0.02 mmol/kg-hr (on single muscle fibre whose initial calcium concentration is 0.9 mmol/kg) occurs after exposure to OCa-ASW (I9). It seems then unlikely that any great depletion of calcium from the SR has taken place. On the other hand, the fact

Table I. Isometric tension, and time to reach plateau tension in fibres bathed for I2 min in OCa-ASW on application of 5 mM-caffeine solution

	Isometric tension ($kgcm^{-2}$)	Time to plateau tension (sec)
ASW bathed fibres	''0.56 ± 0.09	''8 ± 2
Fibres bathed for I2 min in OCa-ASW	'0.49 ± 0.II	'II ± 4

Mean from four (') and five ('') single muscle fibres.

that the rate at which transient light and tension, and resting light decrease can be related to the rate at which calcium leaves the cleft system (20) is of great interest, and seems to role out the depletion of calcium from the SR as the major cause of the disappearance of responsiveness in muscle fibres bathed in OCa-ASW. An action of external calcium either as a direct activator of the contractile system, or as being involved in some step of the excitation-contraction coupling might then be considered. The first proposition appears strongly affected by the results of Caputo and DiPolo using the voltage-clamp technique (2I), and Ashley et al. experimenting on bundles of myofibrils (22). Both papers demonstrate that the calcium which triggers the contractile system comes from intracellular stores, namely the SR.(The results from Table I can also be taken to prove this point.) In addition, the evidence that calcium ion displacement from the transverse tubular elements due to hypertonic solutions results in a loss of mechanical response is in accord with the present results, and would support the notion that calcium ions might be involved in the transmission of membrane potential changes from the T-system to the SR, by occupying sites on the external surface of the cleft system in direct correspondence with the terminal sacs of the SR. This would also explain the disappearence of potassium contracture in fibres bathed for I5 min in OCa-ASW, and its restoration on reapplication of ASW (24), assuming that the events leading to the mechanical response during a membrane depolarization induced electrophysiologically or a potassium depolarization are the same.

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