

Calcium Movements in Muscle

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The movements of calcium in muscle have been followed during contraction and contracture to test the hypothesis that the release of calcium from the surface of the muscle membrane during stimulation initiates the contractile mechanism. Nitrate ion increases the calcium influx during a single twitch and during potassium contracture, and also increases the tension developed. The increased entry of calcium during a potassium contracture is transient and not sustained as is the contracture. Caffeine, which brings about a contracture without depolarization of the membrane and despite the absence of calcium from the medium, causes calcium to be released from the muscle.

THE PRESENT DISCUSSION of calcium movements in muscle will deal with frog sartorius muscle. In the next paper, Dr. Winegrad will consider recent findings on heart muscle.

Calcium has long been proposed as the link between membrane depolarization and contraction.¹⁻⁴ Of all the physiologic ions that have been injected in small quantities, calcium alone causes contraction.^{5, 6} Sandow, in 1952,² made a detailed correlation of the kinetics of the sequence of excitatory and mechanical events (fig. 1*). At 13 C. the rise time of the spike potential is 0.6 msec.; the time from the peak of the spike potential to the onset of latency relaxation is 1.2 msec.; and it is during these time intervals that the muscle is mechanically quiescent. The time interval until the earliest sign of development of tension, the inflexion point of latency relaxation, is 3.5 msec.; and the total time until the onset of tension above the initial tension is 5.4 msec. At 25 C. the corresponding time intervals are approximately 0.2 msec., and 2.5 msec. respectively. It is during the mechanically quiescent period that 2 processes are occurring: (1) membrane depolarization, and (2) an intervening process between the peak of the action potential and the beginning of latency relaxation termed the "spike activation" link.² The size of the spike potential appears to be unrelated to twitch tension for anions that potentiate the twitch,

e.g., Br, NO₃, I, SCN, CH₃SO₄ have been shown to have little effect on the spike.^{3, 7, 8} Hodgkin and Horowicz⁹ have found agreement between the threshold of depolarization necessary for mechanical activity during K-contracture and the degree of depolarization necessary to initiate the spike potential, suggesting that it is the lowering of the membrane potential to a critical level, rather than the height of the spike potential, that is concerned in excitation-contraction coupling.

Under physiologic conditions, the conducted action potential of frog sartorius muscle initiates a process whereby contraction occurs. Membrane depolarization itself is not the most intimate link in the process, for contraction can be brought about without depolarization of the muscle membrane. Thus, caffeine can cause a contracture without any change occurring in the resting membrane potential.¹⁰ Potassium-induced contracture can fail to occur, even though the muscle membrane is depolarized, if external calcium is removed.¹¹ Csapo has shown that treatment of the turtle retractor penis muscle with NaI can bring about greater tension development with smaller membrane depolarization.¹² Hodgkin and Horowicz have demonstrated that replacement of Cl with NO₃ can lower the threshold of membrane depolarization necessary to bring about a contraction.¹³ All of these findings point to another intervening process between membrane depolarization and the initiation of mechanical activity.

Studies on calcium movements in muscle

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*Figure 1 from Sandow: Yale J. Biol. & Med. 25: 176, 1952.² By permission of the journal.

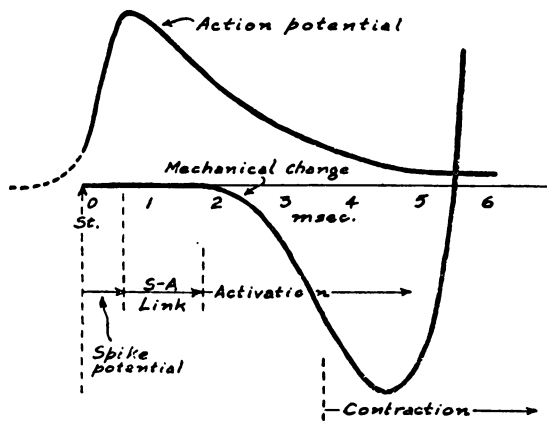


Figure 1

Temporal correlation of excitation and mechanical events during latent period of frog sartorius muscle at 13 C according to Sandow. (From Sandow.²)

conducted by Shanes and Bianchi provide evidence that an increase in calcium influx during depolarization by an action potential, or by an increase in extracellular K, is part of the intermediate process between events in the muscle membrane and mechanical activity of the contractile proteins. Table 1 shows that calcium influx in unstimulated muscle is 0.094 micromicromoles/cm.²sec. Stimulation of the muscle causes additional calcium to enter the muscle, which amounts to 0.2 micromicromoles/cm.² twitch. If one assumes that calcium enters the muscle fibers at a high rate immediately upon depolarization and continues to enter during the period of mechanical quiescence (1.0 msec. at 25 C.), then the resting influx rate of calcium would have increased from 0.1 micromicromoles/cm.² sec. to 200 micromicromole/cm.² sec., an increase of approximately 2,000. If the calcium were considered to enter during the period from membrane depolarization to the appearance of initial tension development (2.5 msec.), then the increase would still be 800-fold.

The amount of calcium entering per twitch and the twitch height is increased 60 per cent by replacing the chloride of Ringer's solution with nitrate. Under these conditions, nitrate has no effect on the unstimulated influx of calcium, showing that nitrate affects only the

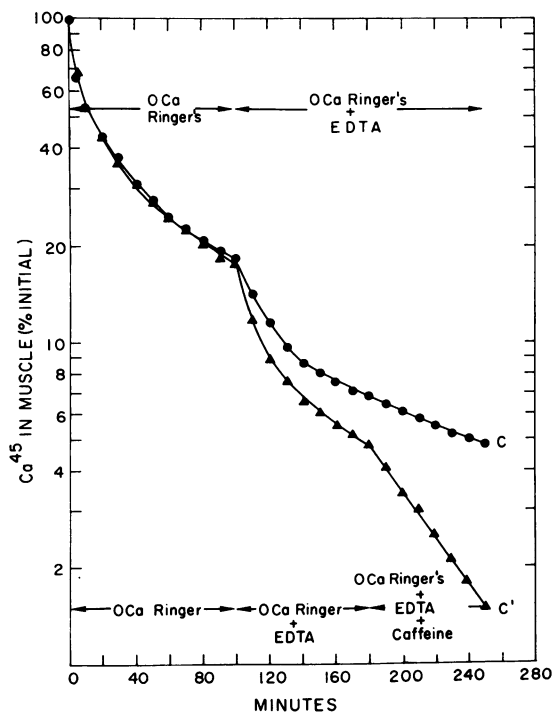


Figure 2

The effect of edathamil (EDTA) and caffeine on the washout of Ca^{45} from a muscle previously soaked for 5 hours in Ca^{45} Ringer's solution. At 100 minutes 0.004 M EDTA is added to the medium, bathing both C and C'. An immediate increase in Ca^{45} release occurs, which tapers off after 140 minutes. At 180 minutes, 0.005 M caffeine is added to muscle C', causing a sustained increase in the release of Ca^{45} from the muscle.

calcium entering during a twitch. Nitrate has been shown to prolong the active state,^{14, 15, 16} which would account for the increased twitch height that is observed in nitrate Ringer's solution. A transitory increase in the ionized calcium level of the muscle fibers, brought about by the increased amount of calcium entering per twitch, could account for the prolongation of the active state. In keeping with this hypothesis, superficial muscle-fiber sites have been shown to bind about 0.1 micromole/Gm. of calcium in nitrate Ringer's solution.¹⁷ The increased binding of calcium and the increased influx during stimulation are in agreement with the suggestion of Shanes⁴ that the enhancement of the twitch height when other halogens or nitrate replaces chloride may be due to an improved binding of

Table 1
Calcium Influx in Frog Sartorius Muscle

Conditions	Influx
Unstimulated	
Ringer's solution	0.094 $\mu\text{mole/cm.}^2$ sec.
Ringer's nitrate solution	0.108 $\mu\text{mole/cm.}^2$ sec.
Stimulated	
Ringer's solution	0.20 $\mu\text{mole/cm.}^2$ twitch
Ringer's nitrate solution	0.32 $\mu\text{mole/cm.}^2$ twitch
Ringer's solution + 20 mM KCl	0.124 $\mu\text{mole/cm.}^2$ sec.
Ringer's solution + 80 mM KCl (after relaxation)	0.056 $\mu\text{mole/cm.}^2$ sec.
Ringer's solution + 80 mM KCl (initial depolarization)	38 $\mu\text{mole/cm.}^2$
Ringer's nitrate solution + 80 mM KNO ₃	60 $\mu\text{mole/cm.}^2$

calcium to the membrane, which in turn contributes to enhanced entry of calcium during stimulation.

Depolarization of the sartorius muscle by 20 mM K, a level just below the threshold for contracture, leads to a sustained increase in calcium influx (table 1). Calcium influx measured in the presence of 80 mM K, after relaxation from the induced contracture, is smaller than calcium influx in 20 mM K, and almost equal to influx in unstimulated muscle. The entry during initial depolarization in 80 mM K amounts to 38 micromicromole/cm.².¹⁸ The presence of nitrate increases the amount to 60 micromicromole/cm.², which is in keeping with the potentiated contracture that is observed.¹⁹ The large transient increase in calcium influx during initial depolarization is consistent with the rapid increase in tension during the first second of KCl contracture observed by Hodgkin and Horowitz.⁹ The relaxation of the phasic muscle during maintained KCl depolarization can be interpreted as a failure to sustain the high rate of calcium influx. Shanes²⁰ has shown that a high rate of calcium entry persists in the slow fibers of the frog rectus abdominis, along with the sustained potassium contracture. Thus, in fast fibers both the increased influx of calcium and the contracture are transient during K depolarization, whereas both are sustained in slow fibers during K depolarization.

Contracture brought about by caffeine has important differences from potassium-induced contracture. Potassium contracture is not sustained and is associated with a transitory increase in calcium influx during the initial

membrane depolarization. Removal of external calcium prevents potassium contracture.¹¹ In contrast, caffeine causes a sustained contracture in frog sartorius without membrane depolarization and in the complete absence of external calcium. The site of caffeine action is on the membrane. Axelsson and Thesleff¹⁰ have shown that only caffeine applied externally to the membrane results in a contracture, while caffeine applied by injection to the muscle interior is without effect. It has also been shown that caffeine markedly increases calcium outflux and influx.¹⁸ Figure 2 shows that even after prior treatment of frog sartorius muscle with edathamil (EDTA), which can remove some superficial, bound calcium as well as calcium in solution, caffeine causes a marked increase in calcium outflux, suggesting that caffeine can bring about the release of calcium from membrane sites and perhaps sarcoplasmic reticulum sites, which in turn results in contracture. The increased calcium outflux may therefore reflect the freeing of bound calcium, which would raise the intracellular calcium ion content and thus induce contracture without the necessity of external calcium or a membrane depolarization.

The release of calcium during stimulation has been observed by Woodward²¹ and confirmed by Shanes and Bianchi.²² Figure 3 clearly demonstrates the release of calcium during tetanic stimulation. Potassium contractures, both isotonic and isometric, increase calcium outflux (fig. 4). The increased outflux during tetanic stimulation is not sustained and the minimum calcium released per twitch is about the same as the amount taken

up per twitch: viz., 0.2 micromicromole/cm.²,²²

Potassium contracture results in a rapid release of calcium, which is at about double the base line rate even after 10 minutes (fig. 4). The increased influx and outflux of calcium in frog sartorius observed with tetanic stimulation or potassium contracture may reflect the same basic process, such as freeing of calcium from the surface, supported by the rapid release of calcium during tetanic stimulation. Two other possible explanations for the increased influx and outflux are: (1) a spatial separation in which different sites of the membrane involve calcium influx and outflux, and (2) a temporal separation of the two fluxes.

From the foregoing, it is evident that calcium influx into the muscle fiber is related to mechanical activity in 2 ways. The enhanced twitch height and contracture in nitrate Ringer's solution is correlated with a larger influx of calcium, and there is a temporal relationship between the duration of increased calcium influx and of mechanical activity. Potassium contractures and a high rate of calcium influx are transitory in phasic muscles, while in slow fibers potassium contractures are sustained as is the high rate of calcium influx. The manner in which calcium brings about activation of the contractile mechanism is still unknown, although from experiments on model systems there appear to be 2 possible modes of action. One would be the inhibition of the relaxing factor system, thus allowing contraction to take place, with relaxation occurring as the ionized calcium is removed; the other would be by a direct action of calcium on actomyosin. Weber²³ has shown that calcium in a concentration of 10⁻⁴ M, which would be equivalent to 5 × 10⁻⁵ M ionized calcium, gives a maximum activation of the highly purified actomyosin ATPase system and also maximum superprecipitation of actomyosin with 2 mM Mg ATP. In the absence of added calcium, no superprecipitation could be measured, and the ATPase activity was reduced to 20 per cent of the maximum activity. There is, however, a large discrepancy between the

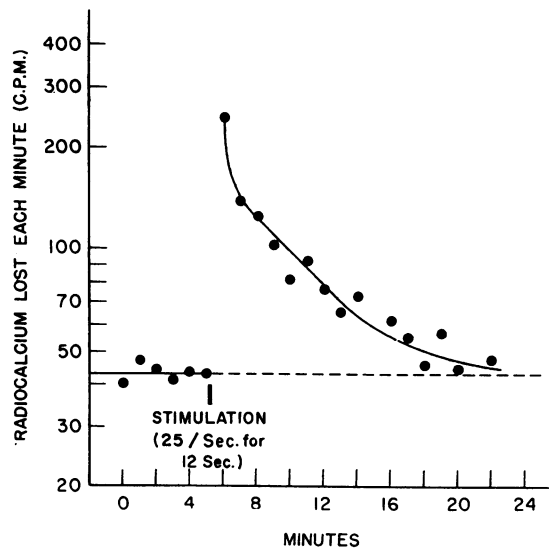


Figure 3

The release of Ca⁴⁵ during a brief tetanic stimulation from frog sartorius muscle that has been previously soaked in Ca⁴⁵ Ringer's solution. Sample collections of the medium bathing the muscle were made at minute intervals in order to obtain the true time course of calcium release.

amount of calcium needed for either inhibition of the relaxing factor system or activation of the actomyosin ATPase system. If the calcium entering per twitch (0.2 micromicromole/cm.²) were uniformly distributed in the muscle fiber water, then the final concentration of ionized calcium would be 10⁻⁷ M, which is too small a concentration by a factor of 100 for both the relaxing factor system and the actomyosin ATPase. The discrepancy is still larger when the number of calcium ions that enter in relation to the actomyosin concentration of muscle is considered. If one estimates 100 mg. of actomyosin for every gram wet weight of muscle and a molecular weight of 500,000, then the actomyosin concentration of frog muscle would be approximately 2 × 10⁻⁴ M, as compared to 10⁻⁷ M for calcium. Much more ionized calcium would be needed than can be accounted for by the calcium entering per twitch, suggesting perhaps that the initial entry of calcium from the membrane can bring about a further release of calcium from binding sites localized in the sarcoplasmic reticulum.

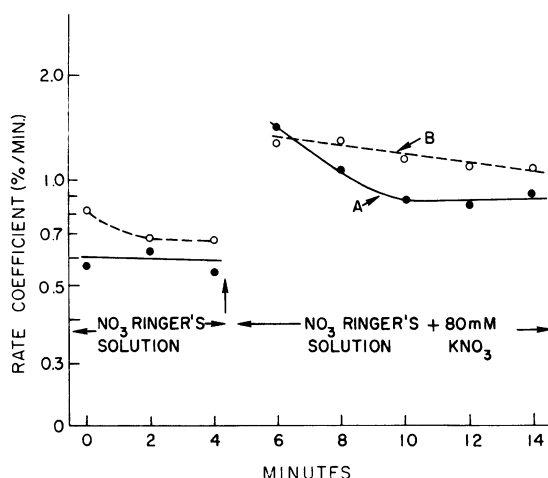


Figure 4

Comparison of the time course of calcium release during isotonic (A) and isometric (B) contraction. The calcium released is plotted as a rate coefficient, i.e., the percentage of the exchangeable calcium in the muscle being released during the 2-minute collection intervals. The contracture occurs when 80 mM KNO_3 is added to the NO_3 Ringer's solution.

Thus, calcium influx is markedly increased during a muscle twitch and potassium contracture in frog sartorius muscle. Nitrate ion, which potentiates both the twitch and potassium contracture, also increases the entry of calcium under these conditions. The caffeine-induced contracture may be accounted for by an increase in the ionized calcium level in the muscle fiber brought about by a direct action of caffeine on calcium-binding sites in the membrane and perhaps those located in the sarcoplasmic reticulum.

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