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COMMENTARY

THE CURRENT VIEW OF THE SOURCE OF TRIGGER CALCIUM IN EXCITATION–CONTRACTION COUPLING IN VERTEBRATE SKELETAL MUSCLE

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The idea that calcium ions play an important role in excitation–contraction (e–c) coupling is not new. In 1883, Ringer [1] demonstrated that the frog heart fails to contract and remains relaxed when calcium ions are absent from its perfusion fluid. By about 15 years later it had been shown that the depolarization of the muscle fibre membrane is the electrical event responsible for initiating the mechanical response [2]. About 30 years after Ringer's work it was shown that when the frog heart is relaxed by perfusion with a calcium-free (0-Ca^{2+}) solution the rhythmic, spontaneous cardiac action potentials are still present in an only slightly modified form [3].

In 1947, Heilbrunn and Wiercinski [4] demonstrated that calcium was the only physiologically occurring cation which would cause shortening when injected in low concentrations into bits of skeletal muscle fibres. This finding was later confirmed under more physiological conditions [5]. One obvious explanation of these findings was that the action potential or membrane depolarization permits or promotes the entrance of calcium ions from the surface to the interior of the muscle fibre and that these ions then initiate the mechanical response. A working hypothesis of this type was proposed in 1952 by Sandow [6].

In 1930 in a review on skeletal muscle physiology, Gasser [7] made the important distinction between contractions initiated by action potentials, and muscle shortening or tension produced by other means. The latter he called contractures to distinguish them from the twitches or tetani called contractions. In studying e–c coupling, we are primarily concerned with determining the mechanism linking the action potential with the mechanical response of the muscle, i.e. contractions. As will be discussed below, the careful and cautious use of contractures has been useful in these investigations. On the other hand, the indiscriminate use of contractures or the unthinking use of experimental conditions liable to produce contractures instead of or in addition to contractions has led to much confusion and has hindered progress in this area.

Development of the trigger calcium hypothesis for e–c coupling

Since the conclusion in the classic study of A. V. Hill [8] that there was not sufficient time for calcium ions entering at the surface of a skeletal muscle to initiate the twitch virtually eliminated for a time any further consideration of a role for calcium in e–c coupling, the modern studies on this subject can be considered to have begun about 20 years ago with the investigations of Bianchi and Shanes [9] and Frank [10, 11]. These studies which reopened the subject were stimulated by the demonstration by Hodgkin and Keynes [12] that an influx of calcium ions accompanies each action potential in the squid giant axon.

Bianchi and Shanes [9], using ^{45}Ca, demonstrated that there was also an influx of calcium ions accompanying action potentials and potassium depolarizations which produce twitches and contractures, respectively, in frog twitch fibres. They also showed that the replacement of chloride by nitrate anions, which increases the twitch tension, also increased the calcium influx per twitch.

In my initial studies, potassium-induced contractures of frog toe muscles were used as a model for the events occurring during e–c coupling and the sodium ions in the bathing solutions were replaced by choline ions since it was known that sodium ions were not required for coupling.* This model was employed to avoid the complicating effects produced by changes in extracellular calcium concentration on excitability. Using this model it was shown that: (1) there was a rapid and complete elimination of the mechanical response in a 0-Ca^{2+} solution [10, 11]; (2) diffusion of calcium ions out of the extracellular spaces of the toe muscle is the rate-limiting step in the elimination of the mechanical response [11]; (3) in the absence of extracellular calcium ions the potassium-induced depolarization is not reduced and the resting membrane potential is only slightly reduced or unchanged at the instant when the mechanical response is completely eliminated [10, 14]; (4) the size of the potassium-induced contracture can be partly reduced by a partial reduction

* It has been suggested in a recent voltage-clamp study [13] that this is not so and that replacement of sodium by lithium ions reduces the strength of depolarization-induced contractures. However, in all previous studies in this area the tensions of potassium-induced contractures are unreduced by the replacement of sodium ions by choline or other monovalent cations unless the membrane potential is greatly altered. Also twitches, even in isolated fibres, are only potentiated slightly by the use of lithium in place of sodium ions in the bathing solution until sufficient lithium ions have entered the cell to depolarize the membrane and make the cell inexcitable (unpublished results).

in the extracellular calcium concentration [11, 15]; and (5) unmodified caffeine-induced contractures can be obtained in muscles kept in a 0-Ca^{2+} solution for a length of time sufficient to eliminate completely the potassium-induced contracture [11]. Since caffeine is able to induce contractures by directly releasing calcium ions from intracellular stores [16, 17], this is a useful means of showing that removal of extracellular calcium ions does not directly depress the intracellular contractile apparatus but rather modifies e–c coupling.

Initially in these studies it was thought that the extracellular calcium ions entering during the action potential served to initiate the mechanical response and, while this may well be the case in some other muscle types and in some invertebrate striated muscles [18], it soon became obvious that this was not the case in vertebrate skeletal muscle. Thus, it was found that the replacement of chloride ions by bromide ions, which potentiated twitches and potassium-induced contractures by an effect at the muscle surface membrane, increased the time required for the loss of the potassium contracture response in a 0-Ca^{2+} solution [15]. It seemed highly unlikely that the bromide ion produces this effect by decreasing the rate of calcium diffusion out of the extracellular spaces of the muscle. Next it was found that several divalent and some trivalent cations and low, subcontracture concentrations of caffeine could restore potassium contractures previously eliminated in a 0-Ca^{2+} solution [17]. However, after prolonged exposure to the 0-Ca^{2+} solution, these ions no longer supported contracture production. However, the latter was restored by reexposing the muscles to calcium ions. This naturally led to a consideration of the role of cellularly 'bound' calcium ions in e–c coupling.

When calculations using the Ca^{2+} influx data of Bianchi and Shanes [9] indicated that insufficient extracellular Ca^{2+} entered per twitch to activate fully the contractile proteins, it was proposed that the Ca^{2+} entering the sarcoplasm during the membrane depolarization serve to produce some other intracellular event which eventually leads to mechanical activation [19]. Since there is evidence that a rise in the sarcoplasmic Ca^{2+} concentration initiates a mechanical response and that most of this Ca^{2+} comes from the calcium stored in the sarcoplasmic reticulum [20], presumably the trigger calcium which enters from the surface serves to stimulate the release of calcium from the sarcoplasmic reticulum. A more detailed and elaborate form of this trigger calcium type of mechanism was later proposed by Bianchi [21, 22]. My most recent model for this type of mechanism [23] will be presented and discussed below.

Subsequent developments in this area

In the 15 years or so that have passed since the initial development of the trigger calcium hypothesis, a large number of studies have been reported in the area of e–c coupling. Unfortunately, it is beyond the scope of this commentary to review all of this work; however, there have been some important findings that do require mention and consideration here.

Although Hill's early objection to a role for calcium in e–c coupling [8] could be set aside by the findings indicating that there was an anatomical system for carrying the electrical information from the outer surface of the muscle fiber to its center [24, 25], the indispensable role played by this system, the t-tubular system, in e–c coupling in vertebrate twitch fibres was only revealed by the results obtained using the glycerol exposure–removal technique [26–29]. Following such a treatment which closes off the surface openings of the t-tubules, action potentials and caffeine contractures still could be produced but twitches and potassium-induced contractures could not. In addition to showing the essential role of the t-tubules, these experimental results also demonstrated the fundamental similarity and the identical site of action of e–c coupling produced by action potentials and potassium depolarizations.

Using skinned muscle fibres in which some of the sarcolemma has been removed, it has been shown more recently that calcium ions can cause a large, rapid release of calcium from the sarcoplasmic reticulum and thereby produce a mechanical response [30–33]. Although the physiological role played by this mechanism has been questioned [34], more recent studies have tended to support a physiological role for this process [35, 36]. Considering the unphysiological status of such preparations, a dispute of this nature is not surprising. Nevertheless the demonstration that calcium ions can induce the release of greater amounts of calcium ions from the sarcoplasmic reticular calcium stores provided an essential piece of evidence supporting the trigger calcium hypothesis. The existence of this release process was earlier predicted by proponents of the trigger calcium hypothesis and, in fact, is required for this mechanism to work.

As mentioned above, several divalent and some trivalent cations at a concentration of 1 mM support the development of potassium-induced contractures in a 0-Ca^{2+} solution [17]. When later it was found in many cells and tissues that higher Mn^{2+} concentrations inhibited inward Ca^{2+} movements, the effects of these higher Mn^{2+} concentrations on e–c coupling were investigated in vertebrate skeletal muscles [37–39]. It was found that 10 mM Mn^{2+} greatly depressed the twitch within 8 sec in isolated fibres [37] and completely blocked the twitch in 2–3 min in whole sartorius muscles [38, 39]. The action potentials were little affected by 10 mM Mn^{2+}. Potassium-induced contractures were more resistant to this block [37, 39], and 15–20 mM Mn^{2+} was required to produce a complete block [38]. Caffeine contractures still occurred in the presence of 10 mM Mn^{2+}.

Similar effects have been observed with La^{3+} another cation known to interfere with calcium movements at cell membranes. At a concentration lower than the cations mentioned above, La^{3+} (0.1 mM) could maintain the production of potassium-induced contractures in a 0-Ca^{2+} solution [40]. Higher concentrations (up to 1 mM) inhibited potassium-induced contractures in the presence of calcium ions [40–42] but not caffeine contractures. La^{3+} (1 mM) also reduced the twitch amplitude without reducing the size of the action potential [41]. Thus, by the use of these cations which interfere with calcium movements at cell membranes, e–c coupling could be greatly inhibited or blocked, without reduc-

ing the action potential, blocking the internal contractile apparatus, or modifying the structure of the triad (i.e. the t-tubule with the two associated terminal cisternae of the sarcoplasmic reticulum) [39].

The final topic to be considered in this section is the recent findings suggesting an irreducible latent period greater than 1.0 msec between the start of the muscle action potential and the start of the release of calcium from the sarcoplasmic reticulum. This minimum latent period has been found in two different lines of research: (1) studies on the mechanism for latency relaxation (the small decrease in tension which precedes the twitch following a stimulus), and (2) optical studies of e–c coupling in skeletal muscles.

In 1966, Sandow [43] proposed that latency-relaxation (LR) was caused by the physical effects accompanying the release of calcium from the sarcoplasmic reticulum. Subsequently, Mulieri [44] and Gilai and Kirsch [45] have confirmed that LR is concurrent with and caused by the release of Ca^{2+} from the sarcoplasmic reticulum. There was a minimum latent period before the start of LR of 1.2 msec in sartorius muscle fibres and 2.2 msec in semitendinosus muscle fibres. Under the conditions of these experiments using a mass stimulation technique, the electrotonic potentials should have reached a maximum by about 0.2–0.3 msec and be gone before or just at the start of LR. Thus, there is a gap of 1–2 msec between the maximum of the electrical potential and the start of the calcium release from the sarcoplasmic reticulum.

In both optical birefringence studies and fluorescence studies using dyes, a signal has been found which corresponds in time to LR and these signals have been interpreted as indicating a change in the membranes of the sarcoplasmic reticulum corresponding to the calcium release process [46]. This interpretation was challenged recently by the finding that the birefringence signal was suppressed by the intracellular injection of EGTA* (a calcium chelator) into frog skeletal muscle fibres [47]. This was interpreted to suggest that the birefringence signal was directly produced by the increase in sarcoplasmic free calcium levels rather than by a sarcoplasmic reticular membrane change associated with the calcium release. However, there is another interpretation of the latter findings which is more consistent with the results in other optical studies, in LR studies and with the trigger calcium mechanism. This is that the intracellular EGTA chelates the small amount of trigger calcium released from the t-tubular membrane into the triadic junction thereby preventing these ions from reaching the terminal cisternae of the sarcoplasmic reticulum and, thus, the calcium release process is not initiated and no birefringence signal is produced.

This minimum latent period of about 1–2 msec between the maximum of the electrical potential and the start of the calcium release from the sarcoplasmic reticulum cannot be explained by a mechanism depending upon the t-tubular electric currents or potentials to initiate directly and maintain the release of calcium from the sarcoplasmic reticulum. The most reasonable explanation for this delay is that it is the time required for a substance released from the membrane of the t-tubule to diffuse across the triadic junction and to act upon the membranes of the sarcoplasmic reticulum. The situation may be viewed as similar to that of synaptic transmission in which there is a delay of about 1 msec when a chemical transmitter is released from the pre-synaptic ending and has to diffuse across the synaptic junctional space to act on the post-synaptic cell but there is no delay in the start of the post-synaptic potential change when the junction is electrically coupled.

Block of e–c coupling by removal of extracellular calcium and some experimental problems

As already mentioned, the demonstration that bathing a skeletal muscle in a 0-Ca^{2+} solution will eliminate e–c coupling before sufficient intracellular calcium is removed to block a mechanical response is one of the main experimental supports for the trigger calcium hypothesis. When using whole muscles with small cross-sections, such as the frog toe muscle, the loss of the potassium-contracture was determined by the rate at which calcium ions diffused out of the extracellular spaces of the muscle [11]. However, when isolated fibres are used and the effect of calcium removal on the twitch is investigated, the loss of the twitch, although rapid (usually 2–5 min) [48–51], is considerably longer than would be expected for the time required for calcium to diffuse out of the t-tubules.

These findings make it clear that, although under ordinary circumstances extracellular calcium ions are required to maintain the surface membrane store of trigger calcium ions, the extracellular calcium ions do not directly serve as the trigger calcium ions. For example, even in large (i.e. 100 μm diameter) single fibres it can be calculated that the calcium concentration in the t-tubules should be essentially zero in less than 1 min in a 0-Ca^{2+} solution (M. N. Oguztoreli, personal communication). Thus, the twitch response can continue for several minutes in the absence of extracellular calcium ions in the t-tubules and only disappears when the store of trigger calcium is lost, presumably by diffusion into the external 0-Ca^{2+} solution.

Even more dramatic separations between the loss of extracellular and the loss of trigger calcium ions can be obtained by pharmacological means. The most obvious way is by the addition of appropriate concentrations of many divalent or trivalent cations, which inhibit the removal of trigger calcium, to the 0-Ca^{2+} solution. This effect, already discussed in relation to potassium-induced contractures, also occurs with contractions [23, 52, 53].

Ordinarily the inhibitory effects of multivalent cations on trigger calcium removal should present no difficulty. However, an effect of this type sometimes subtly complicates studies in this area. This is due to Mg^{2+} which, in mammalian skeletal muscle as in many other tissues, antagonizes Ca^{2+}-mediated effects [54] but in frog skeletal muscle supports mechanical responses for a considerable time in a 0-Ca^{2+} solution [17]. Mg^{2+} is not required in Ringer's solution, and isolated frog skeletal muscle can function for 8 hr or more without this ion. Thus, it usually

* EGTA,ethyleneglycolbis(aminoethylether)tetra-acetate.

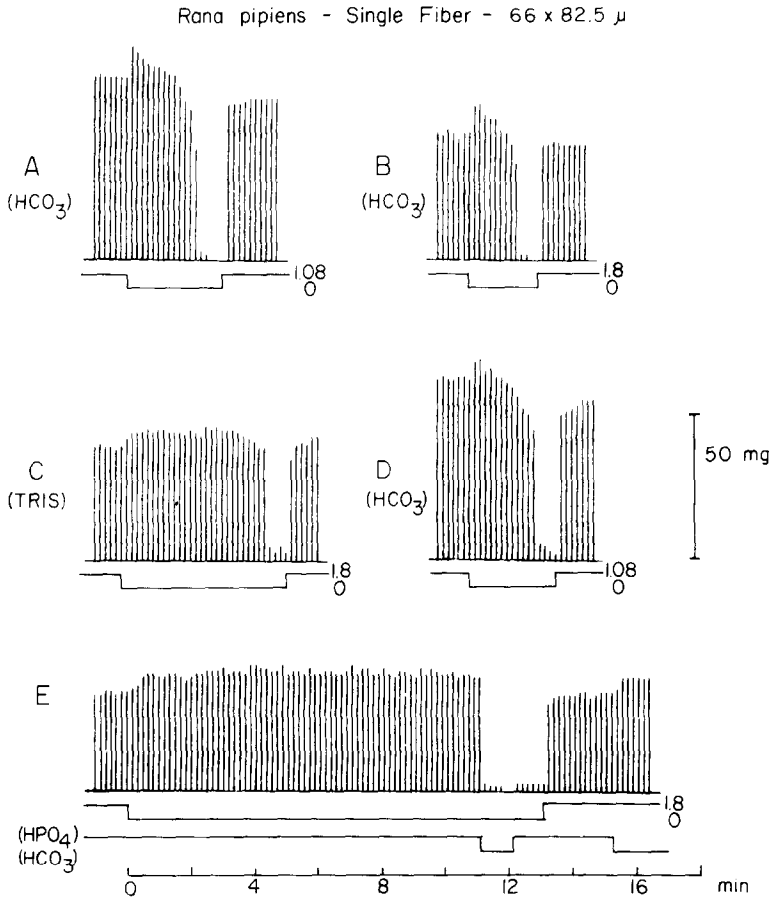

Fig. 1. Elimination of twitch in isolated frog semitendinosus muscle fibre by 0-Ca^{2+} solutions, and antagonism of this effect by Tris or phosphate buffered solutions. The Ca^{2+} concentration (in mM) in the solution bathing the fibre is indicated by lines immediately below each record. Changes in buffer are indicated by a second line below the line for Ca^{2+} in E. Fibre in the solution is indicated at the start of each test for at least 10 min before the start of records. Tests were conducted in the order shown: B, 20 min after A; C, 29 min after B; D, 28 min after C; and E, 28 min after D. With the exception of E, only one type of buffered solution was used for each test. See text for further details. (From Ref. 50.)

is not included in Ringer's solution even though frog plasma does contain 1.2 mM Mg^{2+} [55]. However, some investigators have included Mg^{2+} in their Ringer's solution [56–61]. No doubt it is of benefit to the homeostasis of the frog to have a substance in the plasma which prevents or delays the depletion of trigger calcium ions, and probably there are other, as yet unrecognized substances with such a function in the plasma of all vertebrates. Nevertheless, the addition of Mg^{2+} to frog's Ringer's, by interfering with the removal of trigger calcium ions greatly complicates the interpretation of the results obtained in studies on e–c coupling with frog and probably other amphibian muscles.

The loss of trigger calcium into a 0-Ca^{2+} solution also can be greatly delayed by the use of buffers other than a bicarbonate buffer in Ringer's [50, 51]. The effects of a phosphate and a Tris buffer are illustrated in Fig. 1. This effect was variable, and twitch elimination in isolated fibres in a 0-Ca^{2+} phosphate buffered solution takes from 4.5 to > 35 min [48, 50, 51, 62] but always longer than in a 0-Ca^{2+}, bicarbonate buffered Ringer's solution when compared on the same fibre. The use of Tris instead of bicarbonate also delayed the twitch loss but its effect was not as great as phosphate and the twitch was usually gone in 0-Ca^{2+}, Tris-buffered Ringer's by 8 min [49–51]. Although they have yet to be carefully investigated, it would seem likely that the more exotic buffers occasionally employed (e.g. imidazole, HEPES, PIPES, etc.)* might well have a similar effect on e–c coupling.

It has been mentioned above that replacing Cl^- by Br^- slows down the loss of trigger calcium ions into a 0-Ca^{2+} solution. Presumably a similar effect would be produced by other lyotropic anions. In addition, several organic anions modify e–c coupling, probably by an action on surface membrane bound Ca^{2+} [63]. Anions, both organic and inorganic, are often indiscriminately substituted for Cl^- in Ringer's or in other physiological solutions without any serious consideration of their possible effects on e–c

* HEPES, 4-(2-hydropyethyl)-1-piperazine-ethanesulfonic acid; and PIPES, 1,4-piperazinebis-ethanesulfonic acid.

coupling or their other pharmacological effects. A frequent offender is methylsulfate which has been shown to modify e–c coupling [64, 65].

Another frequently unrecognized problem is that, unless a neuromuscular blocking agent is used, the acetylcholine released from intramuscular nerve endings by electric stimulation or potassium depolarization will interfere with the loss of trigger calcium ions into 0-Ca^{2+} solutions. In potassium contracture experiments this can induce repetitive twitching [66], and it takes longer to eliminate the twitch response in a 0-Ca^{2+} solution than the potassium contracture (unpublished results). More importantly, acetylcholine, like the divalent cations, inhibits the loss of trigger calcium ions into a 0-Ca^{2+} solution [67].

Many other chemicals and drugs interfere with trigger calcium movements and effects. In contrast to the few specific problems mentioned in this section, when these other chemicals are used it is usually recognized that they are producing a pharmacological effect which requires consideration and interpretation. It is beyond the scope of this commentary to go into this large area of the pharmacology of e–c coupling.

Finally, another technical problem which can produce conflicting and confusing results is the use of excessive stimulating currents. Such currents can produce a twitch-like response even when the action potential is blocked by tetrodotoxin or by $choline^+$ replacement of Na^+ in the bathing solution [23]. The mechanism for the production of this response is of necessity different from the normal e–c coupling process and it may be similar to the one responsible for the mechanical responses produced in skinned fibres by strong longitudinal currents [68]. These twitch-like responses are resistant to rapid elimination by removal of extracellular Ca^{2+} and possibly might be due to the direct release of Ca^{2+} from the sarcoplasmic reticulum [69] without the intervention of the normal e–c coupling process.

Alternative mechanisms

Although it is not the purpose of this commentary to analyze alternative hypothetical coupling mechanisms, consideration should be given to the reasons leading some investigators to seek and propose such alternatives. One reason often put forward results from the difficulty that some investigators experience in eliminating rapidly the twitch or contracture in a 0-Ca^{2+} solution because of the pharmacological and physiological factors detailed above which inhibit the loss of trigger calcium ions. Usually the source of this problem is unrecognized by the investigator, but one wonders about investigators working in this area who over the years change their experimental solutions in ways known to inhibit the loss of the mechanical response in 0-Ca^{2+} solutions (e.g. Refs. 61 and 70).

Another source of confusion results from a large but variable membrane depolarization recorded by some workers (but not by others) using intracellular microelectrodes in 0-Ca^{2+} solutions. When it is found, the investigator then proposes that the loss of the mechanical response is due to inexcitability caused by the large membrane depolarization. When tested, however, membrane depolarizations produced by potassium cause a distinctly different pattern of effects on the twitch [71]. In addition, these large depolarizations are not recorded when using a sucrose-gap apparatus, and they have been shown to be a recording artifact produced by the insertion of microelectrodes into the muscle membranes with increased fragility caused by the 0-Ca^{2+} solution [72]. It must be concluded that this variably occurring artifact does not provide an explanation for the loss of e–c coupling in 0-Ca^{2+} solutions.

The first and most enduring alternative mechanism was the suggestion that intracellular currents produced by the surface action potential directly cause the release of Ca^{2+} from the sarcoplasmic reticulum. This mechanism was proposed despite a large body of evidence, starting with the work of Biedermann [2], repeatedly demonstrating that it is the membrane depolarization and not intracellular currents which cause e–c coupling. In the final review of a recent active worker in this area [73], it was concluded that there was insufficient current flow through the membrane of the sarcoplasmic reticulum to cause calcium release from this intracellular store when the surface membrane is depolarized by an action potential (for further discussion see Ref. 23).

Two more recent alternative hypotheses are: (1) that changes in fixed surface membrane charges are involved in e–c coupling [74], or (2) that a surface 'gating mechanism' modifying charge movement causes e–c coupling by a mechanical linkage to the sarcoplasmic reticulum [75]. However, the experiments purporting to support these mechanisms were conducted under such abnormal physiological and pharmacological conditions (e.g. Refs. 76–78) that it is difficult to determine what relation, if any, these findings have to the normal physiological function of skeletal muscle.

A model for the trigger calcium mechanism

Presented in Figs. 2 and 3 is a recent version of the model first proposed by Bianchi [21, 22]. This model distinguishes among several locations and actions of skeletal muscle cell calcium. In the resting cell, the free Ca^{2+} concentration in the myoplasm (Ca_{my}) is below the threshold level for mechanical activation. The free Ca^{2+} concentration in the triadic junction (Ca_{tj}) is in equilibrium with Ca_{my} and is presumably the same. Most of the cell calcium is sequestered in the terminal cisternae of the sarcoplasmic reticulum (Ca_{SR}). The trigger calcium (Ca_{mb}) is shown bound to the intracellular surface of the t-tubular membrane. This would seem to be the most likely location for the trigger calcium because it is rapidly released by depolarization into the triadic junction yet it takes several minutes for it to be depleted by diffusion into a 0-Ca^{2+} solution in the t-tubule lumen. Finally, there is Ca^{2+} (Ca_{ts}) loosely bound to the luminal surface of the t-tubular membrane. The latter is in equilibrium with extracellular Ca^{2+} in the lumen of the t-tubule (Ca_t).

When the t-tubular membrane is depolarized, some of the trigger calcium (Ca_{mb}) is released into the triadic junction. It diffuses across this junction during the latent period to reach and act on the terminal cisternae membrane thereby causing the

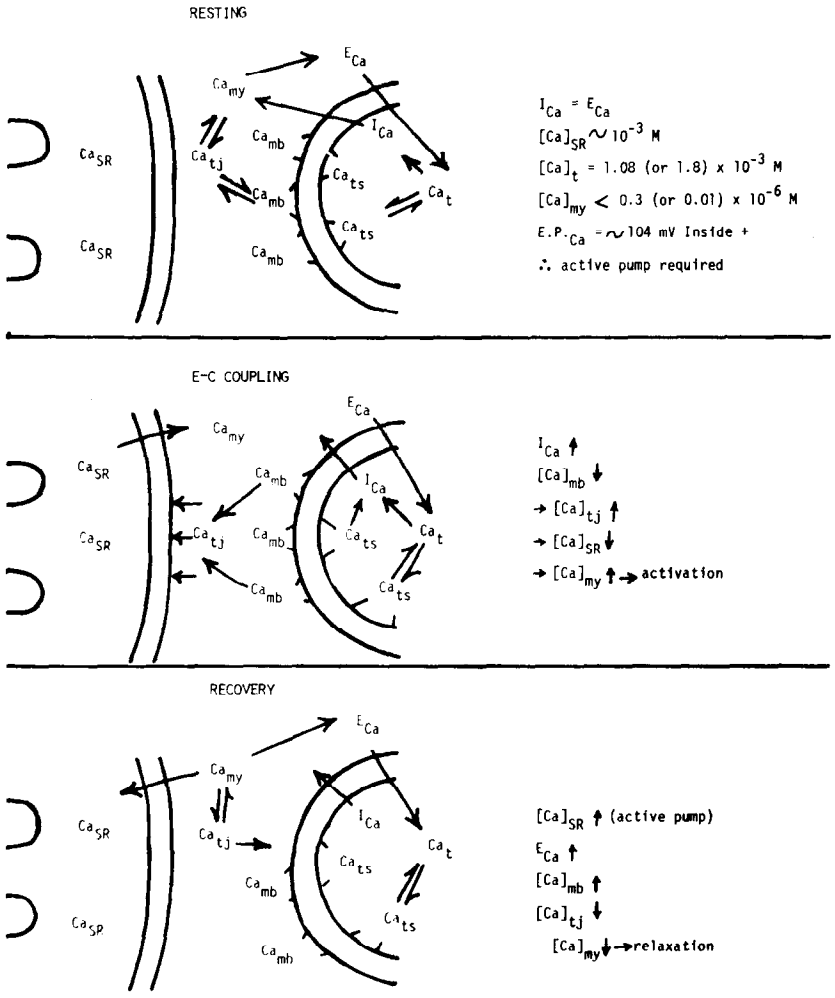

Fig. 2. Working model for the trigger calcium mechanism for e–c coupling: I. Resting, coupling and recovery. T-tubule on right and terminal cisternum on left. Abbreviations: t, t-tubular lumen; ts, luminal surface of t-tubule membrane; mb, t-tubular membrane; tj, triad junction; my, myoplasm; SR, sarcoplasmic reticulum; E, efflux; I, influx; and Ca, calcium. See text for further details. (From Ref. 23.)

release of Ca_{SR} into the myoplasm raising the $[Ca_{my}]$ to above the threshold concentration required to produce a mechanical response.

Recovery (or relaxation) is brought about by active processes pumping the Ca_{my} back into the sarcoplasmic reticulum or out of the fibre. During recovery, the Ca_{mb} also must be restored to resting levels. This is particularly important to the normal physiological functioning of skeletal muscle fibres in which isolated action potentials are rare and activity tends to come in bursts.

The luminal surface Ca_{ts} helps to bind the trigger calcium to the membrane. This has two effects: (1) to inhibit the loss of Ca_{mb} from its binding site and thereby to maintain a sufficient level of Ca_{mb} for coupling, and (2) to control the amount of Ca_{mb} released by depolarization into the triadic junction. When the $[Ca_{ts}]$ is reduced by lowering $[Ca_t]$ (Fig. 3), submaximal depolarization contractures or twitches are potentiated because the binding of Ca_{mb} is weakened and more is released into the triadic junction for a given amount of depolarization. These mechanical responses are only reduced or blocked when the $[Ca_{mb}]$ is reduced to very low levels.

Several multivalent cations, by taking the place of Ca_{ts} at these extracellular binding sites, can prevent the loss of Ca_{mb} into a 0-Ca^{2+} solution and thereby maintain e–c coupling in a 0-Ca^{2+} solution (shown as the Co^{2+} effect in Fig. 3). Not shown is that several of these multivalent cations will potentiate the twitch or potassium contracture response when added to Ringer's in very low concentrations (10^{-6} M), presumably by replacing some of the Ca_{ts} but producing a weaker binding of Ca_{mb}. This will permit more of the Ca_{mb} to be released by the action potential. Finally, several of these multivalent cations can inhibit or block e–c coupling when added to Ringer's in high concentrations. These include Mn^{2+} ($10–20 \times 10^{-3}$ M), La^{3+} (10^{-3} M) and Ca^{2+} itself ($\geq 5 \times 10^{-3}$ M). This suggests that there might be a surplus of these luminal surface binding sites and, when they are more fully occupied, the release of Ca_{mb} by an action potential is inhibited.

In frog ventricular muscle [22] and in some mol-

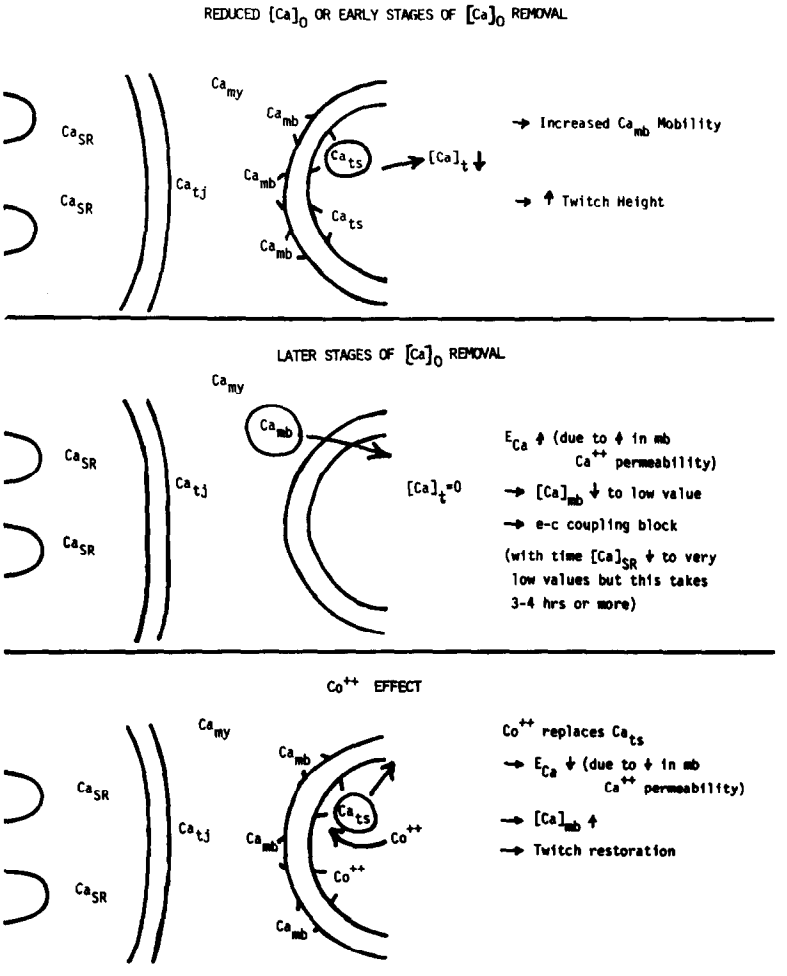

Fig. 3. Working model for the trigger calcium mechanism for e–c coupling: II. Effects of placing the fibre in a 0-Ca^{2+} solution and the mechanism for the divalent ion maintenance of coupling in 0-Ca^{2+}. See Fig. 2 for abbreviations and text for further details. (From Ref. 23.)

luscan smooth muscles [79, 80] it has been demonstrated that the trigger calcium located at the intracellular side of the surface membrane serves as the source of the Ca^{2+} required to raise $[Ca_{my}]$ sufficiently to produce a contraction. By analogy, the possibility has been suggested that in vertebrate skeletal muscle as well, Ca_{mb} might supply the Ca^{2+} for increasing $[Ca_{my}]$ during e–c coupling and that the sarcoplasmic reticulum store serves mainly to maintain the level of Ca_{mb} [22]. However, since it takes several hours with the muscle in a 0-Ca^{2+} solution to deplete Ca_{SR} but only a few minutes to deplete Ca_{mb} [17], this suggestion seems unlikely.

On the other hand, the trigger calcium mechanism as proposed here is very similar to the e–c coupling mechanism recently proposed for mammalian ventricular muscle [81, 82]. The main difference is that in the latter muscle type it is proposed that the trigger calcium is not bound to the surface membrane but is a trans-sarcolemmal influx of extracellular calcium occurring during the plateau of the mammalian action potential.

In conclusion, it must be emphasized that this is only a working model which attempts to incorporate the most recent results in this area of study. Although not a final answer, this mechanism and model incorporate the most reliable results obtained in studies on e–c coupling in vertebrate skeletal muscle during the past century. In addition, this mechanism (and model) is particularly important for pharmacologists because it indicates that one of the most promising areas for study involves the investigation of the effects on membrane Ca^{2+} of a large number of chemical substances known or suspected to modify muscle contraction by an effect on the muscle surface membrane rather than at an intracellular site.

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