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Invited Paper

Appraisal of the physiological relevance of two hypotheses for the mechanism of calcium release from the mammalian cardiac sarcoplasmic reticulum: calcium-induced release versus charge-coupled release

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Abstract

Recent studies correlating the calcium current with, respectively, the clamp-imposed voltage and the calcium current in intact isolated mammalian cardiac myocytes are reviewed. The major findings are the following: [1] With the exception of one group, all investigators agree that a calcium transient is never observed in the absence of a calcium current. In addition, there is a good correlation between voltage dependence of the calcium current and that of the calcium transient, although this correlation may vary among the cardiac tissues from different animal species. [2] Repolarization clamp pulses from highly positive potentials produce a 'tail current' which is associated with a 'tail calcium transient'. [3] The calcium transient is inhibited when the calcium current is blocked by calcium deprivation or substitution, or by the addition of calcium current antagonists, despite the fact that sarcoplasmic reticulum still contains calcium that can be released by caffeine (with inhibition of this release by ryanodine). These three findings are strongly in favor of a calcium-induced release of calcium and against the hypothesis of charge-movement-coupled release of calcium from the sarcoplasmic reticulum. [4] The only finding that would be more in favor of the latter hypothesis (although still reconciliable with the former) is that repolarization occurring before the rapid rise of calcium transient is complete curtails the calcium transient. Thus, the possibility that charge movement might somehow regulate calcium-induced release of calcium cannot be excluded.

The hypothesis of calcium-induced release of calcium from the mammalian cardiac sarcoplasmic reticulum stipulates that the small amount of calcium which crosses the sarcolemma during the mammalian cardiac action potential would be insufficient to activate the myofilaments directly but would trigger release of calcium from the sarcoplasmic reticulum.

In a recent series of articles I gathered as many arguments as I could obtain from experiments in skinned cardiac cells (fragments of single cardiac cells from which the sarcolemma has been removed by microdissection) for the mechanism [1] and physiological relevance [2] of calcium-induced release of calcium from the sarcoplasmic reticulum. The study on the mechanism of calcium-induced release of calcium [1] described the properties of a putative Ca^{2+} channel across the sarcoplasmic reticulum at a time when no direct evidence for such a channel had ever been recorded. The study on the physiological relevance of calcium-induced release of calcium [2] simulated the function of the intact cardiac cell by stimulating a skinned cardiac cell with a simulated transarcolemmal calcium current.

The major thrust of these two areas of research has now shifted away from skinned cardiac cells for the following reasons. First, the recent availability of single channel recordings from sarcoplasmic reticulum incorporated into black lipid membranes [3] diminishes the need of studying the calcium single channel indirectly in skinned cells. Secondly, recent methodological improvements permit the study of physiological relevance of calcium-induced release of calcium directly in intact cardiac cells. The purpose of this brief review is to bring attention to these recent studies in intact cardiac cells. Because these studies are very recent, the review can be complete on this limited subject, and therefore potentially useful despite the space limitation.

Arguments consistent with a calcium-induced release of calcium from the sarcoplasmic reticulum were already available from experiments in multicellular cardiac preparations [4, 5]. However, a new impetus on testing the hypothesis of calcium-induced release of calcium in cardiac cells has stemmed from the availability of preparations of isolated single cardiac cells which permit a better voltage control, a more accurate measurement of calcium current, a more homogenous cell contractile response than in multicellular preparations, and in which the complication of interstertial ion accumulation has been eliminated by the separation into single cells. In addition, the technique of internal perfusion permits modifications of the internal environment that previously were possible only in skinned cells.

A number of hypotheses for the mechanism of calcium release from the sarcoplasmic reticulum alternative to that of calcium-induced release of calcium have been eliminated, including a calcium release induced by a change in pH [6], a sodium-induced release of calcium [7], and a resistive or capacitive coupling between the sarcolemma, or transverse tubules, and the sarcoplasmic reticulum [8]. One possibility still being explored is that of an inositol [1,4,5]-trisphosphate-induced release of calcium from the sarcoplasmic reticulum. However, this process appears to be too slow to be the primary mechanism of calcium release from the cardiac sarcoplasmic reticulum [9]. Another alternative hypothesis to that of calcium-induced release of calcium is that of a charge-coupled release of calcium from the sarcoplasmic reticulum. This hypothesis has been extensively tested in skeletal muscle [10] where it would reconcile the observations of i) the tight control of the calcium release from the sarcoplasmic reticulum by the voltage imposed on the transverse tubular membranes and ii) the lack of resistive or capacitive coupling between transverse tubule and sarcoplasmic reticulum, inasmuch as the coupling between the charges in the transverse tubular membrane and the release sites in the sarcoplasmic reticular membrane should be mechanical rather than electrical. It is only very recently that charge movements have been studied in cardiac muscle [11, 12, 13]. The description of the charge movements in cardiac muscle was not necessary, however, to know that, if such a mechanism occurs in cardiac muscle, the calcium release from the sarcoplasmic reticulum should be directly correlated to the change in membrane potential. This is the rationale for the studies that are reviewed here.

To compare the physiological relevance of the hypothesis of calcium-induced release of calcium to that of charge-coupled release from the sarcoplasmic reticulum, one may relate the amount of calcium released from the sarcoplasmic reticulum to i) the change of potential across the membrane imposed by voltage clamp and ii) the calcium current. Data from skinned cardiac cells indicate that calcium-induced release of calcium is not all or none but graded with the amplitude and rate of the change of free calcium concentration that triggers it [1]. The detailed mechanism of this gradation is complex and requires a functional, if not anatomical, separation between the sites of calcium trigger and of calcium release from the sarcoplasmic reticulum [1]. Thus, a good correlation between calcium release and calcium current would favor the hypothesis of calcium-induced release of calcium since this hypothesis stipulates that the Ca^{2+} release should increase with the amplitude of the calcium current (or any other transarcolemmal calcium influx) that triggers it. In contrast, a good correlation between calcium release and imposed membrane potential would favor the hypothesis of charge-coupled release of calcium since this hypothesis stipulates that the amount of calcium re-

lease should be proportional to the amount of charge movement during the depolarization. The change of free calcium concentration resulting from calcium release can be detected either with calcium sensitive probes or through contraction (tension development or cell shortening) recording.

Isenberg, *et al.* [14] have measured the shortening of bovine myocytes during voltage clamp, and observed a slow component of the contraction with a holding potential near −50 mV and a low-stimulation frequency, and a fast component of contraction with a more negative potential and a higher frequency of stimulation. They observed that when they increased the voltage beyond the calcium reversal potential, this eliminated only the slow component of the contraction but not the fast component. This result could suggest that the fast component was triggered by the voltage per se, perhaps through a charge-coupled process. However, as pointed out by London and Krueger [15], this result is not incompatible with a calcium-induced release of calcium because the conditions under which the fast component of the contraction was observed may have substantially loaded the cell with sodium, and the large positive potential may have caused calcium entry via a sodium-calcium exchange, which could have triggered calcium release from the sarcoplasmic reticulum through a calcium-induced release of calcium.

London and Krueger [15] used single guinea pig ventricular myocytes and recorded the imposed voltage, the calcium current, and the extent and velocity of shortening with a photodiode array. They observed a very good direct correlation between the amplitude of the twitch and the calcium current, and this for two very different types of calcium currents: the calcium current associated with depolarization and the 'tail current' associated with repolarization. In contrast, when the voltage increased beyond the reversal potential for calcium, there was no longer calcium release from the sarcoplasmic reticulum as shown by the lack of resulting contraction. These findings are incompatible with a charge-coupled mechanism of excitation-contraction coupling and consistent with a calcium-induced release of calcium.

The first studies using fura-2 to detect Ca^{2+} release from the sarcoplasmic reticulum and to evaluate the relationship between this Ca^{2+} release and voltage versus Ca^{2+} current were done independently by two laboratories in Baltimore, those of Dr. Lederer [16, 17] and Dr. Weir [18, 19] and one laboratory in Philadelphia, that of Dr. Morad [20, 21].

Cannell, *et al.* [16, 17] have done their study of the voltage dependence of the calcium current versus that of the calcium transient in voltage-clamped ventricular cells from the rat. They observed that the calcium current activates at −40 mV whereas the calcium transient is already activated at −50 mV. Thus, it would seem possible to obtain a calcium transient in the absence of a calcium current. This indeed would be a very strong argument against calcium-induced release of calcium. However Talo *et al.* [22] also using rat ventricular myocytes, note that a small calcium current induced by low depolarization can easily escape detection and be interpreted as leakage current. In addition, Callewaert, *et al.* [20] demonstrated in the same rat ventricular preparation an excellent correlation between the calcium current and the calcium transient provided that the latter is measured 25 ms after the depolarization pulse. Another finding of Cannell *et al.* [16, 17] that could be consistent with a charge-coupled induced release from the sarcoplasmic reticulum is that repolarization before the rapid rise of calcium concentration is complete turns off the calcium transient. However, as pointed out by Barcenas-Ruiz and Wier [18] and Beuckelmann and Wier [19], this could be explained by an increase in the rate of calcium extrusion by sodium-calcium exchange which is known to occur at more negative potential. Finally, like other investigators, including London and Krueger [15], Barcenas-Ruiz and Wier [18] and Beukelmann and Wier [19], Cannell, *et al.* [16, 17] observe that a strong repolarization from a highly positive potential is followed by a 'tail calcium current' accompanied by a 'tail calcium transient.' This is considered by others [15, 18, 19] as a strong argument against a charge-coupled release of calcium. However, Cannell *et al.* [16, 17] propose a modification (not yet supported by any data) of this charge-coupled release hypothesis which could, in their opinion, explain, the 'tail calcium current' and 'tail calcium

transient': upon repolarization, the calcium channel would return from a voltage-dependent inactivated state to an open state back to the resting state sufficiently slowly for calcium to be released during the very brief passage to the open state.

The study by Beukelmann and Wier [19] expands the earlier study by Barcenas-Ruiz and Wier [18]. Thus, these studies will be analyzed together. The article by Beukelmann and Wier [19] presents the most complete study published on this subject and, since its discussion thoroughly refers to previous articles, the readers are encouraged to read this article as a primary source of information for this rationale, comparing the calcium transient to, respectively, the calcium current and the imposed voltage. Barcenas-Ruiz and Wier [18] and Beukelmann and Wier [19] used a detection of the calcium release with fura-2 in voltage-clamped guinea pig ventricular myocytes. They used the calcium antagonist verapamil and the calcium agonist Bay K8644 to identify the calcium current and ryanodine to identify the calcium release. They demonstrated that the voltage dependence and the pharmacology of the calcium current and of the calcium transient elicited by depolarization are very consistent. Both phenomena have a bell-shaped dependence on membrane voltage. This contrasts with the lack of correlation observed in rat ventricular cells reported by Cannell *et al.* [16, 17]. It is possible that some of the differences are related to species differences in the study by Cannell *et al.* [16, 17] were done in rat ventricular cells. However, other investigators [20] observed a very good correlation in the rat ventricular cell as well. This excellent correlation is consistent with the hypothesis of calcium-induced release of calcium from the sarcoplasmic reticulum. Barcenas-Ruiz and Wier [18] and Beukelmann and Wier [19] also observed that repolarization from a membrane potential greater than +30 mV induces a 'tail current' during the time after repolarization when the calcium gradient for the calcium influx is increased and the calcium conductance is not yet deactivated. They observed that this 'tail current' is accompanied by a 'tail calcium transient' which is abolished by ryanodine and, therefore, generated by calcium release from the sarcoplasmic reticulum. The occurrence of these 'transients' is one of the strongest arguments against the charge-coupled release of calcium from the sarcoplasmic reticulum since this repolarization should have 'shut off' the calcium release by moving back the coupling charge. Indeed, in skeletal muscle where charge-coupled release of calcium could play a physiological role, repolarization after a brief depolarization pulse does not cause a calcium release [10]. The only experimental result that could be, at first sight, in favor of a charge-coupled release of calcium from the sarcoplasmic reticulum is that a repolarization before the rapid rise of calcium is complete curtails the calcium transient. But this could be explained by an increase of calcium extrusion by sodium-calcium exchange, which is known to occur at more negative potential, as previously explained.

Callewaert, *et al.* [20] used simultaneous recordings of cell shortening and fura-2 fluorescence to have two indexes of the calcium transients during calcium release from the sarcoplasmic reticulum in rat ventricular myocytes. They observed that the quality of the correlation between the calcium current and the calcium transient depends upon the time at which the calcium transient was measured. They estimated that the most meaningful measurement of the calcium transient for this correlation was 25 ms after the depolarizing clamp pulse, because the calcium current peaks 15 to 25 ms after this pulse. Under these conditions the time dependence of the calcium transient was almost superimposed to that of the calcium current. On the other hand, the voltage dependence of the peak of the calcium signal had a broader maximum than the voltage dependence of the calcium current. This result is similar to the result reported by Cannell, *et al.* [16, 17] with the exception, however, that Callewaert, *et al.* [20] never observed calcium transients not accompanied by a calcium current. Callewaert, *et al.* [20] also observed 'tail calcium transients' upon repolarization from highly positive potentials. As previously indicated, this is a strong argument against a charge-coupled release of calcium from the sarcoplasmic reticulum.

In an even more direct demonstration, Näbauer, *et al.* [21] used a new method for extremely rapid changes of the extracellular solution superfusing rat ventricular myocytes to demonstrate in a compelling manner that the influx of calcium through

the calcium channel is a mandatory link in the process which couples depolarization of the sarcolemma to the calcium release from the sarcoplasmic reticulum. The entry of either sodium or barium in the absence of a significant calcium influx through the calcium channel failed to trigger calcium release. Thus, these experiments bring a very strong argument against the intramembrane charge movement as a mechanism of calcium release from the sarcoplasmic reticulum.

The studies using indo-1 instead of fura-2 to detect the calcium release from the sarcoplasmic reticulum were done in the laboratories of Dr. Laketta in Baltimore [22, 23] and Dr. Houser in Philadelphia [24].

Spurgeon, *et al.* [23] have monitored simultaneously the fluorescence of indo-1 and the cell shortening to obtain two sensors of the calcium transient. They found that the same depolarization by voltage clamp that produces a calcium transient in the presence of 1 mM extracellular calcium fails to produce such a transient in the presence of 2 mM nitrendipine which blocks the calcium current. However, under these conditions of calcium current blockage by nitrendipine, calcium can still be released by caffeine. This release is abolished by pretreatment with ryanodine, which demonstrates that it takes place from the sarcoplasmic reticulum. Thus, these experiments by Spurgeon, *et al.* [23] directly demonstrate in a compelling manner a block of the calcium-induced release of calcium by removing the sarcolemmal calcium influx.

In a study on feline isolated ventricular myocytes, duBell and Houser [24] observed that, in general, when the calcium current increased or decreased, so did the calcium transient. However, the voltage dependence of the calcium current was more bell shaped than that of the calcium current. Yet duBell and Houser [24] never observed a calcium transient in the absence of calcium current.

In summary, recent studies of the relationship between the calcium transient and respectively the voltage and the calcium current gave results which render it very difficult to accept the charge-coupled release hypothesis for the mechanism for calcium release from the sarcoplasmic reticulum. However, a definitive negative conclusion should await more complete studies on the characteristics of the charge movement in cardiac cells [11, 12, 13]. The data are, in contrast, generally in agreement with the hypothesis of calcium-induced release of calcium. Most investigators find that in all cardiac tissues studied (guinea pig, rat, and cat) there is a good consistency between the voltage dependence of the calcium current and of the calcium transient. The only exception is the study by Cannell *et al.* [16, 17] which is partly reconciled by the data obtained in the same tissues by other investigators [20, 22]. The 'tail transients' are strongly in favor of a calcium-induced release of calcium. Finally, experiments using calcium deprivation, calcium substitution, or calcium current blockage demonstrate that the calcium current is essential to trigger calcium release from the sarcoplasmic reticulum. The only problem with this hypothesis of calcium-induced release of calcium from the sarcoplasmic reticulum is the curtailment of the calcium release by a repolarization, which can be explained by the operation of the sodium-calcium exchange [19]. This would require, however, that the transsarcolemmal calcium influx through sodium-calcium exchange be fast enough to trigger calcium release from the sarcoplasmic reticulum, which is not yet established. Thus, one cannot exclude the alternative possibility, suggested by Channel *et al.* [17], that charge movement, which may be the primary mechanisms of excitation-contraction coupling in skeletal muscle [10], may play a more minor role of regulator or controller of calcium-induced release of calcium in cardiac muscle.

The hypothesis of the calcium-induced release of calcium should be submitted to additional tests in intact cardiac cells, as it is now possible, for instance, by flash photolysis of caged calcium compounds [25, 26]. An equally important problem is that the hypothesis of calcium-induced release of calcium cannot be accepted until a detailed mechanism is proposed. Thus, an evaluation of the physiological relevance of calcium-induced release of calcium cannot be dissociated from progress in understanding of the mechanism of this process. This was the conclusion of the last series of experiments in skinned cardiac cells [1, 2] and this still remains true at the present time. However, the best methods for this bi-directional progression have shifted. Experiments in skinned cardiac cells are no longer

the most efficient approach to study either the mechanism or the physiological relevance of this process inasmuch as direct approaches are now available: single channel recordings for the study of mechanism or experiments in intact cardiac cells for the study of the physiological relevance.

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