Commentary

Excitation Contraction Coupling in Cardiac Muscle: Is there a Purely Voltage-dependent Component?

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It is well established that excitation contraction (EC) coupling in cardiac myocytes is mediated by the entry of calcium ions (Ca^{2+}) from the bathing medium into the cell cytoplasm (Fabiato, 1985), which then triggers calcium-induced calcium release (CICR) from the sarcoplasmic reticulum (SR). More recently, it was proposed that a second, quite separate, EC-coupling process, mediated by an effect of membrane potential per se on SR Ca^{2+} release, also operates in cardiac myocytes (Ferrier and Howlett, 1995; Hobai et al., 1997; Howlett and Ferrier, 1997; Howlett et al., 1998, 1999; Ferrier et al., 1998, 2000; Mason and Ferrier, 1999; Zhu and Ferrier, 2000; for review see Ferrier and Howlett, 2001). Although a substantial body of diverse experimental observations has been reported in support of this voltage-sensitive release mechanism (VSRM; Ferrier and Howlett, 2001), it remains highly controversial (Nabauer et al., 1989; Wier and Balke, 1999; Piacentino et al., 2000; Sipido, 2003; Trafford and Eisner, 2003).

One reason for reservations regarding VSRM is that it is said to be observable only under very restricted conditions: very negative holding potentials, temperatures near 37°C, a fully loaded SR, and, for internally dialyzed cells, in the presence of agents that promote protein kinase A (PKA)-mediated phosphorylation. These conditions all increase either L-type Ca^{2+} currents ($I_{Ca(L)}$) or SR Ca^{2+} release. Hence, conditions purported to demonstrate VSRM could, instead, just be enhancing CICR. A further problem is that whereas nominally Ca^{2+}-free bathing media fully abolish contraction, contraction is restored with as little as 50 μM external Ca^{2+} (Ferrier and Howlett, 1995). Ferrier and Howlett (1995) have taken this result to indicate that a small amount of external Ca^{2+} is required as a kind of cofactor for VSRM, as opposed to actual Ca^{2+} entry. However, the observation could equally well indicate that only little Ca^{2+} entry is needed to trigger SR Ca^{2+} release, especially under conditions of high SR load. Moreover, much of the evidence presented in support of VSRM amounts to the repeated claim, under a variety of conditions and in a number of different contexts, that contraction is observed in the absence of detectable inward Ca^{2+} current. If a Ca^{2+} current actually was present, but just unrecognized, much of the presumptive evidence for VSRM would vanish. Consequently, despite the many observations reported to be in support of VSRM, it remains unclear whether VSRM operates at all in cardiac myocytes.

In this issue, Griffiths and MacLeod (2003) make a solid contribution to the resolution of this controversy, by showing that inward Ca^{2+} currents are present under conditions similar to those in some previous studies where Ca^{2+} currents were reported to be absent. Specifically, Griffiths and MacLeod show that: (a) nifedipine-sensitive difference currents can be demonstrated for small depolarizing voltage steps even when $I_{Ca(L)}$ is not obvious in the unsubtracted current traces; (b) inward $I_{Ca(L)}$ can be unmasked by block of outward current components; and (c) repolarization-activated tail contractions can be seen during hyperpolarizing post pulses, demonstrating that conducting, unblocked Ca^{2+} channels may be present even when inward current during the test step is not evident. These new findings present a challenge to VSRM, as they call into question the core claim behind VSRM, that contractions can be observed in the absence of inward Ca^{2+} currents. It therefore is of interest to examine some of the observations reported in support of VSRM in the light of the new results of Griffiths and MacLeod (2003)—as well as other relevant observations. While a number of the observations presented as demonstrating or suggesting VSRM are consistent with CICR, others are less obviously so.

Evidence supporting VSRM has been reported primarily by a single group, that of G.R. Ferrier, S.E. Howlett, and their collaborators. Some of the key observations of the Ferrier-Howlett group are:

(1) Ferrier and Howlett report that contractions can be initiated at membrane potentials negative to those at which $I_{Ca(L)}$ apparently is activated and that the contractions are increased by holding potentials negative to −40 mV, in the range of −65 to −70 mV (Ferrier and Howlett, 1995; Ferrier et al., 1998; Mason and Ferrier, 1999). Ferrier and Howlett interpreted these results as indicating that there must be a VSRM, which is inactivated at holding potentials positive to −40 mV.

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Griffiths and MacLeod (2003) confirm that more negative holding potentials do shift the contraction threshold in the negative direction. However, Griffiths and MacLeod (2003) show that there is also a negative shift in the potential at which $I_{Ca(L)}$ is first detectable (as did Ferrier and Howlett [1995] themselves). Rather than promoting recovery from inactivation of the putative VSRM, more negative holding potentials simply increase $I_{Ca(L)}$. The $I_{Ca(L)}$ availability curve is just not saturated at −40 mV. Moreover, Griffiths and MacLeod (2003), in addition to their own experiments, cite a number of previous studies showing that $I_{Ca(L)}$ can be elicited at test potentials negative to −40 mV. Hence the core claim for VSRM, that contractions are seen in the absence of inward Ca^{2+} current, is not supported when more stringent methods for detecting Ca^{2+} current are employed.

(2) Ferrier and Howlett have reported multiple times that Ca^{2+} channel blockers (verapamil, Cd^{2+}, nifedipine, Ni^{2+}) suppress $I_{Ca(L)}$ substantially (or completely) with little or no effect on contraction or the Ca^{2+} transient at more negative potentials (Ferrier and Howlett, 1995; Hobai et al., 1997; Howlett and Ferrier, 1997; Ferrier et al., 1998; Howlett et al., 1998; Zhu and Ferrier, 2000). The block of $I_{Ca(L)}$, but not contraction, was seen for voltage steps to −40 mV from a holding potential of −65 mV (conditions selective for the putative VSRM: recovery of VSRM from inactivation has occurred, and there is only little $I_{Ca(L)}$ activation), but not for steps to 0 mV from a 200–300-ms prestep to –40 mV (conditions selective for CICR: VSRM is inactivated, and there is about maximal $I_{Ca(L)}$). Ferrier and Howlett took these results to indicate that there must be a VSRM and that it has a pharmacological profile distinct from CICR. However, using block of outward current components and demonstration of repolarization activated tail contractions, Griffiths and MacLeod (2003) show that $I_{Ca(L)}$ persists under conditions where the Ferrier and Howlett group had taken it to be fully blocked (by 100 or 300 μM Cd^{2+}, 2.5 μM nifedipine, and 200 μM or sometimes 5 mM Ni^{2+}). Griffiths and MacLeod (2003) find that $I_{Ca(L)}$ is not completely blocked by 200 μM Cd^{2+} or 60 μM nifedipine. They found 12 mM Ni^{2+} would fully suppress $I_{Ca(L)}$ and that such concentrations also fully suppress contraction under all conditions tested. Griffiths and MacLeod (2003) also cite previous studies showing that both dihydropyridine and heavy metal cations block of Ca^{2+} channels is voltage dependent, being less effective at both more negative holding potentials and more negative test steps. The important point is that a distinct pharmacological profile for the putative VSRM has not been demonstrated.

(3) Ferrier and Howlett report that at more negative holding potentials, in the range of −55 to −65 mV, the contraction-voltage curve becomes sigmoid and approximately plateaus near −20 mV (Ferrier and Howlett, 1995; Howlett et al., 1998, 1999; Ferrier et al., 1998, 2000; Mason and Ferrier, 1999). This is in contrast to the bell-shaped contraction-voltage curve obtained at holding potentials of −40 mV, which rises to a maximum and then declines again at more positive potentials. In the case of the bell-shaped curve the contraction amplitude is approximately proportional to $I_{Ca(L)}$ amplitude, consistent with CICR. In the case of the sigmoid curve it is not, and Ferrier and Howlett have taken this as strong evidence for VSRM. However, Griffiths and MacLeod (2003) were unable to reproduce the conversion of the contraction-voltage curve from bell-shaped to sigmoid with more negative holding potentials. The curve remained approximately bell-shaped at all holding potentials tested (see also Beuckelmann and Wier, 1988). This is an unresolved issue, however, as bell-shaped contraction voltage curves are not always seen (see DISCUSSION in Griffiths and MacLeod, 2003). There may be a broad maximum, and the reasons for this may be complex. Gain (the amount of SR Ca^{2+} release produced by a given $I_{Ca(L)}$) is not constant over the whole voltage range, and reverse mode Na^{+}-Ca^{2+} exchange may play a role at large positive potentials (Sipido et al., 1997).

(4) Ferrier and Howlett report that under conditions of internal cell dialysis, such as whole cell patch clamp recording, VSRM is selectively lost. It is said to be restored with internal adenosine 3′, 5′-cyclic monophosphate (cAMP), which promotes PKA-mediated protein phosphorylation (Hobai et al., 1997; Ferrier et al., 1998). Ferrier et al. (1998) report that cAMP increases contraction amplitude substantially under VSRM-selective conditions, but without any accompanying increase in $I_{Ca(L)}$. Under CICR-selective conditions, contraction and $I_{Ca(L)}$ both increase moderately. Ferrier et al. (1998) take these findings as further evidence for VSRM (increase in contraction but not $I_{Ca(L)}$) and a demonstration of its distinctness from CICR (CICR does not require cAMP). As noted by Griffiths and MacLeod (2003), cAMP is known to increase $I_{Ca(L)}$ amplitude, SR Ca^{2+} content, and, hence, gain. Because of this increase in gain and the instances in which the Ferrier-Howlett group did not detect increases in $I_{Ca(L)}$, it becomes difficult to assess Ferrier and Howlett's interpretation. One would need to have the Ferrier and Howlett group's observations replicated using the rigorous methods of Griffiths and MacLeod (2003) to assess the $I_{Ca(L)}$ amplitude.

(5) Zhu and Ferrier (2000) report that the purported selective loss of VSRM induced by internal dialysis can be restored by activation of Ca^{2+}-calmodulin–dependent kinase II. That is, internal calmodulin is said to, like cAMP, increase the contraction amplitude without an increase in $I_{Ca(L)}$ under VSRM-selective conditions.

Under CICR-selective conditions both contraction and $I_{Ca(L)}$ increase somewhat, presumptively demonstrating the distinct nature of VSRM vs. CICR. But again, the evidence for VSRM is based entirely on the failure to detect, or detect changes in, small amplitude Ca^{2+} currents. Reports of such failures must remain in question until replicated with more rigorous methods for detecting small currents.

(6) Mason and Ferrier (1999) reported that tetracaine, which is believed to suppress SR Ca^{2+} release, strongly blocks contraction at very negative and at very positive test potentials, with very little effect near 0 mV. These experiments were done at a holding potential of −65 mV, so that the putative VSRM is available. Mason and Ferrier (1999) interpret these findings as demonstrating distinct VSRM and CICR processes. They attribute contraction both at very negative and very positive potentials primarily to VSRM as $I_{Ca(L)}$ amplitude is small in these ranges. $I_{Ca(L)}$ is maximal near 0 mV. However, Ca^{2+} entry is small both at very negative and very positive potentials, and any intervention that hinders EC coupling would be expected to be more effective in suppressing contraction at these potentials. That a blocker of EC coupling is more effective in blocking contraction at some, as compared with other, voltage ranges is not of itself evidence for distinct EC coupling processes.

(7) As noted above, the Ferrier and Howlett group has reported distinct pharmacological profiles for CICR as compared with the putative VSRM, which they take as evidence for two distinct EC-coupling mechanisms. The Ca channel blockers, verapamil, Cd^{2+}, nifedipine, and Ni^{2+} are said to preferentially block CICR (Ferrier and Howlett, 1995; Hobai et al., 1997; Ferrier et al., 1998, 2000; Howlett et al., 1998, 1999; Zhu and Ferrier, 2000), while ryanodine, thapsigargin, and tetracaine are said to preferentially block VSRM (Ferrier and Howlett, 1995; Ferrier et al., 1998; Howlett et al., 1998; Mason and Ferrier, 1999). These attributions are all based on whether block appears to be more effective at more negative (ryanodine, thapsigargin, tetracaine) or more positive (Ca^{2+} channel blockers) voltage ranges. As discussed in point 2 and by Griffiths and MacLeod (2003), Ca^{2+} channel block is voltage dependent, being less effective at more negative potentials. And, as discussed in point 6, a more effective block of contraction at more negative potentials where $I_{Ca(L)}$ is small is not surprising. Hence, the "distinct pharmacological profiles" could equally well be attributed to distinct properties of the blocking agents rather than to distinct EC coupling processes.

(8) Howlett et al. (1998) report that increased SR Ca^{2+} loading increases contraction amplitude more effectively under putative VSRM-selective conditions than under CICR-selective conditions. They took this to support the presence of two distinct EC-coupling processes. However, under VSRM-selective conditions the $I_{Ca(L)}$ whole cell current amplitude is greater (because of the more negative holding potentials), the $I_{Ca(L)}$ single channel current amplitude is greater (more negative test steps) and gain is higher. Thus, CICR can fully account for the observed effect. Again, differential effects obtained at different voltage ranges do not necessarily imply distinct EC coupling processes. Howlett et al. (1998) also report that identical contraction-voltage curves, presumed to arise entirely from the putative VSRM component, can be obtained by two quite separate treatments, ryanodine exposure and reduced SR Ca^{2+} loading, which is taken as support for a genuine VSRM process. Using a holding potential of −65 mV, so that VSRM and CICR are both available, a contraction-voltage curve is constructed. A standard train of conditioning pulses to maintain SR Ca^{2+} loading is presented before each holding potential/test step sequence. The experiment is repeated in the presence of ryanodine, which is said to preferentially block the VSRM component (see point 7), and a difference contraction-voltage curve is obtained. In the second series of experiments, ryanodine is not used, but the amplitude of the conditioning pulses is reduced so that SR Ca^{2+} loading is reduced, which also is said to preferentially reduce VSRM. A difference contraction-voltage curve is again obtained. These two difference contraction-voltage curves are virtually superimposable point for point, which would tend to support the idea of a distinct VSRM component. But one would need to replicate these experiments.

(9) Howlett et al. (1999) report that in the cardiomyopathic hamster model, contraction is more suppressed under VSRM-selective than under CICR-selective conditions, which is taken as evidence for a distinct VSRM component. However, as noted in point 8, VSRM-selective conditions are exactly those where contraction is most sensitive to changes in gain, which makes it very difficult to interpret this finding definitively.

(10) Ferrier et al. (2000) report that contractions typically express a sustained component in addition to the usual phasic component. The sustained component is reported to be evident over the whole range over which phasic contractions are seen. It was interpreted to be a VSRM-mediated process because of its relative insensitivity to Cd^{2+} block and because its amplitude is maximal where $I_{Ca(L)}$ is fully inactivated. This sustained component, however, is different from the phasic VSRM component in that it does not inactivate. These are again experiments that one would like to see replicated. The same is true for the remaining two points below.

(11) Howlett et al. (1998) report that the midpoint of the steady-state inactivation curve for the VSRM contraction component is 25–30 mV more negative than that for

$I_{Ca(L)}$. The VSRM contraction is fully inactivated at potentials where $I_{Ca(L)}$ is at 90% of its maximum availability. This finding is not obviously reconcilable with CICR.

(12) Howlett et al. (1998) also report that restitution of the VSRM component of contraction proceeds with a time constant nearly twofold slower than that for $I_{Ca(L)}$ recovery from inactivation. Hence, $I_{Ca(L)}$ is available when VRSM-mediated contraction is not. This finding is also not obviously reconcilable with CICR.

In conclusion, much of the evidence presented in support of VSRM is ambiguous. A number of effects attributed to VSRM can be equally well attributed to CICR when unrecognized Ca^{2+} currents, voltage-dependent gain, and other issues are taken into account. Griffiths and MacLeod (2003) have made a clear contribution to this question by showing that inward Ca^{2+} current does accompany contraction under conditions similar to those for which some earlier reports have said that it does not. Thus, a single EC coupling mechanism, CICR, can account for a considerable body of experimental observations.

It is very difficult, however, to demonstrate conclusively that there is no VSRM-mediated component of EC coupling operating in parallel with CICR. The possibility must be left open. And, while the body of experimental evidence apparently supporting VSRM has been appreciably reduced by the careful experiments of Griffiths and MacLeod (2003), some observations remain. A fuller resolution of this question will await further experimental studies.

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