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Topical Review

Regulation of Current Flow through Ryanodine Receptors by Luminal Ca^{2+}

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Introduction

Ca^{2+} release from intracellular stores occurs through two main classes of intracellular membrane channel; the sarcoplasmic reticulum (SR) Ca^{2+}-release channel/ryanodine receptor (RyR), or the inositol 1,4,5-trisphosphate ($InsP_3$) receptor. Under physiological conditions the opening of both types of channel is thought to be triggered by a signal which interacts with the cytosolic channel face. Ca^{2+} release, once triggered, is controlled by a number of other factors and intracellular ligands, most of which act at the cytosolic side of the channels. Over quite a long period of time and with a variety of experimental preparations it has become increasingly apparent that luminal Ca^{2+} may play a role in regulating the Ca^{2+} release through RyR in both cardiac and skeletal muscle. The purpose of this review is to concentrate on the effects of luminal [Ca^{2+}] on the gating of RyR, the mechanisms involved in these effects and to discuss how such changes may regulate Ca^{2+} release from intracellular stores during excitation-contraction (EC) coupling.

Evidence for a RyR-Mediated Effect of Luminal Ca^{2+}

The first suggestion that the luminal SR [Ca^{2+}] may influence the release of Ca^{2+} came from the work of Endo (for review *see* Endo (1977) [5]) with skinned skeletal fibers. It was shown that a certain ''threshold'' loading of the SR was required before Ca^{2+} release could be triggered. In skinned cardiac cells, Fabiato & Fabiato [7] demonstrated that the magnitude of Ca^{2+}-induced Ca^{2+}-release (CICR) was increased as the Ca^{2+} loading of the SR was increased. Fabiato later proposed two types of CICR in skinned cardiac cells: (i) a time- and Ca^{2+}-dependent activation which is triggered by the rapid increase of [Ca^{2+}] at the cytoplasmic side of the SR and (ii) the spontaneous release of Ca^{2+} which requires Ca^{2+} overload of the SR and may be initiated by Ca^{2+} binding to an intraluminal site [6].

Studies of isolated cardiac myocytes are consistent with the earlier findings indicating that the luminal [Ca^{2+}] may play a role in controlling SR Ca^{2+} release during EC-coupling [2, 10, 16, 34, 35]. This is confirmed by the observations that cardiac cells exhibit spontaneous Ca^{2+} release and Ca^{2+} waves [22] under conditions of SR Ca^{2+} overload.

Further information about the regulatory effects of luminal Ca^{2+} has been derived from studies of Ca^{2+}-release from isolated SR vesicles. The results indicate that both the amount of Ca^{2+} released and the rate constants of release depend on the luminal [Ca^{2+}] [4, 14, 19]. In agreement with the earlier work on skinned cells [5, 7], isolated SR studies also demonstrated that a certain level of SR Ca^{2+} loading is necessary before Ca^{2+}-induced Ca^{2+}-release (CICR) can occur [9, 21] and suggested that the binding of Ca^{2+} to intraluminal sites regulated SR Ca^{2+} release.

Role of Calsequestrin in SR Ca^{2+}-Release

Calsequestrin is one of the major Ca^{2+}-binding proteins in the sarcoplasmic reticulum and a number of studies have implicated a role for this protein in the Ca^{2+}-release process. By measuring the fluorescence intensities of the conformational probe N-[7-(dimethylamino)-4-methyl-

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3-coumarinyl] maleimide (DACM) attached to proteins in the SR membrane including RyR, Ikemoto and coworkers originally suggested that the binding of Ca^{2+} to calsequestrin produced a conformational change in calsequestrin which subsequently caused a conformational change in RyR leading to variations in SR Ca^{2+} release [14]. In later studies [13, 23] it was proposed that agonists binding to RyR cause conformational changes in the channel which lead to the dissociation of Ca^{2+} from calsequestrin and a subsequent elevation in intraluminal free [Ca^{2+}] and increased release of Ca^{2+} from the SR. Gilchrist et al. [9] also proposed a role for calsequestrin in the Ca^{2+}-release dependence of isolated SR vesicles on luminal Ca^{2+} content. The experiments of Donoso et al. [4] demonstrated that the free intravesicular Ca^{2+} was less than 10% of the total amount of Ca^{2+} released by ATP-induced Ca^{2+}-release in triads isolated from rabbit or frog skeletal muscle and that most of the vesicular Ca^{2+} is bound to calsequestrin. Most of the Ca^{2+} released from the vesicles, therefore, must have first dissociated from calsequestrin. Donoso et al. [4] report that Ca^{2+} dissociation from calsequestrin is far more rapid (< 1 msec) than the Ca^{2+} release process which occurs over several milliseconds. In contrast with these experiments, it has been suggested that most of the SR Ca^{2+} is free and does not dissociate from calsequestrin before release from SR vesicles [39]. It is important to establish how calsequestrin buffers luminal Ca^{2+} in vivo as without this knowledge the range of free SR [Ca^{2+}] that occurs physiologically and the time course of such changes cannot be determined. From the Ca^{2+}-flux studies it is not yet clear if the conformational change in calsequestrin resulting when Ca^{2+} dissociates can directly regulate RyR gating (as suggested by Ikemoto and coworkers [14]) or if another protein is involved which links RyR with calsequestrin or if luminal Ca^{2+} directly regulates the channel. It is quite possible that more than one mechanism of action may be responsible for the effects of luminal Ca^{2+} on SR Ca^{2+} release in intact cells.

The above studies point to a regulatory effect of luminal Ca^{2+} occurring at the level of RyR but without directly monitoring the gating and conductance of RyR it is difficult to predict how luminal Ca^{2+} can affect the flow of Ca^{2+} current through the channel. The following section describes what is known about the effects of luminal Ca^{2+} on the gating and conductance of single RyR incorporated into planar phospholipid bilayers.

Effects of Luminal Ca^{2+} on the Single-Channel Properties of RyR Reconstituted into Planar Phospholipid Bilayers

Conductance

The effects of luminal [Ca^{2+}] on the Ca^{2+} current through the sheep cardiac RyR under physiological ionic conditions has been predicted using a computer model based on Eyring rate theory [37]. Figure 1 illustrates the calculated Ca^{2+} current flowing from the luminal to the cytosolic side of RyR at 0 mV over the range of luminal [Ca^{2+}] that might be expected to occur physiologically. The prediction assumes that, in the cell, no change in SR membrane potential occurs and that Ca^{2+} binding to calsequestrin does not lead to a conformational change in RyR that affects ion conduction through the channel. Maximal Ca^{2+} current is 3.46 pA and half maximal current is obtained at a luminal [Ca^{2+}] of 1.78 mM. Although ion conduction through the skeletal RyR has not been modeled, evidence suggests that it is very similar to that of the cardiac isoform and therefore luminal [Ca^{2+}] may be expected to control Ca^{2+} current in a similar manner.

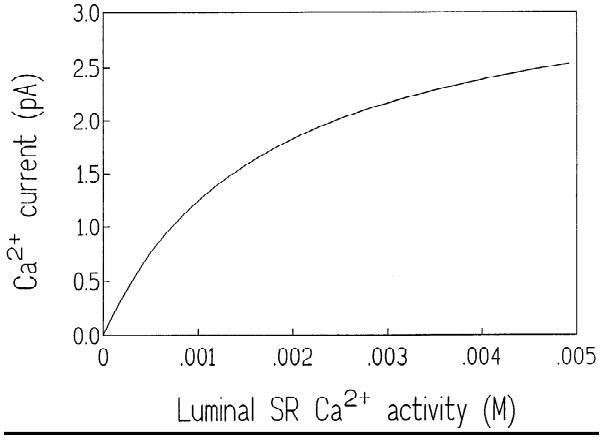

Fig. 1. Predicted variation in Ca^{2+} current through the sheep cardiac SR Ca^{2+}-release channel with varying intraluminal Ca^{2+} activity. Current was calculated using the rate theory model described in Tinker et al. [36]. For the purpose of the calculation it was assumed that the ionic activities of K^+ and Mg^{2+} in both the cytosol and the SR lumen were 120 mM and 0.5 mM respectively and that cytosolic Ca^{2+} was at a level that would not have a significant effect on ion conduction. Modified from Tinker et al. (1993).

Gating

Before we consider the effects of luminal Ca^{2+} we must first understand how cytosolic Ca^{2+} and other agonists regulate RyR channel gating. The gating of RyR when activated solely by cytosolic Ca^{2+} is characterized by very brief open events (mean open lifetimes ⩽1 msec [1, 28, 32]. This is true for both cardiac and skeletal isoforms although the lifetimes of cardiac channels are slightly longer [1, 25, 28, 30, 32]. An important observation concerning the activation of rabbit and sheep skeletal RyR [30, 32] and sheep and human cardiac RyR [12, 28] is that activation of the channels by cytosolic Ca^{2+} alone is generated by an increase in the frequency of

channel opening. No significant increases in mean open time are observed although at peak open probabilities in sheep cardiac channels there is a trend towards a slight increase in open time duration [28]. Canine cardiac channels are regulated differently by cytosolic Ca^{2+}. The open lifetimes are approximately 10-fold longer than those of sheep or human cardiac RyR and activation by cytosolic Ca^{2+} alone involves significant increases in open lifetime duration [25].

Other ligands, for example those acting at the caffeine or ATP site, can activate RyR in the absence of activating levels of cytosolic Ca^{2+} [18, 27, 41]. As is the case when Ca^{2+} is the sole ligand, other ligands cannot, alone, fully open the channel. Lifetime analysis of such Ca^{2+}-independent gating demonstrates that long open events occur which are unlike the brief channel openings observed when Ca^{2+} is the sole ligand, indicating that a different gating mechanism is operating. A third gating mechanism appears to exist whereby the synergistic activation of RyR by cytosolic Ca^{2+} plus a second ligand (for example, caffeine or ATP analogues) can fully activate the channel [18, 27, 41].

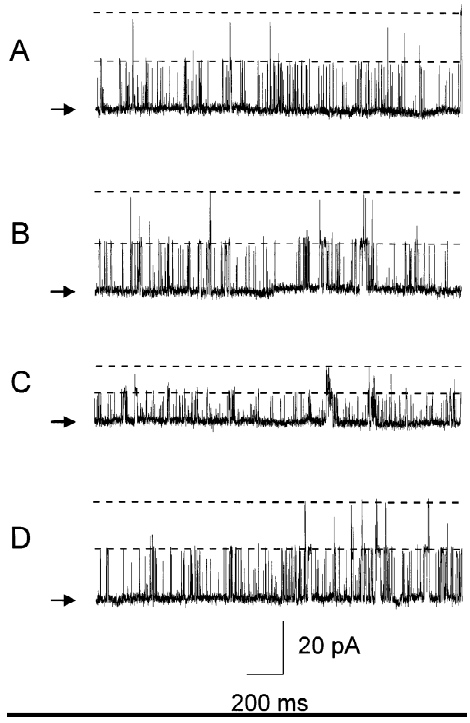

Fig. 2. Current fluctuations through sheep cardiac native RyR incorporated into planar phosphatidylethanolamine lipid bilayers in symmetrical 250 mM Cs^+ and voltage-clamped at −40 mV. Two channels have incorporated into the bilayer. The arrows indicate the closed channel level and the dotted lines indicate the open channel levels. At this holding potential current flow is in the luminal to cytosolic direction. In the first trace (*A*), both the cytosolic and luminal [Ca^{2+}] are 10 μM. Channel openings are very brief and P_o is low as is typical for channels activated by cytosolic Ca^{2+} in the absence of other activating ligands. Sequential changes to the luminal [Ca^{2+}] were made as follows: (*B*) 100 μM, (*C*) 2 mM, (*D*) approximately 30 nM (addition of 12 mM EGTA). No change in P_o occurs. At millimolar luminal [Ca^{2+}] a reduction in current amplitude can be observed which reflects the greater affinity of the conduction pathway for Ca^{2+} than for Cs^+. The baseline fluctuations result from the gating of SR K^+ channels which incorporate into the bilayers together with RyR but which have only a low Cs^+ conductance.

Effects of Luminal Ca^{2+} on the Gating of the Cardiac RyR

In the sheep cardiac RyR activated solely by micromolar cytosolic Ca^{2+}, it was demonstrated that increasing the luminal [Ca^{2+}] from picomolar levels to 4 mM did not alter P_o [29] (Fig. 2). Additionally, the relationship between P_o and membrane potential was unaltered. The lack of effect of luminal Ca^{2+} on P_o is an important experimental observation as it indicates that the Ca^{2+} which flows through the channel from the luminal to the cytosolic channel face does not have access to the cytosolic Ca^{2+} bindings sites. The experiments were conducted at both positive and negative holding potentials at a cytosolic-free [Ca^{2+}] (10 μM) below the EC_{50} value for Ca^{2+} activation of the sheep cardiac channel. It would therefore be expected, that if Ca^{2+} flowing from the lumen of the SR had access to the cytosolic Ca^{2+} binding sites, at negative holding potentials and at high millimolar luminal [Ca^{2+}], an increase in P_o would occur. Recent experiments by Lukyanenko et al. [16] find the same inability of changes in luminal [Ca^{2+}] to affect the P_o of canine cardiac RyR activated solely by cytosolic Ca^{2+}. This result is especially interesting since the P_o changes resulting from Ca^{2+}-activation of channels from dog occur over a much greater range than the cytosolic Ca^{2+}-induced P_o changes in sheep channels.

From the results of the above experiments alone it would appear that luminal Ca^{2+} has no regulatory effect on channel gating. However, activating ligands other than Ca^{2+} can induce gating mechanisms distinct from the mechanism of activation by cytosolic Ca^{2+} alone. This is true for a number of ligands but has been better characterized for agents which bind to the caffeine or ATP activation sites on the channel [18, 27, 32, 41]. It was therefore investigated if luminal Ca^{2+} could modify the sheep cardiac RyR gating when the channel was activated by a ligand inducing a different gating scheme to that induced by Ca^{2+} alone. Sulmazole, an agonist at the caffeine binding site on RyR, was used to activate the channel in the absence of activating cytosolic Ca^{2+} but similar effects were observed with ATP (*unpublished observations*). The effect of sulmazole was critically dependent on the luminal [Ca^{2+}]. As the luminal [Ca^{2+}] was increased from micromolar to millimolar levels, an increase in the duration and the frequency of channel openings was observed. Figure 3 illustrates the

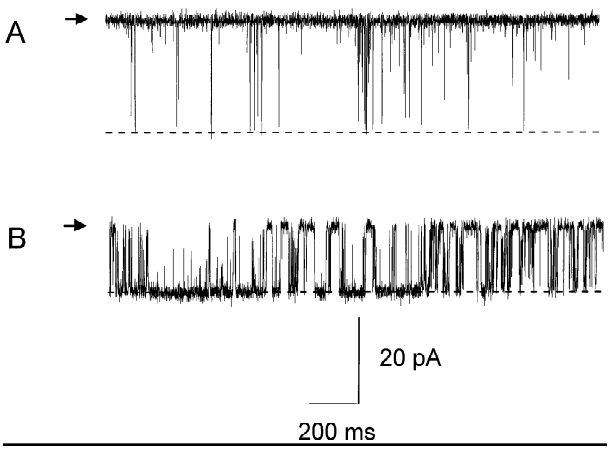

Fig. 3. Recordings from a typical sheep cardiac single purified channel in symmetrical 210 mM K^+, voltage-clamped at +40 mV. The arrows indicate the closed channel level and the dotted lines indicate the fully open level. Current flow is in the cytosolic to luminal direction. In trace (*A*) a subactivating [Ca^{2+}] (approximately 100 pM) is present at the cytosolic channel face and 10 μM Ca^{2+} is present on the luminal side of the channel. The channel is activated by 5 mM cytosolic sulmazole (which binds to the caffeine site). At this holding potential and luminal [Ca^{2+}], P_o is very low. In trace (*B*) the luminal [Ca^{2+}] has been increased to 2 mM and this results in a marked increase in P_o which is characterized by long open events. As in Fig. 2, a decrease in current amplitude occurs at this concentration of luminal Ca^{2+}.

marked increase in P_o that results from an increase in luminal [Ca^{2+}]. The voltage dependence of P_o was also altered as the luminal [Ca^{2+}] was increased. At low luminal [Ca^{2+}], the long open events and increase in P_o were more marked at negative holding potentials than at positive potentials. The most obvious explanation for the voltage dependence of the sulmazole effect is that luminal Ca^{2+} has access to cytosolic Ca^{2+} binding sites. However, there are two main pieces of evidence which indicate that this may not be the mechanism. (i) When the channel is activated solely by cytosolic Ca^{2+}, luminal Ca^{2+} has no effect. (ii) Williams & Holmberg [41] demonstrated that activation of the channel by sulmazole alone produced gating characterized by long open events. In the presence of cytosolic Ca^{2+}, gating was characterized by shorter events. The very long open events resulting when the channel is activated by sulmazole in the absence of cytosolic Ca^{2+} but in the presence of luminal Ca^{2+} indicate that Ca^{2+} is not binding to the cytosolic activation sites. It was therefore concluded that luminal Ca^{2+} may bind to sites distinct from the cytosolic Ca^{2+} binding sites and which could be present within the channel pore or on the luminal face of the channel.

What was surprising about these experiments was that the effects of luminal Ca^{2+} depended on the mechanism of channel activation. Luminal Ca^{2+} had no effect on channels activated solely by cytosolic Ca^{2+} but controlled the gating of channels activated solely by sulmazole. A similar result was also obtained by Lukyanenko et al. [16] with dog cardiac channels, who demonstrated that luminal Ca^{2+} had no effect when the channel was activated solely by cytosolic Ca^{2+} but in the presence of a second ligand (ATP) could increase P_o. The mechanisms underlying these observations are yet to be resolved. One possible explanation is that the conformation of the channel activated solely by cytosolic Ca^{2+} is such that the sites to which luminal Ca^{2+} binds in the presence of a second ligand, are inaccessible. In the cardiac bilayer experiments it is likely that the luminal Ca^{2+}-induced P_o effects were mediated by a direct effect of Ca^{2+} on the channel since similar effects were observed with native and purified channels [29] but a regulatory role of calsequestrin on channel gating in vivo must also be considered.

Implications of the Single-Channel Studies to Cardiac EC-coupling and Spontaneous Ca^{2+}-release from the SR

An unknown but important factor in the analysis of the contribution of the luminal Ca^{2+} content to SR Ca^{2+} release is the free luminal [Ca^{2+}] and how this changes during the contractile cycle. Electron probe analysis gives an estimate of the total end-systolic and end-diastolic SR [Ca^{2+}] as approximately 1.1 and 2.4 mM respectively [40] however, there may be difficulties in extrapolating these values to the working cell. Also unknown is the extent to which SR Ca^{2+} is bound to calsequestrin, at what stage the buffering capacity of calsequestrin is reached and whether activation of RyR during systole leads to a conformational change in calsequestrin causing the rapid (<1 msec [4]) dissociation of Ca^{2+} and a large but unknown rise in [Ca^{2+}] at the luminal face of RyR.

The effects of luminal Ca^{2+} on single sheep and dog cardiac RyR incorporated into planar phospholipid bilayers indicate that direct effects of luminal Ca^{2+} may modulate SR Ca^{2+}-release both during normal EC-coupling and under conditions such as Ca^{2+} overload of the SR. In the presence of high luminal [Ca^{2+}] (up to 50 mM) but in the absence of other cytosolic activating ligands, the cardiac RyR does not open [27, 29, 41]. Therefore, luminal Ca^{2+} cannot itself trigger the channel to open. In the cell, however, other activating ligands are present, which do not activate the channels under normal conditions at diastolic cytosolic [Ca^{2+}]. Under conditions of Ca^{2+} overload, the marked increase in P_o which luminal [Ca^{2+}] exerts on RyR activated by adenine nucleotides (and other channel activators) may be enough to lower the threshold for channel activation sufficiently to produce channel openings at diastolic Ca^{2+} levels. Thus although luminal Ca^{2+} does not actually trigger the opening of RyR, it shifts the activation of

the channel to a much lower cytosolic [Ca^{2+}]. This may be a possible explanation for the spontaneous Ca^{2+}-release events which can be observed in overloaded cells. Once the spontaneous events have been triggered, the raised luminal [Ca^{2+}] would be expected to result in a larger than normal flow of Ca^{2+} through the channel due to the increased SR Ca^{2+} gradient [37] and the increased P_o [16, 29]. Such a large release of SR Ca^{2+} may activate neighboring groups of RyR causing Ca^{2+} waves to propagate through the cell.

Bassani et al. [2] showed that at high loading conditions of the SR small increases in Ca^{2+} content caused large increases in the fractional SR Ca^{2+} release. Similarly, Lukyanenko et al. [16] observed an increase in frequency and a fourfold increase in amplitude of Ca^{2+} sparks for an estimated 30% increase in SR Ca^{2+} content. The stimulatory effects of luminal Ca^{2+} on the opening of the cardiac RyR [16, 29] could provide a large contribution to this effect if the free luminal [Ca^{2+}] in the vicinity of RyR was increased in the range from 10 μM to millimolar levels. The EC_{50} for luminal Ca^{2+} potentiation of P_o in cardiac RyR activated synergistically by Ca^{2+} plus ATP is not known but the value for sheep skeletal RyR activation is approximately 100 μM. This value is likely to be altered particularly by the cytosolic free Ca^{2+}, Mg^{2+} and adenine nucleotide levels but possibly by other factors such as pH and phosphorylation state of the channel. An increase in free luminal [Ca^{2+}] under physiological ionic conditions also increases the Ca^{2+} current through RyR with an apparent K_D of 1.78 mM although if intracellular Mg^{2+} levels are raised (for example during ischaemia) the K_D also increases. Therefore increases in luminal [Ca^{2+}] that affect gating will also affect the amplitude of the Ca^{2+} current and both parameters would be expected to contribute to the increased Ca^{2+}-release observed in single cells with high SR load. It is not possible to attribute the exact proportion that each parameter would contribute to the overall increase in SR Ca^{2+} release during SR Ca^{2+} overload or during the excitation-contraction cycle, simply because we cannot be sure of the luminal [Ca^{2+}] close to RyR and how this is regulated by calsequestrin. We also have to consider that conformational changes in calsequestrin caused by increases and decreases in Ca^{2+} binding [14] may also directly modify channel gating and provide a third mechanism for increased release of SR Ca^{2+} with overloaded SR.

Effects of Luminal Ca^{2+} on the Skeletal RyR

Single channel studies also demonstrate that the gating of the skeletal RyR channel can be regulated by luminal [Ca^{2+}], however the reported effects of luminal Ca^{2+} are diverse and the mechanism for the effect of luminal Ca^{2+} is unresolved. Early studies with purified rabbit skeletal channels reconstituted into bilayers reported that increasing luminal [Ca^{2+}] led only to a decrease in P_o; no increase in P_o was observed [17]. A similar result was obtained with native skeletal channels from the pig [8]. Interestingly, high luminal Ca^{2+} did not inactivate RyR from skeletal muscle SR of a homozygous recessive pig model of malignant hypothermia (MH). MH is an inherited disease which is characterized by abnormal muscle contractures in response to certain pharmacological agents and this appears to result from loss of regulation of the cell [Ca^{2+}]. In both experiments the cytosolic [Ca^{2+}] was maintained at 10 μM and current flow was in the cytosolic to luminal channel direction. The results of these experiments were explained in terms of a low affinity Ca^{2+} binding site being present within the channel pore which is equally accessible from the luminal and from the cytosolic side of the channel.

More recent studies indicated that increases in luminal [Ca^{2+}] could also increase the P_o of RyR isolated from skeletal muscle SR [11, 30, 31, 38]. Tripathy & Meissner [38] and Herrmann-Frank & Lehmann-Horn [11] used purified rabbit skeletal RyR and demonstrated that increasing the luminal [Ca^{2+}] increased P_o at low concentrations but reduced P_o at higher concentrations. This effect of luminal Ca^{2+} was voltage dependent and both groups came to the conclusion that luminal Ca^{2+} acts by binding to cytosolic Ca^{2+} activation and inhibition sites. Using the model for ion conduction in the sheep cardiac RyR [37], it was suggested that the effect of luminal Ca^{2+} on skeletal RyR gating was correlated with the flux of Ca^{2+} through the channel [38]. In earlier studies, the effect of luminal Ca^{2+} on native sheep skeletal RyR was investigated [30, 31]. In contrast to the experiments using purified rabbit skeletal RyR [11, 38] luminal Ca^{2+} had no effect on the P_o of channels activated solely by cytosolic Ca^{2+}. These key experiments indicated that luminal Ca^{2+} was not acting at the cytosolic activation or inhibition sites. However, marked activation of channels activated by cytosolic Ca^{2+} plus ATP (and related compounds) or by ATP in the absence of activating cytosolic [Ca^{2+}] was observed. As for the cardiac channel [29], the presence of a second ligand which induces gating kinetics distinct to that induced by cytosolic Ca^{2+}, rendered the channel sensitive to luminal [Ca^{2+}]. The effect of luminal Ca^{2+} on channels activated by cytosolic Ca^{2+} plus ATP did not exhibit voltage dependence and therefore it was suggested that luminal Ca^{2+} was binding to sites on the luminal side of the channel [30].

Unfortunately, key experiments which should provide clues as to the mechanism of the luminal Ca^{2+} effect give different results in different laboratories using purified or native channels. Clearly, the mechanism for activation and inhibition of the skeletal RyR by luminal Ca^{2+} is still disputed, however, the results from all

groups demonstrate that luminal Ca^{2+} does cause a marked effect on gating. The experiments by Kawasaki & Kasai [15] highlight a further complication of the effects of luminal Ca^{2+}. They observed a Ca^{2+}-dependent increase in the P_o of skeletal RyR by adding calsequestrin to the luminal side of the bilayer. As for cardiac cells, the contribution of luminal Ca^{2+} in regulating the gating and the amplitude of the Ca^{2+} current through skeletal RyR *in situ* is not known. This requires a greater understanding of the fluctuations of luminal $[Ca^{2+}]$ that RyR is exposed to and of the role of calsequestrin.

Physiological Consequences of the Effects of Luminal Ca^{2+} in Skeletal Muscle

As in cardiac muscle, before any regulatory effect can be attributed to the luminal $[Ca^{2+}]$ a crucial consideration that must be addressed is the free luminal $[Ca^{2+}]$ in the vicinity of RyR at rest and how this changes during contraction. Again this is an unknown variable. Studies have been carried out which assess the amount of Ca^{2+} in the SR. Using electron probe analysis, Somlyo et al. [33] estimated approximately 1.5 mM Ca^{2+} in the SR terminal cisternae from frog muscle at rest and that approximately 40% is still present after 1.2-sec tetanus. This estimate may include some Ca^{2+} re-accumulated by the SR. More recently a study of skinned rat and toad muscle fibers suggested that the SR Ca^{2+} content can be more than 80% depleted by T-tubule depolarization or by caffeine application [24]. These studies suggest that RyR may be exposed to large variations in free luminal $[Ca^{2+}]$ during a twitch over a range similar to that predicted by Cannell & Allen [3]. The study by Owen et al. [24] demonstrated that the force response to depolarization was dependent on SR Ca^{2+} content, however, from these results we cannot determine if this relationship results from a regulatory effect of luminal $[Ca^{2+}]$ on RyR gating or whether it just reflects the change in RyR current amplitude that will occur with a change in free luminal $[Ca^{2+}]$. As previously discussed, isolated SR vesicle studies indicate that interactions between calsequestrin and RyR may play a crucial role in regulating SR Ca^{2+} release. A greater knowledge of such interactions would greatly improve our understanding of the mechanisms of SR Ca^{2+} release.

An indication that the luminal $[Ca^{2+}]$ may play a regulatory role in skeletal muscle EC-coupling comes from examining Ca^{2+}-release mechanisms in tissue from MH. In SR vesicles isolated from MH tissue, the threshold for CICR was much lower than that from normal SR vesicles [20]. Single channel studies reported that high luminal $[Ca^{2+}]$ could reduce the P_o of RyR from normal but not from MH tissue [8]. Ion handling in normal and MH RyR appears to be similar [26] and therefore the above studies suggest that a regulatory effect of luminal $[Ca^{2+}]$ on skeletal RyR gating may be lost.

Summary

Single-channel studies unequivocally demonstrate that changes in luminal $[Ca^{2+}]$ cause marked changes in conductance and gating although the precise mechanisms involved in the luminal Ca^{2+}-induced changes in gating have not been fully resolved. Evidence from isolated vesicle studies and single cell work also point to a regulatory role for luminal Ca^{2+} in the control of SR Ca^{2+}-release for muscle contraction. Outstanding issues which remain to be addressed include: (i) What are the changes in free $[Ca^{2+}]$ that occur at the luminal face of the RyR channels during EC-coupling. (ii) Does luminal Ca^{2+} affect gating by binding to luminal or cytosolic sites on RyR. (iii) What are the direct and indirect effects of calsequestrin on RyR gating.

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