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Is ryanodine receptor a calcium or magnesium channel? Roles of K^+ and Mg^{2+} during Ca^{2+} release

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Abstract

The ryanodine receptor (RyR) is a poorly selective channel that mediates Ca^{2+} release from intracellular Ca^{2+} stores. How RyR's selectivity between the physiological cations K^+ , Mg^{2+} , and Ca^{2+} affects single-channel Ca^{2+} current amplitude is examined using a recent model of RyR permeation. It is found that K^+ provides the vast majority of the countercurrent (through RyR itself) that is needed to prevent the sarcoplasmic reticulum (SR) membrane potential from changing and stopping Ca^{2+} release. Moreover, intra-pore competition between Ca^{2+} and Mg^{2+} defines single RyR Ca^{2+} current amplitude. Since both $[Mg^{2+}]$ and $[Ca^{2+}]_{SR}$ can change during pathophysiological conditions, the RyR unitary Ca^{2+} current amplitude during Ca^{2+} release may change significantly due to this Ca^{2+}/Mg^{2+} competition. Compared to the classic action of Mg^{2+} on RyR open probability, these Ca^{2+} current amplitude changes have as large or larger effects on overall RyR Ca^{2+} mobilization. A new aspect of RyR divalent versus monovalent selectivity is also identified where this kind of selectivity decreases as divalent concentration increases.

INTRODUCTION

During excitation/contraction (EC) coupling in striated muscle, the role of the ryanodine receptor (RyR) is to conduct a large current of Ca^{2+} out of the sarcoplasmic reticulum (SR). This “simple” job is, in fact, accomplished by a complex combination of single-channel RyR permeation and gating. Alteration of either will change how much Ca^{2+} is mobilized. The RyR is a poorly-selective, Mg^{2+} -modulated, Ca^{2+} -activated channel. Its unitary Ca^{2+} current amplitude (i_{Ca}) amplitude and open probability (P_o) varies with SR Ca^{2+} load ($[Ca^{2+}]_{SR}$) and Mg^{2+} concentration ($[Mg^{2+}]$). Load varies as Ca^{2+} is released/resequestered from/by the SR. Cellular Mg^{2+} levels are normally stable, but do vary in many different physiological circumstances.

The Mg^{2+} regulation of RyR P_o has been studied at length over the last two decades, but alterations in RyR Ca^{2+} current amplitude by changes in $[Mg^{2+}]$ has received less attention. In this paper, we show how unitary Ca^{2+} current amplitude—and therefore presumably Ca^{2+} release in situ—is defined by the selectivity and permeation properties of RyR for the physiological cations Ca^{2+} , Mg^{2+} , and K^+ . To do this, we use a recent model of ion permeation through RyR [1, 2] to first describe RyR's high Mg^{2+} affinity and weak K^+

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affinity, as well as analyze how these ions influence Ca^{2+} release. We conclude that i_{Ca} depends on relatively independent actions of K^+ and Mg^{2+} within the RyR pore. The $[\text{K}^+]$ establishes the effective countercurrent amplitude and the $[\text{Mg}^{2+}]$ determines the unitary Ca^{2+} current amplitude. We find that the effects of $[\text{Mg}^{2+}]$ on RyR i_{Ca} are comparable in magnitude to the effects of $[\text{Mg}^{2+}]$ on RyR P_0 . This implies that, in physiological conditions where $[\text{Mg}^{2+}]$ is abnormal, RyR function is altered in nearly equal measure by Mg^{2+} -induced changes in P_0 and i_{Ca} .

RyR SELECTIVITY IN THE PHYSIOLOGICAL IONIC REGIME

RyR is Mg^{2+} selective in cells

EC coupling occurs with the SR membrane potential near 0 mV [3] and within a relatively defined set of ionic concentrations. The standard physiological RyR permeable cation concentrations in resting muscle are approximately 120 mM K^+ , 8 mM Na^+ , and 1 mM Mg^{2+} in both the cytosol and the SR lumen. There is ~100 nM Ca^{2+} in the cytosol and 1 mM Ca^{2+} in the SR. In heart, pathological conditions (e.g., heart failure, ischemia, and hypertension) can alter resting intra-SR Ca^{2+} load and cytosolic $[\text{Mg}^{2+}]$, as exemplified by the studies in Table 1. Those studies come from different species (e.g., human as well as rat and dog) and thus may not all occur in any one given species. However, these studies show that these pathological disturbances typically increase or decrease $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Mg}^{2+}]$ by approximately a factor 2 from normal levels. Therefore, we consider 0.5 to 2 mM as a typical range over which $[\text{Mg}^{2+}]$ and $[\text{Ca}^{2+}]_{\text{SR}}$ can vary. Cytosolic Na^+ levels may increase 2–3 fold during these cardiac pathophysiological states [4, 5]. In this paper, however, we do not include Na^+ because its RyR permeation properties are very similar to K^+ and K^+ is substantially more abundant in cells.

Ca^{2+} is unique in that it is the only one of these cations that is not symmetrically distributed across the SR membrane; the SR Ca^{2+} ATPase generates a trans-SR Ca^{2+} gradient. The symmetric Mg^{2+} and asymmetric Ca^{2+} distributions have important ramifications for RyR ion selectivity. This was observed by Kettlun et al. [6], where symmetric Mg^{2+} (1 mM) application reduced current amplitude carried by 10 mM luminal Ca^{2+} by ~30%. This is a huge action considering Mg^{2+} and Ca^{2+} are conducted nearly equally by the RyR [7]. It implies that Mg^{2+} preferentially occupies the RyR pore, enough to substantially limit the flow of Ca^{2+} current [8]. The Mg^{2+} enjoys preferential occupancy likely because of its symmetric distribution.

The Kettlun et al. [6] result seems to contradict the results of Tinker and Williams [7] who showed the permeability ratios among divalent cations is 1. The explanation is that Mg^{2+} was applied symmetrically by Kettlun et al. [6] and it was not by Tinker and Williams [7]. Symmetric application gives Mg^{2+} a significant advantage over Ca^{2+} and in effect makes the RyR a Mg^{2+} -selective channel under physiological conditions. Another significant consequence of this situation is that it makes i_{Ca} very weakly dependent on $[\text{K}^+]$. This was also observed by Kettlun et al. [6].

The experiments of Kettlun et al. [6] demonstrate how RyR's selectivity properties affect Ca^{2+} current amplitude in vitro. Since the RyR selectivity filter is unlikely to be dramatically different in situ, the competition of Ca^{2+} and Mg^{2+} for the pore in cells is likely to be similar to that found in single-channel experiments. Therefore these experiments give important insight into the interactions of Mg^{2+} and Ca^{2+} . However, to quantify these interactions and their consequences requires a model. The model we use in this paper [1, 2, 8–10] (which is described in Appendix A) has reproduced RyR current/voltage experiments in more than 160 different ionic conditions, including those of Kettlun et al. [6]. It also predicted specific anomalous mole fraction effects and their concentration dependence

before these were experimentally verified [1, 2, 8, 10]. The unusually broad experimental verification of this RyR permeation model lends substantial confidence to its predictions.

Mg²⁺ is the most prevalent ion in the RyR pore

The amount of each ion species present in the open RyR pore under resting physiological conditions was predicted using the model. Figure 1 plots the concentrations of the permeating ions along the pore axis. The concentration for each ion species at x is proportional to the probability of finding that ion within the channel cross-section at location x . These profiles show that Mg²⁺ is the most prevalent ion in the RyR pore. In fact, the concentration of Mg²⁺ in the selectivity filter ($10 < x < 25$ Å) is ~3.5 times that of Ca²⁺. Moreover, in the cytosolic vestibule ($0 < x < 10$ Å), Mg²⁺ and K⁺ have almost the same concentration and these are well above that of Ca²⁺. This is because Ca²⁺ is only present in abundance on the luminal side of the pore while the other ions are symmetrically distributed (i.e., abundant on both sides).

How this changes over the typical pathophysiological range of ion concentrations (e.g., low or high [Mg²⁺] or [Ca²⁺]_{SR}) is shown in Fig. 2. The number of K⁺ (*dashed lines*), Mg²⁺ (*dotted lines*), and Ca²⁺ (*solid lines*) ions in the selectivity filter are plotted as [Ca²⁺]_{SR} (Fig. 2A) or symmetric [Mg²⁺] (Fig. 2B) are varied. When the cations are at resting physiological levels (thin vertical lines; Fig. 2), there are on average ~1.5 Mg²⁺ ions and less than 0.5 Ca²⁺ or K⁺ ions. When [Mg²⁺] increases (Fig. 2B), it displaces even more Ca²⁺ and K⁺ from the selectivity filter. Note that symmetric Mg²⁺ must fall to <0.5 mM in order for there to be an equal number of Mg²⁺ and Ca²⁺ ions in the selectivity filter (Fig. 2B). With 1 mM symmetric Mg²⁺ present, luminal Ca²⁺ must rise to 4 mM in order for there to be an equal number of Mg²⁺ and Ca²⁺ ions in the filter (Fig. 2A). Thus, the concentrations of the other RyR permeable ions must be raised substantially for them to compete equally with Mg²⁺ for occupancy of the pore. The preferential access Mg²⁺ enjoys (because it is symmetrically distributed) effectively gives the RyR pore a higher apparent affinity for Mg²⁺ than Ca²⁺. From this point of view, this makes the RyR a Mg²⁺ selective channel. From a current point of view, however, RyR is a Ca²⁺ selective channel because the net Ca²⁺ current will always be larger than the net Mg²⁺ current. Since the conductances (and diffusion coefficients) of Ca²⁺ and Mg²⁺ are nearly identical (Appendix A), this is solely because Ca²⁺ is asymmetrically distributed

K⁺ is not displaced from the pore

While [Mg²⁺] and [Ca²⁺]_{SR} have a substantial effect on Ca²⁺ concentration in the selectivity filter, neither has a large effect on K⁺ occupancy of the selectivity filter over their typical physiological ranges (0.5 to 2 mM). The K⁺ occupancy varies only from ~0.35 and 0.6 (Fig. 2) as the divalent concentrations vary across this physiological range. Surprisingly, even with 5 mM Mg²⁺, K⁺ has not been displaced from the pore and its occupancy in the selectivity filter is 2.5 times that of Ca²⁺ (Fig. 2B). Also, K⁺ occupancy is relatively insensitive to increasing SR Ca²⁺ load over the physiological range (Fig. 2A). It seems as if the divalents can displace each other very effectively, but only weakly displace K⁺ as bath divalent concentration increases. That is, the channel's divalent versus monovalent selectivity seems to weaken as [Mg²⁺] and/or [Ca²⁺]_{SR} increase.

This phenomenon is actually a prediction of the model. In a recent paper [2], we used our RyR permeation model to determine the thermodynamic basis for RyR's Ca²⁺ selectivity. We reported that two kinds of electrostatic factors determined Ca²⁺ versus K⁺ selectivity, namely

1. the mean electrostatic potential (the long-time average of the electrostatic potential) that draws ions into the filter and which Ca^{2+} “feels” more strongly because of its higher valence;
2. the screening component that describes the superior ability of a divalent cation (over monovalents) to be coordinated (screened) by the negatively-charged glutamate residues in the selectivity filter.

These factors contribute equally at low (symmetric) $[\text{Ca}^{2+}]$, but at high millimolar $[\text{Ca}^{2+}]$ the pore becomes more and more charge neutral, leaving only the second of the two energetic advantages of the divalent over the monovalent [2]. Therefore, when the concentration of divalent is *high*, *selectivity is low*, a phenomenon we call DiHi-SelLo.

DiHi-SelLo occurs because the mean electrostatic potential decreases at high divalent concentrations. Reducing one of the two factors that make RyR divalent selective decreases its divalent selectivity. The model also indicates that divalent versus monovalent selectivity is not reduced if monovalent concentrations are raised and that monovalent versus monovalent selectivity does not change at high monovalent concentrations (data not shown). Thus, DiHi-SelLo is not simply an action of high ionic strength, but rather a divalent cation specific effect.

We demonstrate the DiHi-SelLo effect experimentally in Fig. 3. In symmetric K^+ , the model predicts the *net* current through RyR will be reduced when Ca^{2+} is added to the luminal bath ($I_{+\text{Ca}}$) when compared to the *net* current with no Ca^{2+} present ($I_{0\text{Ca}}$) [2, 8]. The fractional reduction of current (FRC) can be defined as $(I_{0\text{Ca}} - I_{+\text{Ca}}) / I_{0\text{Ca}}$ and used as an indicator of Ca^{2+} versus K^+ selectivity. Low FRC implies low Ca^{2+} current (the currents $I_{0\text{Ca}}$ and $I_{+\text{Ca}}$ are almost the same) and therefore low Ca^{2+} pore occupancy and selectivity (versus K^+). Conversely, high FRC implies high Ca^{2+} selectivity. We measured FRC as $[\text{K}^+]$ was increased while keeping the ratio of K^+ to Ca^{2+} concentration ($[\text{K}^+]:[\text{Ca}^{2+}]_{\text{SR}}$) constant at 100:1 (see Appendix B for experimental details). Under these conditions, one would expect the FRC to be constant as $[\text{K}^+]$ increases because the ratio of $[\text{K}^+]$ to $[\text{Ca}^{2+}]$ is constant. (In fact, one might even expect the increased Ca^{2+} current from the asymmetric application of Ca^{2+} to increase the FRC.) However, as predicted by the model, higher concentrations of Ca^{2+} are *less* effective at reducing K^+ current than lower concentrations (Fig. 3). The observed counterintuitive decrease in FRC is consistent with the DiHi-SelLo concept.

On the other hand, this does not occur with symmetrically added 1:1 mixtures of K^+ and Li^+ . Li^+ was chosen here because RyR's affinity for monovalent cations is greatest for Li^+ [2] and high Li^+ levels reduce K^+ currents. A 1:1 ratio with K^+ was used because much more Li^+ is needed to reduce K^+ current to a similar extent as Ca^{2+} . The effect of Li^+ is, however, very different than the effect of Ca^{2+} ; even at ionic strengths twice that in the Ca^{2+} experiment, there is no change in FRC (Fig. 3, inset). This is consistent with DiHi-SelLo being a divalent action.

DiHi-SelLo then explains the results shown in Fig. 2 that K^+ is never fully eliminated from the RyR selectivity filter. As either $[\text{Mg}^{2+}]$ or $[\text{Ca}^{2+}]_{\text{SR}}$ are increased, the divalents are less and less effective at displacing K^+ from the pore. Instead, Ca^{2+} and Mg^{2+} displace each other much more effectively.

RYR SELECTIVITY AND Ca^{2+} RELEASE

We explored the physiological ramifications of K^+ remaining in the pore at high $[\text{Mg}^{2+}]$ or $[\text{Ca}^{2+}]_{\text{SR}}$ and the competition of Ca^{2+} and Mg^{2+} for the pore. Specifically, we evaluated how these influence RyR self-countercurrent and SR membrane potential, as well as unitary RyR

Ca^{2+} current amplitude. If the selectivity characteristics of RyR do not change substantially in situ, then the phenomena described below are likely to occur during Ca^{2+} release as well.

Countercurrent

Any release of Ca^{2+} from the SR lasting more than a few milliseconds requires a countercurrent to prevent the SR membrane potential from reaching the equilibrium (Nernst) potential for Ca^{2+} (E_{Ca}) where net Ca^{2+} efflux ceases. The countercurrent could be either anions moving out of the SR or cations moving in. We have previously shown that RyR itself conducts sufficient countercurrent in the form of K^+ and Mg^{2+} to keep the SR membrane potential near 0 mV [9]. This is possible because the RyR's poor Ca^{2+} versus K^+ selectivity permits substantial K^+ and Mg^{2+} counterfluxes while Ca^{2+} is being released. We showed that the open RyR in physiological solutions would establish an SR membrane potential of about -2 mV (the RyR reversal potential V_{rev}). Moreover, robust RyR Ca^{2+} release occurs when cytosolic K^+ is exchanged for Cs^+ and Cl^- for large anions like aspartate [11–14]. This is consistent with RyR carrying its own countercurrent because the SR K^+ or Cl^- channels conduct these substitute ions (Cs^+ and large anions) poorly [15, 16]. Also, Kamp et al. [17] showed that proton fluxes can account for only a small fraction (5–10%) of the necessary charge compensation during RyR Ca^{2+} release, and thus we do not consider H^+ countercurrent.

While both K^+ and Mg^{2+} passing through the RyR can contribute to the countercurrent, they do not contribute equally. One might expect that Mg^{2+} provides the bulk of the countercurrent since Mg^{2+} is almost always the most abundant ion in the selectivity filter (Fig. 2). It is, however, K^+ that provides the vast majority of the countercurrent, as shown in Fig. 4. This is because K^+ has a 5- to 10-fold larger conductance through RyR than Mg^{2+} [7, 18]. This higher conductance overcomes the <4.7 -fold larger number of Mg^{2+} in the selectivity filter (Fig. 2). Note that the ratio of Mg^{2+} to K^+ in the filter is limited to ~ 4.7 by the DiHi-SelLo mechanism because it ensures that K^+ will never be completely displaced from the pore, even at high divalent concentrations.

SR membrane potential

The SR membrane potential is determined by all the ions moving through open channels in the SR membrane, including SR K^+ and Cl^- channels. Open SR K^+ and Cl^- channels essentially clamp the SR membrane potential at zero when RyRs are closed. We previously shown experimentally and theoretically that open RyR's alone (no SR K^+ and Cl^- channels open) would clamp the SR membrane potential close at about -2 mV [9]. Using the same equivalent circuit analysis we applied before [9], Fig. 5 shows how SR membrane potential (V_{SR}) changes over the typical pathophysiological ranges of luminal Ca^{2+} and/or cellular Mg^{2+} (i.e., 0.5 to 2 times normal). This analysis, which assumes only the RyRs are open, indicates that V_{SR} will always between -1 and -5 mV. If the SR K^+ channel conducts significant K^+ current during Ca^{2+} release, then its effect would be to move the potential closer to 0. Indeed, this might be the role the SR K^+ channel plays during such pathological challenges.

It is important to remember that the large self-RyR countercurrent during Ca^{2+} release is a direct consequence of RyR selectivity. As we have shown, having Mg^{2+} on both sides of the membrane greatly increases RyR's apparent affinity for Mg^{2+} . A similar finding holds for K^+ [6]. This high affinity for K^+ and Mg^{2+} ensures that these ions are always present in the pore and therefore can produce countercurrent. In fact, as long as one of these ion species is symmetrically distributed, V_{SR} will never deviate far from zero. Even in the extreme case of 0 cytosolic K^+ and otherwise normal physiological ion concentrations, the symmetric 1 mM Mg^{2+} would provide enough countercurrent to keep V_{SR} far from E_{Ca} (~ -17 mV).

RyR unitary Ca^{2+} current amplitude

By far the largest—and physiologically most important—ramification of RyR's selectivity is on unitary Ca^{2+} current amplitude. Selectivity determines how many Ca^{2+} are in the selectivity filter and this is roughly proportional to i_{Ca} [2]. Over the likely pathophysiological range of luminal Ca^{2+} and/or cellular Mg^{2+} , our RyR permeation model shows that Ca^{2+} occupancy and i_{Ca} are defined by the competition between Ca^{2+} and Mg^{2+} with K^{+} playing only a minor role. It also shows Mg^{2+} is the most abundant ion in the RyR selectivity filter and K^{+} occupancy of the filter remains relatively constant due to the DiHi-SelLo effect. This is consistent with experiments where small changes in $[\text{Mg}^{2+}]$ have large effects on i_{Ca} while large additions of K^{+} (in the presence of millimolar Mg^{2+}) have a relatively small action [6].

The model allows us to compute i_{Ca} over the salient ranges of $[\text{Mg}^{2+}]$ and $[\text{Ca}^{2+}]_{\text{SR}}$, as shown in Fig. 6A. As the divalent concentrations vary, there are large changes in i_{Ca} due to the changing balance of Mg^{2+} and Ca^{2+} occupancy of the pore (Fig. 2). However, the effect of the two divalents are very different. Not surprisingly, an increase in SR Ca^{2+} load leads to an almost proportionate increase in i_{Ca} and an increase in $[\text{Mg}^{2+}]$ leads to a decrease in i_{Ca} . The action of high $[\text{Mg}^{2+}]$ is smaller than that of elevated $[\text{Ca}^{2+}]$. This is because of RyR's high effective Mg^{2+} affinity. Since the open pore is largely occupied by Mg^{2+} , there is relatively little Ca^{2+} for high $[\text{Mg}^{2+}]$ to displace and thus Ca^{2+} current is reduced modestly by high $[\text{Mg}^{2+}]$. On the other hand, high Ca^{2+} can displace some of the abundant Mg^{2+} , increasing the Ca^{2+} current substantially.

The changes in $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Mg}^{2+}]$ that may occur during the pathological conditions are sufficient to alter the balance of divalents in the RyR pore and thus sufficient to alter i_{Ca} . Indeed, the changes in i_{Ca} are surprisingly large, as shown in the percent change of i_{Ca} in Fig. 6B. Increases and decreases of Ca^{2+} current amplitude of >50% are possible. This is possible when $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Mg}^{2+}]$ change in opposite directions. Then, Ca^{2+} current will change more than if only one concentration changed. For example, if $[\text{Ca}^{2+}]_{\text{SR}}$ increases and $[\text{Mg}^{2+}]$ decreases, then Ca^{2+} current will increase because the Ca^{2+} driving force increases and because there is less competition between Ca^{2+} and Mg^{2+} , allowing more Ca^{2+} to enter the pore. On the other hand, if $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Mg}^{2+}]$ move in the same direction, then the change in $[\text{Mg}^{2+}]$ tends to mitigate the change in Ca^{2+} current made by the SR Ca^{2+} load. For example, if both $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Mg}^{2+}]$ decrease, then Ca^{2+} current amplitude will decrease because SR Ca^{2+} load decreased, but the decrease in $\text{Ca}^{2+}/\text{Mg}^{2+}$ competition will allow more Ca^{2+} into the pore, offsetting some of the Ca^{2+} current decrease.

The experiments of Kettlun et al. [6] are consistent with this analysis; the model merely allows us to approximately quantify the results. The extrapolation of this analysis to in vivo Ca^{2+} release then depends on how similar RyR's selectivity between K^{+} , Mg^{2+} , and Ca^{2+} is in cells and bilayers. Many studies have established that conditions in vivo (e.g., the presence of calsequestrin and ATP) regulate RyR-mediated Ca^{2+} release in intact muscle cells [19–22]. To our knowledge, these conditions affect P_0 , but do not substantively change RyR selectivity between the physiological cations (e.g., [23]). Therefore, the phenomena of $\text{Ca}^{2+}/\text{Mg}^{2+}$ competition described here should still substantively affect Ca^{2+} release on the permeation level.

Many of the in vivo agents (including Mg^{2+}) change Ca^{2+} release because they modulate RyR gating. The action of Mg^{2+} on overall RyR Ca^{2+} mobilization is almost always interpreted solely in terms of P_0 change. For example, the recent comprehensive experimental manipulations of intracellular free $[\text{Mg}^{2+}]$ on sparks by Gusev and Niggli [24] never even considered the action of $[\text{Mg}^{2+}]$ on RyR i_{Ca} . Therefore, for comparison, Fig. 6B also shows two published effects of Mg^{2+} -induced changes in single RyR P_0 (dashed line).

The Mg^{2+} and Ca^{2+} dependence of P_o is very complicated. It is influenced by which side of the membrane the ions are on (cytosolic or luminal) [20, 25–27], the presence or absence of ATP [19, 20] or calsequestrin [21], among other factors [22]. Thus, Fig. 6B is necessarily a rather rough ballpark comparison. The Mg^{2+} actions on P_o are about the same magnitude as the Mg^{2+} action on i_{Ca} (at 1 mM Ca^{2+} load). These curves for P_o do not include the effects of cellular substances like ATP because we are interested in showing the general sizes of the effects. However, if such substances increase RyR P_o (e.g., ATP [19]), the changes in P_o due to Mg^{2+} are reduced and therefore smaller than those shown in Fig. 6B.

While these single-channel results on both permeation and gating are approximations to what occurs in vivo, our results indicate that the action of Mg^{2+} on single RyR i_{Ca} can be of the same magnitude as the action of Mg^{2+} on single RyR P_o . Because these two independent mechanisms are both present, future studies should at least consider the role of Mg^{2+} on Ca^{2+} current due to $\text{Ca}^{2+}/\text{Mg}^{2+}$ competition in the pore.

CONCLUSION

We have calculated ionic currents through an open RyR as $[\text{Mg}^{2+}]$ and $[\text{Ca}^{2+}]_{\text{SR}}$ vary over their possible cellular concentrations. This was done using a well-tuned RyR permeation model that both accurately reproduces and predicts a wide range of single-RyR experimental data (including the DiHi-SelLo prediction in Fig. 3). The ideas presented here are based on the ionic selectivity properties of RyR, which are not expected to change significantly in situ. All that is required is that RyR remains a “poor” calcium channel. We report that K^+ and Mg^{2+} permeation through the RyR have two very different and mostly independent actions on SR Ca^{2+} release: RyR K^+ current carries the vast majority of the countercurrent during SR Ca^{2+} release, while the $\text{Ca}^{2+}/\text{Mg}^{2+}$ competition for the RyR pore largely defines the unitary Ca^{2+} current amplitude.

These roles of K^+ and Mg^{2+} RyR permeation are due to their valence and their symmetric distribution across the SR membrane. For Mg^{2+} , its valence means it can compete effectively with Ca^{2+} and its preferential access to the pore (i.e., symmetric distribution) assures it out-competes Ca^{2+} . Consequently, high Mg^{2+} occupancy determines how much Ca^{2+} is in the pore and therefore i_{Ca} amplitude. For K^+ , it is at a lower concentration in the pore than Mg^{2+} , but its valence means that it is bound less tightly than the divalents in the highly-charged selectivity filter. Consequently, it moves more easily through the pore, allowing it to mediate a large countercurrent during Ca^{2+} release.

These roles of K^+ and Mg^{2+} are ramifications of RyR being a “poor” Ca^{2+} channel, as compared to the highly selective voltage-dependent dihydropyridine receptor (DHPR). If the RyR had the same selectivity as the DHPR, then neither K^+ nor Mg^{2+} would be in its pore and it could not carry its own countercurrent [9]. Thus, self-mediated countercurrent is an advantage of a “poorly” selective RyR pore. The obvious disadvantage is a smaller Ca^{2+} current because Mg^{2+} competes with Ca^{2+} in the pore. Without this inter-ion competition, i_{Ca} would be ~2 times larger under physiological conditions [6]. But, highly selective calcium channels like DHPR that exclude Mg^{2+} typically have ~10 times smaller Ca^{2+} conductances than the RyR [28]. This is largely because of their relatively narrow pore and high Ca^{2+} affinity [29], so even with $\text{Ca}^{2+}/\text{Mg}^{2+}$ competition, single RyR i_{Ca} is still relatively large. Thus, RyR’s “poor” selectivity is overall advantageous and fundamental to its physiological role of mediating efficient SR Ca^{2+} release.

Acknowledgments

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APPENDIX A: SUMMARY OF THE MODEL

The PNP/DFT model of RyR [1, 2] describes the electrodiffusion of ions through the pore of an open tetrameric RyR channel. Each RyR subunit is modeled with the five glutamates and aspartates that mutation experiments have shown significantly affect either K^+ permeation or Ca^{2+} selectivity [30–32]. The model differs from other published RyR permeation models (e.g., barrier models [33]) in that it uses the most recent theories of electrolytes in confined geometries to describe the interactions of ions with each other and, most importantly, with the charged amino acids of the protein. It is these latter interactions that generate RyR selectivity [2].

It is important to note that model parameters are not adjusted in order to replicate the experimentally observed RyR selectivity. Rather, selectivity is an *output* of the model based solely on how the ions interact with the protein charges. In fact, the only model parameter that was ever adjusted is the pore diffusion coefficient that is unique for particular ions (Li^+ , Na^+ , K^+ , Rb^+ , Cs^+ , Mg^{2+} , and Ca^{2+}). The value for those diffusion coefficients were initially defined in early work [4] and have never been changed thereafter. In the selectivity filter, the diffusion coefficients are $6.91 \times 10^{-11} \text{ m}^2/\text{s}$ for K^+ , $0.42 \times 10^{-11} \text{ m}^2/\text{s}$ for Mg^{2+} , and $0.41 \times 10^{-11} \text{ m}^2/\text{s}$ for Ca^{2+} .

The currents in different experimental conditions (ionic concentrations and voltages) are determined solely by the physics of electrolytes included in the model. This includes the drift-diffusion of the ions down their electrochemical gradient where the ions are charged, hard spheres with fixed size. Indeed, it is the size of the ions that generates RyR selectivity as ions and protein charges interact within the selectivity filter where permeant ion concentrations can reach $\sim 20 \text{ M}$ [1, 2]. The selectivity filter is so crowded because it contains bulky amino acids with negative charge in a very small volume (a charge density of $\sim -13 \text{ M}$) and this attracts cations to similarly high concentrations. At such high concentrations, it is more difficult for larger sized ions to find empty space to occupy. Therefore small, highly-charged cations like Ca^{2+} and Mg^{2+} are favored over monovalents. Among monovalents, smaller ones like Li^+ are favored over larger ones like Cs^+ [2].

To date, the model has accurately predicted RyR current in more than 160 different ionic concentrations (including of divalent and monovalent cation mixtures) over a wide range of applied voltages [1, 2, 8–10]. To stringently evaluate this model, several a priori model predictions were made and subsequently experimentally verified without the predictions known to the experimentalists [1, 2, 8–10]. These predictions include the reduction in divalent cation selectivity at high divalent concentrations (Fig. 3), net current in mixtures of three monovalent cations over a wide range of concentrations [10], and anomalous mole fraction effects in mixtures of Na^+ and Cs^+ [1] and in mixtures of Ca^{2+} and Li^+ , Na^+ , K^+ , or Cs^+ [2, 8], including their concentration and voltage behavior [8]. This unprecedented level of RyR permeation model validation instills substantial confidence that the ionic currents calculated by this model accurately reflect those carried by the real channel.

APPENDIX B: MATERIALS AND METHODS

Heavy SR microsomes were prepared from rat ventricular muscle using previously described methods [34]. Planar lipid bilayers were composed of a 5:4:1 mixture (50 mg/ml in decane) of bovine brain phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine. Bilayers were formed across a $100 \mu\text{m}$ diameter hole in a Teflon

partition separating two compartments. One compartment (cis) was virtually grounded and filled with a HEPES-Tris solution (250 mM HEPES, 120 mM Tris, pH 7.4). The other compartment (trans) was initially filled with HEPES-Ca solution (250 mM HEPES, 50 mM $\text{Ca}(\text{OH})_2$, pH 7.4). Then, 500 mM CsCl, 2 mM CaCl_2 , and 5–15 μg heavy SR microsomes were added to the cis chamber. Channel incorporation always resulted in the cytosolic side of the RyR2 channel facing the cis compartment [35]. Immediately upon observing single channel activity, the cytosolic and luminal solutions were replaced to establish our test conditions.

The test cis and trans solutions (pH 7.4) contained equal monovalent concentration (100, 250, or 500 mM K^+). These were added as HEPES or methansulfonate salts. The trans solution contained 1, 2.5, or 5 mM CaCl_2 to maintain a $[\text{Ca}^{2+}]:[\text{K}^+]$ ratio of 1:100. The free Ca^{2+} concentration in the cis solution (unbuffered) was always between 1 and 5 μM , as verified by Ca^{2+} electrode measurements. For the Li^+/K^+ monovalent experiments, the test cis and trans solutions (pH 7.4) contained equal monovalent concentration (100, 250, or 500 mM K^+ and 100, 250, or 500 mM, respectively, Li^+) added as HEPES or methansulfonate salts.

Single channel recordings were made at room temperature (20–22°C). Analysis was done using pCLAMP9 software (Molecular Devices, Sunnyvale, CA). Currents were sampled at 50 $\mu\text{s}/\text{pt}$ and filtered at 1 kHz (4-pole Bessel; dead time $\sim 200 \mu\text{s}$). Current amplitude was determined from all-points histograms.

Figure 1.
(color online) Concentration profiles of K^+ (dotted lines), Mg^{2+} (dashed lines), and Ca^{2+} (solid lines) under resting physiological conditions ($[K^+] = 120$ mM, $[Mg^{2+}] = 1$ mM, $[Ca^{2+}]_{SR} = 1$ mM).

Figure 2. (color online) The occupancy of the model RyR selectivity filter by K^+ (dotted lines), Mg^{2+} (dashed lines), and Ca^{2+} (solid lines). The thin vertical lines indicate the resting physiological concentrations. (A) SR Ca^{2+} load $[Ca^{2+}]_{SR}$ is varied. $[K^+] = 120$ mM and $[Mg^{2+}]$ is indicated. (B) $[Mg^{2+}]$ is varied. $[K^+] = 120$ mM and $[Ca^{2+}]_{SR}$ is indicated.

Figure 3.

The DiHi-SelLo effect. FRC (see text) at -20 mV is plotted as a function of symmetric $[K^+]$ while $[K^+]:[Ca^{2+}]_{SR}$ is kept constant at 100:1. $[Ca^{2+}]_{cyto}$ is $1-5 \mu M$. (*Inset*) FRC is plotted as a function of symmetric $[K^+]$ while $[Li^+]:[K^+]$ is kept constant at 1:1. Symbols are the experimental results and lines are the model predictions, computed before the experiments were performed.

Figure 4.

(color online) The percent of countercurrent carried by K⁺. In all cases, V_{rev} (the SR membrane potential in the absence of other channel types) was calculated for the indicated ionic conditions. At that voltage, Ca²⁺ current is outward ($i_{Ca} > 0$) and the K⁺ and Mg²⁺ countercurrents are inward ($i_K < 0$, $i_{Mg} < 0$). $|i_K / i_{Ca}|$ is plotted. (*Left*) SR Ca²⁺ load [Ca²⁺]_{SR} is varied. [K⁺] = 120 mM and [Mg²⁺] is indicated. (*Right*) [Mg²⁺] is varied. [K⁺] = 120 mM and [Ca²⁺]_{SR} is indicated.

Figure 5.

(color online) The SR membrane potential, which is the RyR reversal potential because we assume that the RyR is the only channel in the SR membrane (the worst case scenario) [9].

(Left) SR Ca^{2+} load $[Ca^{2+}]_{SR}$ is varied. $[K^+] = 120$ mM and $[Mg^{2+}]$ is indicated. (Right)

$[Mg^{2+}]$ is varied. $[K^+] = 120$ mM and $[Ca^{2+}]_{SR}$ is indicated.

Figure 6.

(color online) (A) Unitary Ca^{2+} current (i_{Ca}). (B) Percent change in i_{Ca} . (*Left*) SR Ca^{2+} load $[\text{Ca}^{2+}]_{\text{SR}}$ is varied. $[\text{K}^{+}] = 120$ mM and $[\text{Mg}^{2+}]$ is indicated. (*Right*) $[\text{Mg}^{2+}]$ is varied. $[\text{K}^{+}] = 120$ mM and $[\text{Ca}^{2+}]_{\text{SR}}$ is indicated. In panel B, the dashed lines are the percent change in P_o from the application of Mg^{2+} . The long-dashed line is from Qin et al. [21] where Mg^{2+} was applied on the luminal side of RyR2. The short-dashed line is from Laver et al. [42] where Mg^{2+} was applied on the cytosolic side of RyR1.

Table 1

SR Ca^{2+} load and intracellular $[\text{Mg}^{2+}]$ in cardiac muscle under three pathological conditions (ischemia, hypertension, and heart failure).

	ischemia	hypertension	heart failure
$[\text{Ca}^{2+}]_{\text{SR}}$	↓ [36]	↑ [37]	↓ [38]
$[\text{Mg}^{2+}]$	↑ [39]	↓ [40]	↓ [41]

↑ indicates an increase compared to normal physiological levels and ↓ a decrease. Literature citations are given for each case.