Calcium Activation of Ryanodine Receptor Channels—Reconciling RyR Gating Models with Tetrameric Channel Structure

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Despite its importance and abundance of experimental data, the molecular mechanism of RyR2 activation by calcium is poorly understood. Recent experimental studies involving coexpression of wild-type (WT) RyR2 together with a RyR2 mutant deficient in calcium-dependent activation (Li, P., and S.R. Chen. 2001. *J. Gen. Physiol.* 118:33–44) revealed large variations of calcium sensitivity of the RyR tetramers with their monomer composition. Together with previous results on kinetics of Ca activation (Zahradníková, A., I. Zahradník, I. Györke, and S. Györke. 1999. *J. Gen. Physiol.* 114:787–798), these data represent benchmarks for construction and testing of RyR models that would reproduce RyR behavior and be structurally realistic as well. Here we present a theoretical study of the effects of RyR monomer substitution by a calcium-insensitive mutant on the calcium dependence of RyR activation. Three published models of tetrameric RyR channels were used either directly or after adaptation to provide allosteric regulation. Additionally, two alternative RyR models with Ca binding sites created jointly by the monomers were developed. The models were modified for description of channels composed of WT and mutant monomers. The parameters of the models were optimized to provide the best approximation of published experimental data. For reproducing the observed calcium dependence of RyR tetramers containing mutant monomers (a) single, independent Ca binding sites on each monomer were preferable to shared binding sites; (b) allosteric models were preferable to linear models; (c) in the WT channel, probability of opening to states containing a Ca^{2+}-free monomer had to be extremely low; and (d) models with fully Ca-bound closed states, additional to those of an Monod-Wyman-Changeaux model, were preferable to models without such states. These results provide support for the concept that RyR activation is possible (albeit vanishingly small in WT channels) in the absence of Ca^{2+} binding. They also suggest further avenues toward understanding RyR gating.

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INTRODUCTION

The SR calcium release channel (also known as the ryanodine receptor, RyR) is composed of four identical subunits (Lai et al., 1989). It forms characteristic foot structures (Inui et al., 1987) that span the gap between t-tubules and junctional SR in skeletal and cardiac muscle (Block et al., 1988; Sun et al., 1995). The massive cytoplasmic domain of RyR has fourfold rotational symmetry as revealed by electron cryomicroscopy and image reconstruction (for review see Wagenknecht and Samsó, 2002). This domain contains binding sites for numerous cytoplasmic regulators of RyR activity, including calcium and ATP (for review see Meissner, 2002, 2004).

In the physiological context, the most important regulator of the cardiac RyR isoform (RyR2) is cytoplasmic calcium. RyR2 activation by calcium forms the basis of calcium-induced calcium release, a mechanism responsible for excitation–contraction coupling in cardiac muscle (Fabiato, 1985). The activation of the RyR by calcium ions has been the subject of extensive experimental work (for review see Coronado et al., 1994; Meissner, 2004). When studied in planar lipid bilayers, RyR2s exhibit a steady-state calcium dependence with EC_{50} of ~1 μM (in the absence of Mg and ATP; Coronado et al., 1994; Meissner, 2004). Kinetic studies with application of fast photolytically generated Ca^{2+} spikes showed a steep calcium dependence of activation, implying that binding of several Ca^{2+} ions ($n \sim 4$) must take place for transition of the resting channel to an open state (Zahradníková et al., 1999). These and other studies led to construction of numerous gating models of the channel (Ashley and Williams, 1990; Sitsapesan and Williams, 1994; Tang and Othmer, 1994; Sachs et al., 1995; Schiefer et al., 1995; Zahradníková and Zahradník, 1996; Keizer and Levine, 1996; Villalba-Galea et al., 1998; Stern et al., 1999; Fill et al., 2000; Saftenku et al., 2001; Sobie et al., 2002; Villalba-Galea et al., 2002; Rosales et al., 2004). These models, while adequate in describing phenomenological aspects of RyR gating, lack clear-cut means of attributing

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Abbreviations used in this paper: a, allosteric; AG, adaptation gating; EMG, extended minimal gating; HM, H-mode; JBS, joint binding site; M, mutant; MWC, Monod-Wyman-Changeux; QM, quarter mutant; SBS, shared binding sites; SM, semi mutant; TM, three-quarter mutant; WT, wild-type.

particular gating transitions to calcium binding at the respective calcium binding sites on the RyR, or, in other words, lack realistic structural context. To our knowledge, there are only three published models of RyR that take into account the tetrameric structure of RyR when describing its calcium activation: the adaptation gating model (AG; Cheng et al., 1995), our extended minimal gating model (EMG; Zahradníková et al., 1999), and a subset of EMG containing only the fast-access states of the H-mode (HM; Dura et al., 2003). In contrast, models of other channel types often possess direct correlates between channel structure and function (Marks and Jones, 1992; Bezprozvanny and Ehrlich, 1993; Goulding et al., 1994; Edelstein et al., 1996; Galzi et al., 1996; Varnum and Zagotta, 1996; Cox et al., 1997; Tibbs et al., 1997; Mak et al., 2003).

The homotetrameric structure of RyR (a), its fourfold rotational symmetry (b), and clear experimental evidence that binding of several calcium ions is required for RyR2 activation (c) all suggest that RyR2 activation by calcium is an allosteric process. Therefore, it may be treated according to the allosteric concept of Monod et al. (1965) in analogy to the elegant treatment of RyR1 activation by the DHPR voltage sensors (Rios et al., 1993). Models describing activation of RyR2 by calcium might carry a structurally distinct Ca^{2+} binding site on each monomer. Alternatively, each monomer might contribute partially to the formation of calcium sensor(s) shared by several monomers (as suggested by Li and Chen, 2001).

Recently Li and Chen (2001) have probed the molecular basis of RyR2 calcium-dependent activation by coexpressing wild-type (WT) RyR2 together with an RyR2 mutant deficient in calcium-dependent activation (E3987A). Importantly, five different channel variants were observed, each with a distinct calcium sensitivity that differed by up to several orders of magnitude. The five variants supposedly represented different combinations of WT and mutant monomers in the channel tetramer. The new dimension of these studies, the variation of calcium sensitivity with monomer composition of the RyR tetramer, provides an opportunity to test and compare RyR models with different mechanisms of activation by Ca^{2+}.

In the present study, we analyzed the effect of monomer substitution by its calcium-insensitive mutant counterpart on the calcium dependence of activation of the resulting RyR tetramers using the three published tetrameric models of RyR, and several new models with various implementations of the channel calcium sensor(s). We show, using mathematical modeling, that for description of RyR gating in mixtures of WT and mutant monomers, models in which four independent calcium binding sites are responsible for the calcium-dependent activation of the channel are preferable to models in which two or four subunits create a joint calcium binding site. In the optimal model, calcium binding and channel opening were allosterically coupled. Opening of the WT channel to states with partially occupied binding sites had very low probability. The model also required inclusion of further, fully calcium-bound closed states, in addition to the states representing the open and closed conformations of the channel at all configurations of calcium binding site occupation. These results provide new insights into the molecular mechanisms of RyR activation by calcium and offer important clues regarding RyR regulation in health and disease.

MATERIALS AND METHODS

Optimization of Models

Calculations of the theoretical calcium dependence of channel open probability for individual RyR variants corresponding to different combinations of WT and mutant (M) monomers in individual gating schemes were performed in Mathematica (Ver. 5.0, Wolfram Research) as described previously (Zahradníková and Zahradník, 1996; Zahradníková et al., 1999), by solving the system of equations describing the equilibria between channel states via their dissociation constants. The resulting formulae, expressing channel open probability as a function of monomer composition and calcium concentration, are presented in the electronic supplement (available at http://www.jgp.org/cgi/content/full/jgp.200509328/DC1). They were compiled, and their variable parameters were fitted in OriginPro (Ver. 7.0, OriginLab) to the data taken from Fig. 7 D of Li and Chen (2001). Only the three most sensitive RyR variants were taken into account because of the experimentally observed overlap between calcium activation and calcium inactivation range of the two least sensitive variants (Li and Chen, 2001) that prevented reliable parameter estimation.

Statistical Analysis

The values of χ^2 were determined from the sum of squares of differences between experimental data and model prediction, and from the experimental variance. The quality of data description by the models was characterized by the χ^2 test (Press et al., 1992) as described previously (Zahradníková et al., 1999). The significance of differences between the models was assessed by means of the F-test (Press et al., 1992).

Dose–response parameters of the data and the models were approximated by the Hill equation. The 95% confidence interval for the parameters was assumed to be equal to ± 2.5 times their standard error of the fit.

Notation

Channel gating schemes are shown in a simplified notation (Fig. 1). Closed states (C) are depicted as squares, and open states (O) are depicted as circles. All channel transitions are reversible and obey the principle of detailed balance. The indexes of individual states, denoting the number of bound calcium ions or a particular mode of activity, are shown as numbers/letters inside the squares/circles. When two types of calcium binding sites are present, the first index corresponds to the binding site that undergoes mutation. Calcium-dependent channel transitions are depicted as thick lines, which are solid if the transition is affected by mutation, and dotted, if the transition is unaffected by mutation. Other transitions between states are depicted as thin lines.

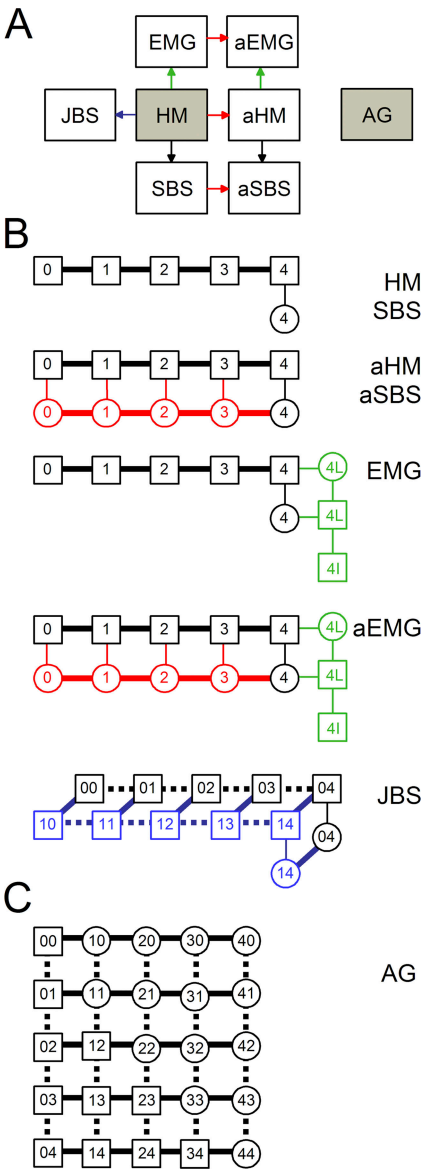

Figure 1. Candidate RyR gating models. (A) Relationships between models. HM, H-mode model (Dura et al., 2003); aHM, allosteric H-mode model; EMG, extended minimal gating model (Zahradníková et al., 1999); aEMG, allosteric EMG model; SBS, shared binding sites model; JBS, joint binding site model; aSBS, allosteric SBS model; AG, adaptation gating model (Cheng et al., 1995). Models HM and AG (shaded rectangles) are unrelated. The relationships between linear and allosteric (red), basic and extended (green), and independent and shared binding site models (black) are shown as arrows in the respective colors. The JBS model was derived from the HM model by adding an additional, joint calcium binding site (blue arrow). (B) Gating schemes of wild-type channel models. Squares and circles denote closed and open states, respectively. States and transitions specific for allosteric, extended, and joint binding site models are shown in red, green, and blue, respectively. Thick solid lines denote Ca^{2+}-dependent transitions potentially affected by the mutation. Dotted lines denote calcium-dependent transitions unaffected by the mutation. Thin lines denote calcium-independent transitions. Numerals (or the first numerals) denote the number of Ca^{2+} ions bound to the site(s) affected by the mutation. The second numeral, where applicable, denotes the number of Ca^{2+} ions bound to the site(s) unaffected by the mutation. The letters L and I denote states of the L and I mode (Zahradníková et al., 1999), respectively. The arrows characterizing the direction of the transitions are omitted. All equilibria between channel states were described using dissociation constants (i.e., the reciprocal values of the respective equilibrium constants).

Online Supplemental Material

The gating schemes of tested channel models with 0, 1, and 2 mutant monomers are presented in the online supplemental material together with the relationships between parameter values and steady-state probabilities of individual channel states (available at http://www.jgp.org/cgi/content/full/jgp.200509328/DC1). The fitting functions, describing the open probability of channel models as a function of parameter values and calcium concentration are also presented.

RESULTS

In the present study we analyzed three previously published tetrameric RyR models, two new simple models with shared calcium sensor(s), and where possible, their allosteric counterparts. Calculations of the calcium dependence of RyR2 activation for tetramers containing zero to two M monomers were based on modification of these gating models as detailed below.

Selection of Models for the Study

The relationships between individual models are shown in Fig. 1 A. The models are described in the order of increasing complexity. A detailed description of model parameters and their relationships to probability of channel states is presented in the online supplement (available at http://www.jgp.org/cgi/content/full/jgp.200509328/DC1). The simplest model, the HM model (Dura et al., 2003), contains four independent Ca^{2+} binding sites, one on each monomer. When all binding sites are occupied by Ca^{2+}, the channel can make the transition into the open state. These transitions result in the gating scheme shown in Fig. 1 B (top). The gating of the channel can be fully described by two parameters: K_{Ca}, the microscopic Ca^{2+} dissociation constant of individual binding sites; and K_{O4}, the dissociation constant of the open state (O_4, depicted as 4 inside a circle) relative to the last closed state (C_4, depicted as 4 inside a square) and thus determines the maximum open probability of the channel (see the online supplemental material). The conceptual scheme of calcium binding sites within the tetrameric structure of the channel in relation to monomer composition is depicted in the first row of Fig. 2 (top). The effect of mutation on channel gating was implemented as a decrease in the affinity of the M Ca^{2+} binding site (yellow

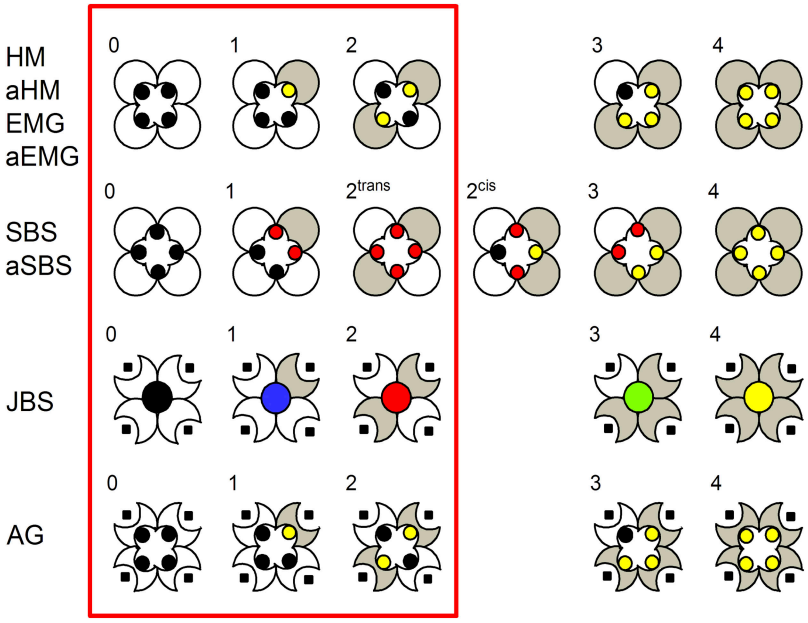

Figure 2. Schematic description of calcium binding sites in RyR channel models containing zero to four mutant subunits. Symbols next to individual tetramer variants denote the number of mutant monomers. The variants are arranged in the order of decreasing calcium affinity. Wild-type and mutant monomers are shown in white and gray, respectively. The variants used in the study are delineated by a red rectangle. The calcium binding sites affected by the mutations are depicted in the inner part of the tetramer. In the JBS model, the joint calcium binding site is represented by the central circle. Extent of mutation (i.e., different affinity) of the binding site is represented by its color: black, wild type (WT); blue, quarter mutant (QM); red, semi mutant (SM); green, three-quarter mutant (TM); yellow, full mutant (M). Calcium binding sites that do not undergo mutation, if present, are depicted at the outer perimeter of the tetramer as black squares. Please note that even with equivalent and independent monomers, Ca^{2+} binding sites of trans and cis double mutants (2^{cis} and 2^{trans}, respectively) differ in SBS and aSBS models.

circles) with no change in the remaining equilibria (see supplemental data for detailed descriptions of all gating schemes of channels containing mutant monomers).

The Monod-Wyman-Changeux (MWC; Monod et al., 1965) allosteric principle describes the effect of conformational changes on the monomers (in our case, calcium binding) on the concerted conformational change of the whole protein (in our case, channel opening), which are considered separate but allosterically coupled steps. Extension of the HM model by application of the MWC principle resulted in the allosteric HM model (aHM; "a" standing for allosteric will be used as a prefix for the sake of consistency in all allosteric models). The model has four independent Ca^{2+} binding sites, one on each monomer, with Ca^{2+} dissociation constants K_{Ca}. In contrast to the HM model, the channel can open independently of the occupation of Ca^{2+} binding sites, resulting in four additional open states with respect to the HM model (Fig. 1 B, second row; O_0–O_3, denoted as numbers inside circles). The tendency for opening at different levels of Ca^{2+} binding is determined by two thermodynamic constants. K_{O0} is the dissociation constant of the calcium-free open state (O_0) relative to the calcium-free closed state (C_0), and the allosteric parameter f is equal to the ratio of calcium dissociation constants of the open states relative to those of closed states (the smaller the value of f, the lower the microscopic calcium dissociation constant of open states relative to closed states). Maximum open probability is unequivocally determined by the value of $K_{O4} = f^4 K_{O0}$ (see the online supplemental material). Therefore at a constant value of maximum open probability in the limiting case of $f \to 0$, the aHM model becomes equivalent to the HM model ($K_{O0} \to \infty$). Again, the effect of mutation (Fig. 2, top) was expressed as a decrease in the affinity of the corresponding Ca^{2+} binding site (yellow circles) without any change in the remaining equilibria.

The shared binding sites (SBS) model was created from the HM model by postulating that the four Ca^{2+} binding sites of the channel are each created by sharing residues of two neighboring monomers. The gating scheme of the wild-type channel has two parameters and is identical to that of the HM model (Fig. 1 B, top). Upon combination of WT and M monomers (Fig. 2, second row), semi mutant (SM) binding sites (red circles) form in addition to WT (black circles) and M binding sites (yellow circles). The effect of mutation was implemented as a progressive decrease in the affinity of the corresponding SM and M Ca^{2+} binding sites (red and yellow circles) without any change in the remaining equilibria.

The allosteric SBS (aSBS) model was created from the SBS model by allowing the channel to open independently of the occupation of Ca^{2+} binding sites (i.e., the relationship between the SBS and aSBS models is the same as the relationship between the HM and aHM models). The gating scheme of the wild-type channel has three parameters and is identical to that of the aHM model (Fig. 1 B, second row). Upon combination of WT and M monomers (Fig. 2, second row), SM binding sites (red circles) form in addition to WT (black circles) and M binding sites (yellow circles). At a constant value of maximum open probability, the aSBS model becomes equivalent to the SBS model in the limiting case of $f \to 0$. The effect of mutation was implemented as a progressive decrease in the affinity of the correspond-

ing SM and M Ca^{2+} binding sites (red and yellow circles) without any change in the remaining equilibria.

The EMG model, developed for description of single-channel kinetics and modal behavior of the RyR (Zahradníková et al., 1999), is an extension of the HM model containing additional, fully calcium-occupied open and closed states that constitute the two additional activity modes, the L and I modes (Zahradníková and Zahradník, 1996; Zahradníková et al., 1999; Fig. 1 B, third row). The calcium dependence of RyR activation is described by five parameters. As in the HM model, the microscopic Ca^{2+} dissociation constant of individual binding sites on the monomers is determined by the parameter K_{Ca}, and the parameter K_{O4} defines the dissociation constant of the open state relative to the last closed state. Three additional dissociation constants K_{OL}, K_{CL}, and K_{CI} (see the online supplemental material) define the equilibria involving states of the L and I modes of activity, O_L, C_L, and C_I (corresponding to states O_2, C_5, and I in the notation of Zahradníková et al., 1999). For the purpose of the present study, the most important effect of these additional states is a decrease in the maximal open probability of the channel. The EMG model becomes equivalent to the HM model as the probabilities of the L and I modes approach 0 (i.e., as the dissociation constants K_{OL}, K_{CL}, and K_{CI} approach infinity). In this model, the effect of mutation was implemented as a decrease in the affinity of the corresponding Ca^{2+} binding site (yellow circles in Fig. 2, first row) without any change in the remaining equilibria.

The allosteric EMG (aEMG) model was created from the EMG model by allowing the channel to open independently of the occupation of Ca^{2+} binding sites (i.e., the relationship between the EMG and aEMG models is the same as the relationship between the HM and aHM models; Fig. 1 B, fourth row). The calcium dependence of RyR activation in the aEMG model is described by six parameters. As in the aHM model, the microscopic calcium dissociation constant of the calcium binding sites on the monomers is defined by the parameter K_{Ca}, K_{O0} is the dissociation constant of the calcium-free open state (O_0) relative to the calcium-free closed state (C_0), and the allosteric parameter f is equal to the ratio of calcium dissociation constants of the open states relative to those of closed states. The additional dissociation constants K_{OL}, K_{CL}, and K_{CI} (see online supplemental material) are analogous to those of the EMG model. At a constant value of the maximum open probability, the aEMG model becomes equivalent to the EMG model in the limiting case of $f \rightarrow 0$. It becomes equivalent to the aHM model as the probabilities of the L and I mode approach 0. The effect of mutation was implemented as a decrease in the affinity of the M calcium binding site (yellow circles in Fig. 2, first row) without any change in the remaining equilibria.

The joint binding site (JBS) model (Fig. 1 B, fifth row) was derived from the HM model by adding a fifth Ca^{2+} binding site, created jointly by all four monomers. The model is fully described by four parameters. Calcium binding to the channel is characterized by the parameters K_{Ca}, the dissociation constant of the joint Ca^{2+}-binding site, and K_{Ca}^{I}, the microscopic dissociation constant of the independent Ca^{2+}-binding sites on individual monomers. The parameters K_{O0} and K_{O1} are dissociation constants of the open states when the joint calcium binding site is empty and occupied, respectively.

The four independent Ca^{2+} binding sites, corresponding to those of the HM model, are not affected by the mutation (squares in Fig. 2, third row). The replacement of each WT monomer by an M monomer was assumed to progressively decrease the calcium affinity of the joint binding site (blue, red, green, and yellow circle, respectively) without affecting any other equilibria. The common feature of the JBS and aHM models is that the channel can open in the presence as well as in the absence of Ca^{2+} binding to the binding site that is affected by the mutation.

The AG model of Cheng et al. (1995) is not directly related to the other models described above. It has been conceived to describe the mechanism of RyR adaptation. It has two types of Ca^{2+} binding sites (Fig. 1 B, bottom). Ca^{2+} binding to the O sites (first index), characterized by the microscopic dissociation constant K_{Ca}, leads to channel activation. Ca^{2+} binding to the A sites (second index), characterized by the microscopic dissociation constant K_A, leads to channel adaptation, i.e., to channel closure. Individual Ca^{2+} binding sites are independent. The channel is open when at least one of the monomers has Ca^{2+} bound to its O site, and when the number of occupied A sites is less than or equal to the number of occupied O sites. The published parameter values are $K_{Ca} = 0.5$ μM and $K_A = 0.1$ μM (Cheng et al., 1995). The effect of mutations in individual monomers on the calcium binding sites is shown in Fig. 2 (bottom). The A sites are denoted as solid squares. The effect of mutation was implemented as a decrease in the affinity of the O sites (yellow circles) without any change in the remaining equilibria. As the maximum open probability of this model is by definition equal to 1, for the purpose of this study it was assumed that there is a separate, independent process that limits P_O^{max} to the experimentally observed value of 0.85 (in analogy to Mak et al., 2003).

Derivation of Models Containing Mutant Subunits

The calcium-binding equilibria (in the case of allosteric models, the calcium-binding equilibria of the resting state of the channel) were described using microscopic dissociation constants of individual calcium binding

TABLE I

The Parameters and Goodness of Fit of Model Gating Schemes

Parameter	HM	aHM	SBS	aSBS	EMG	aEMG	JBS	AG
K_{Ca}^{WT} (μM)	0.29 ± 0.03	0.39 ± 0.04	0.30 ± 0.03	0.66 ± 0.13	1.36 ± 0.08	0.60 ± 0.09	1.18 ± 0.16	0.50 ± 0.15
K_{Ca}^{QM} (μM)							21.2 ± 4.3	
K_{Ca}^{SM} (μM)			39.8 ± 4.0	89 ± 14			670 ± 99	
K_{Ca}^{M} (μM)	112 ± 12	215 ± 40	15000[a]	15000[a]	1423 ± 135	471 ± 125		1379 ± 235
f		0.12 ± 0.02		0.27 ± 0.03		0.046 ± 0.020		
K_{O0}		851[b]		33.21[b]		10800[b]	100[a]	
K_{O1}							0.176[b]	
K_{O4}	0.176[b]	0.176[b]	0.176[b]	0.176[b]	0.00219[b]	0.048[b]		
K_{OL}					0.5[a]	0.5 [a]		
K_{CL}					0.0333[a]	0.89 ± 0.52		
K_{CI}					3[a]	3[a]		
K_{Ca}^{I} (μM)							0.1[a]	
K_A								0.47 ± 0.13
Variable	HM	aHM	SBS	aSBS	EMG	aEMG	JBS	AG
P_O^{min}	0	0.0011	0	0.029	0	0.0001	0	0
χ^2	160.9	94.7	196.0	113.6	132.6	82.0	97.5	170.6
df	74	72	74	73	74	71	73	73
P	2.2×10^{-8}	0.038	5.7×10^{-13}	0.0017	3.4×10^{-5}	0.17	0.029	8.7×10^{-10}

Errors are shown as standard error of the fit. P_O^{min}, open probability in the absence of Ca^{2+}; χ^2, chi-square value; df, degrees of freedom; P, probability that the differences between data and model are due to random deviations.

[a]Constant parameters.

[b]Parameters calculated from the remaining parameters and from the value of $P_O^{max} = 0.85$.

sites. Models of channels containing M monomers were derived by postulating that the calcium affinity of the mutant binding site(s) is decreased, while calcium binding to the wild-type binding sites remains intact. In the case of the JBS, SBS, and aSBS models, the calcium affinities of quarter mutant (QM), SM, three-quarter mutant (TM), and M binding sites were considered independent.

In the case of HM, aHM, EMG, aEMG, and AG models, the binding sites are located on individual monomers. Therefore, the equilibria between channel states differing in the number of bound calcium ions (Eq. 1),

$$S_{i,j} + Ca^{2+} \Leftrightarrow S_{i+1,j}, \qquad S_{i,j} + Ca^{2+} \Leftrightarrow S_{i,j+1}, \tag{1}$$

where $S_{i,j}$ denotes the state with i and j Ca^{2+} ions bound to the WT and M monomers, respectively, were calculated using probability factors for association and dissociation of calcium (Eq. 2):

$$\frac{[S_{i,j}] \cdot [Ca^{2+}]}{[S_{i+1,j}]} = K_{Ca}^{WT} \cdot \frac{n-i}{i+1}, \qquad \frac{[S_{i,j}] \cdot [Ca^{2+}]}{[S_{i,j+1}]} = K_{Ca}^{M} \cdot \frac{m-j}{j+1}, \tag{2}$$

where n and m stand for the number of WT and M monomers, respectively (m + n = 4).

In the case of the SBS and aSBS models, the binding sites are jointly formed by two neighboring monomers. Therefore, upon combination of WT and M monomers, three types of binding sites, WT, SM, and M, are formed. The equilibria between channel states differing in the number of bound calcium ions (Eq. 3),

$$S_{i,j,k} + Ca^{2+} \Leftrightarrow S_{i+1,j,k}, \qquad S_{i,j,k} + Ca^{2+} \Leftrightarrow S_{i,j+1,k}, \qquad S_{i,j,k} + Ca^{2+} \Leftrightarrow S_{i,j,k+1}, \tag{3}$$

where $S_{i,j,k}$ denotes the state with i, j, and k Ca^{2+} ions bound to the WT, SM, and M binding sites, respectively, were therefore calculated using probability factors for association and dissociation of calcium (Eq. 4):

$$\frac{[S_{i,j,k}] \cdot [Ca^{2+}]}{[S_{i+1,j,k}]} = K_{Ca}^{WT} \cdot \frac{n-i}{i+1}, \tag{4a}$$

$$\frac{[S_{i,j,k}] \cdot [Ca^{2+}]}{[S_{i,j+1,k}]} = K_{Ca}^{SM} \cdot \frac{m-j}{j+1}, \tag{4b}$$

$$\frac{[S_{i,j,k}] \cdot [Ca^{2+}]}{[S_{i,j,k+1}]} = K_{Ca}^{M} \cdot \frac{l-k}{k+1}, \tag{4c}$$

where n, m, and l stand for the number of WT, SM, and M binding sites, respectively (l + m + n = 4).

In the case of the JBS model, the binding site is jointly formed by all four monomers. Therefore, upon

combination of WT and M monomers, five types of binding sites, WT, QM, SM, TM, and M, are formed. The equilibria between channel states with empty and occupied joint calcium binding site (Eq. 5),

$$S_{0,j}^{XX} + Ca^{2+} \Leftrightarrow S_{1,j}^{XX}, \quad (5)$$

where $S_{i,j}^{XX}$ denotes the state of the RyR tetramer with the binding site XX and with i Ca^{2+} ions bound to the joint binding site and j Ca^{2+} ions bound to the binding sites corresponding to those of the HM model, was calculated as

$$\frac{[S_{0,j}^{XX}] \cdot [Ca^{2+}]}{[S_{1,j}^{XX}]} = K_{Ca}^{XX}, \quad (6)$$

$$XX \in \{WT, QM, SM, TM, M\}.$$

Approximation of Experimental Data by the Models

The HM Model. The gating of individual channel variants was described by optimizing the parameters K_{Ca}^{WT} and K_{Ca}^{M}, the microscopic Ca^{2+} dissociation constants of individual WT and M binding sites. The parameter $K_{O4} = 0.176$ was determined from the preset value of $P_O^{max} = 0.85$ (Li and Chen, 2001). Parameters of the optimal model are given in Table I. The calcium sensitivity of the M monomer was decreased ~400× relative to the WT monomer.

The effect of consecutive substitutions of the WT monomers by M monomers on the calcium dependence of RyR open probability is depicted in Fig. 3 (top left) overlaid on the data points replotted from Fig. 7 D of Li and Chen (2001). It can be seen that while the calcium dependence of variants with $n_M = 0$ and $n_M = 2$ is well described by the model, the model exhibits significant deviations from the experimentally observed behavior for the variant with $n_M = 1$.

The aHM Model. The gating of individual channel variants was described by optimizing the parameters K_{Ca}^{WT} and K_{Ca}^{M}, the microscopic Ca^{2+} dissociation constants of individual WT and M binding sites, and the allosteric parameter f, characterizing the calcium affinity of open states relative to that of closed states. The parameter $K_{O0} = 0.176/f^4$ was determined from the preset value of $P_O^{max} = 0.85$ (Li and Chen, 2001). Parameters of the optimal model are given in Table I. The calcium sensitivity of the M monomer was decreased ~500× relative to the WT monomer.

The effect of consecutive substitutions of the WT monomers by M monomers on the calcium dependence of RyR open probability is depicted in Fig. 3 (top right). It can be seen that the calcium dependence of all variants is quite satisfactorily described by the model. For the WT channel, the open state with four Ca^{2+} ions bound was predominant at Ca^{2+} concentrations above the apparent calcium dissociation constant, but at lower values of Ca^{2+} ~50% of open probability was due to open states with three or less Ca^{2+} ions bound (black hatched area). The open probability in the absence of Ca^{2+} was $P_O^{min} = 0.0011$. Open states with less than four bound Ca^{2+} ions became quite prominent in the variant with $n_M = 1$ (red hatched area), while their abundance in the variant with $n_M = 2$ (green hatched area) was similar to that of the WT channels.

It was not possible to describe the observed changes in calcium sensitivity of mutant channels if K_{Ca}^{M} was set equal to K_{Ca}^{WT} and the mutation was supposed to produce only changes in the allosteric parameter f. If both

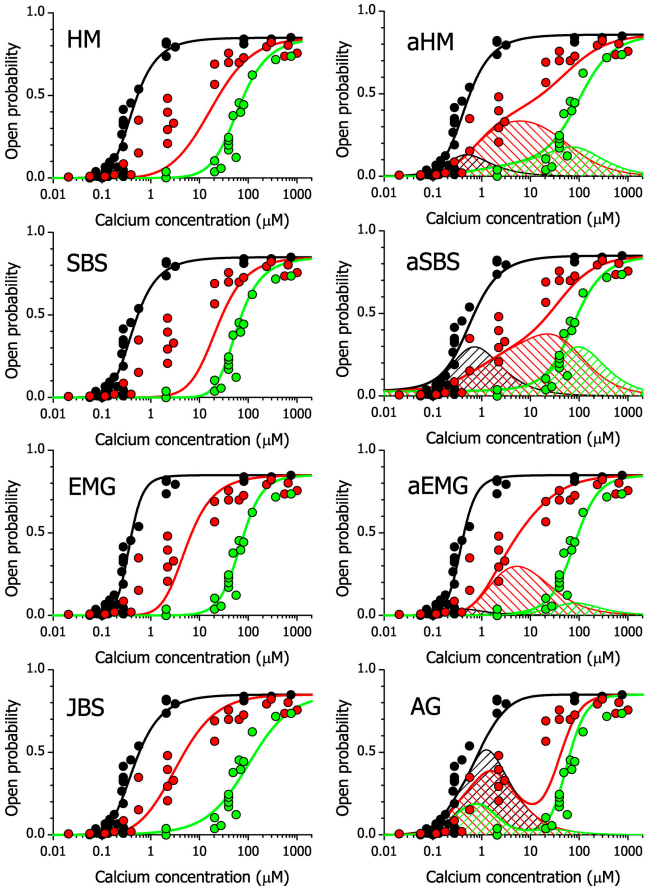

Figure 3. Description of calcium-dependent activation of the RyR tetramers by the studied models. Symbols: the data from Li and Chen (2001) replotted as black, red, and green circles, for $n_M = 0$, 1, and 2, respectively. Thick lines are predictions of the optimal RyR gating models (black, red, and green for $n_M = 0$, 1, and 2, respectively). HM, the H-mode model; aHM, the allosteric H-mode model; SBS, the shared binding sites model; aSBS, the allosteric shared binding sites model; EMG, the extended minimal gating model; aEMG, the allosteric extended minimal gating model; JBS, the joint binding site model; AG, the adaptation gating model. Where applicable, the probability of opening to the states containing less than four calcium ions (black, red, and green, respectively, for $n_M = 0$, 1, and 2) is shown as hatched areas.

K_{Ca} and f were allowed to vary between WT and M monomers, the quality of fit was not improved (unpublished data).

The SBS Model. The gating of individual channel variants was described by optimizing the parameters K_{Ca}^{WT}, K_{Ca}^{SM}, and K_{Ca}^{M}, the microscopic Ca^{2+} dissociation constants of individual WT, SM, and M binding sites. The parameter $K_{O4} = 0.176$ was determined from the preset value of $P_O^{max} = 0.85$ (Li and Chen, 2001). Six different RyR variants (Fig. 2, second row) formed upon combination of WT and M monomers. A satisfactory fit of the calcium-dependent open probabilities of RyR variants could be obtained only for $K_{Ca}^{M} > 15$ mM, and under these conditions the calcium sensitivity of $WT_2M_2^{trans}$ (Fig. 2, second row, third panel), containing four semimutant binding sites, was significantly higher than that of $WT_2M_2^{cis}$ (fourth panel), which contained one fully mutant and three wild-type binding sites. In the examined calcium range of 0.01–2 mM, neither WT_1M_3 nor M_4 activated significantly, so that this model produced five levels of apparent calcium sensitivity similar to those observed by Li and Chen (2001; unpublished data). Parameters of the optimal model are given in Table I. The calcium sensitivity of the SM binding sites was decreased ~130× relative to the WT binding sites.

The effect of consecutive substitutions of the WT monomers by M monomers ($n_M \leq 2$) on the calcium dependence of RyR open probability is depicted in Fig. 3 (second row left). It can be seen that while the calcium dependence of variants with $n_M = 0$ and $n_M = 2$ is well described by the model, the model exhibits significant deviations from the experimentally observed behavior for the variant with $n_M = 1$.

The aSBS Model. The gating of individual channel variants was described by optimizing the parameters K_{Ca}^{WT}, K_{Ca}^{SM}, and K_{Ca}^{M}, the microscopic Ca^{2+} dissociation constants of individual WT, SM, and M binding sites, and the allosteric parameter f, characterizing the calcium dissociation constants of open states relative to that of closed states. The parameter $K_{O0} = 0.176/f^4$ was determined from the preset value of $P_O^{max} = 0.85$ (Li and Chen, 2001). As with the SBS model, six different RyR variants (Fig. 2, second row) formed upon combination of WT and M monomers which, however, produced only five levels of calcium sensitivity in the examined range of calcium concentrations. Parameters of the optimal model are given in Table I. The calcium sensitivity of the SM binding sites was decreased ~130× relative to the WT binding sites.

The effect of consecutive substitutions of the WT monomers by M monomers on the calcium dependence of RyR open probability is depicted in Fig. 3 (second row right). The optimal model was significantly activated in the absence of calcium ($P_O^{min} = 0.029$). It can be seen that at low Ca^{2+} all variants exhibit significant deviations from the experimentally observed behavior due to this basal activation, while the activity of channels is relatively well described by the model at higher calcium concentrations. For the WT channel, open probability at Ca^{2+} concentrations below the apparent calcium dissociation constant was mostly due to open states with three or less Ca^{2+} ions bound (black hatched area), and the open state with four Ca^{2+} ions bound started to predominate only at $[Ca^{2+}] > 1$ μM. Open states with less than four bound Ca^{2+} ions were still more prominent in the variant with $n_M = 1$ (red hatched area), and their abundance in the variant with $n_M = 2$ (green hatched area) was similar to that of the WT channels.

The EMG Model. The gating of individual channel variants was described by optimizing the parameters K_{Ca}^{WT} and K_{Ca}^{M}, the microscopic Ca^{2+} dissociation constants of individual WT, SM, and M binding sites. The parameters K_{OL}, K_{CL}, and K_{CI} were kept identical to the published ones (Zahradníková et al., 1999). The parameter $K_{O4} = 0.00219$ was determined from K_{OL}, K_{CL}, and K_{CI} and from the preset value of $P_O^{max} = 0.85$ (Li and Chen, 2001). Parameters of the optimal model are given in Table I. Calcium sensitivity of the M monomer was decreased ~1000× relative to the WT monomer.

The effect of consecutive substitutions of the WT monomers by M monomers on the calcium dependence of RyR open probability for selected values of P_O^{max} is depicted in Fig. 3 (third row left). It can be seen that while the calcium dependence of variants with $n_M = 0$ and $n_M = 2$ is well described by the model, the model exhibits significant deviations from the experimentally observed behavior for the variant with $n_M = 1$ at calcium concentrations below the apparent K_{Ca}.

The aEMG Model. In addition to parameters of the aHM model, the aEMG model contains parameters K_{OL}, K_{CL}, and K_{CI}. The value of K_{CI} had only minor effects on the calcium dependence of open probability, while optimizing either both K_{OL} and K_{CL}, or either one of these parameters in isolation produced similar results (unpublished data). Therefore, for description of activity of individual RyR variants, we optimized the parameters K_{Ca}^{WT}, K_{Ca}^{M}, f, and K_{CL}, while the parameters K_{OL} and K_{CI} were kept identical to the published values (Zahradníková et al., 1999). The value of $K_{O0} = 0.225K_{CL}/(f^4[3.4 + 0.825K_{CL}])$ was calculated from the values of the remaining parameters. Parameters of the optimal model are given in Table I. Calcium sensitivity of the M monomer was decreased ~800× relative to the WT monomer.

The effect of consecutive substitutions of the WT monomers by M monomers on the calcium depen-

dence of RyR open probability for selected values of P_O^{max} is shown in Fig. 3 (third row right). It can be seen that the calcium dependence of all variants is quite satisfactorily described by the model. For the WT channel, the open state with four Ca^{2+} ions bound (O_{40}) was predominant at $Ca^{2+} > 0.2$ μM, i.e., at all calcium concentrations that evoked significant activation of the channel. Open states with less than four Ca^{2+} ions bound did not contribute substantially to open probability in the WT channel (black hatched area in Fig. 3), while they became quite prominent in the variant with $n_M = 1$ (red hatched area in Fig. 3, contributing >50% of total P_O at $Ca^{2+} < 5$ μM) and (to a lesser extent) in the variant with $n_M = 2$ (green hatched area in Fig. 3, contributing >50% of total P_O at <30 μM). The open probability in the absence of Ca^{2+} was $P_O^{min} = 0.0001$.

As with the aHM model, it was not possible to describe the observed changes in calcium sensitivity of mutant channels if K_{Ca}^{M} was set equal to K_{Ca}^{WT} and the mutation was supposed to produce only changes in the allosteric parameter f. If both K_{Ca} and f were allowed to vary between WT and M monomers, the quality of fit was not improved (unpublished data).

The Joint Binding Site (JBS) Model. For description of activity of individual RyR variants, we optimized the K_{Ca}^{J} parameters characterizing the dissociation constant of the wild-type, quarter mutant, and semi mutant joint Ca^{2+}-binding site (K_{Ca}^{WT}, K_{Ca}^{QM}, and K_{Ca}^{SM}, respectively), at different combinations of parameter values for K_{Ca}^{I} and K_{O0}. The parameter $K_{O1} = 0.176$ was determined from the preset value of $P_O^{max} = 0.85$ (Li and Chen, 2001). Parameters of the optimal model are given in Table I. Only models with $K_{O0} \geq 20$, in which the maximum open probability in the absence of Ca^{2+} binding to the joint binding site was <0.05, provided a satisfactory fit to the data. As a result, the calcium dependence of open probability of the channel was only weakly dependent on the parameter K_{Ca}^{I}. At $K_{Ca}^{I} = 0.1$ μM and $K_{O0} = 100$, the calcium dissociation constant of channel variants with one or two mutant subunits was decreased ~15 and ~500 times, respectively, relative to that of the WT channel.

The effect of consecutive substitutions of the WT monomers by M monomers on the calcium dependence of RyR open probability for selected values of P_O^{max} is shown in Fig. 3 (bottom left). It can be seen that the calcium dependence of all variants is quite satisfactorily described by the model.

The AG Model. The gating of individual channel variants was described by optimizing the calcium dissociation constants of wild-type and mutant monomers, K_{Ca}^{WT} and K_{Ca}^{M}, and the parameter K_A. Parameters of the optimal model are given in Table I.

The effect of consecutive substitutions of the WT monomers by M monomers on the calcium dependence of RyR open probability is shown in Fig. 3 (bottom right). Model channels containing M monomers displayed nonmonotonic calcium dependence, with calcium-dependent inactivation above 1 μM, caused by Ca^{2+} binding to the A sites of the channel, followed by a second increase in P_O above 10 μM due to Ca^{2+} binding to the O sites of the M monomer(s). Such a bimodal calcium dependence was not observed experimentally (Li and Chen, 2001), and is especially at odds with the data of the channel variant with $n_M = 2$. In this model, Ca^{2+} binding to the O sites of the channel leads to channel opening if the corresponding A sites are not occupied by Ca^{2+}, and therefore open probability at a wide range of Ca^{2+} concentrations was due to open states with three or less Ca^{2+} ions bound for all channel variants (black, red, and green hatched areas for $n_M = 0$, 1, and 2, respectively).

Dose–Response Parameters of RyR Variants in the Tested Models. In seven out of eight models, substitution of WT monomers in the RyR tetramer resulted in a progressive decrease in calcium sensitivity. Individual models differed in the relative effect of single and double substitution on the resulting calcium sensitivity. They also differed in the effect of monomer substitution on the steepness of the calcium dependence of open probability. In this section, we compared the predicted apparent calcium dissociation constants and Hill slopes of the three tested WT/M variants in individual models. The AG model displayed nonmonotonic calcium dependence of P_O in channels containing mutant monomers and therefore could not be analyzed by this method.

Fig. 4 summarizes the predicted dose–response parameters of models compared with those of the data (Li and Chen, 2001). Parameters significantly different from those of the data are marked by asterisks. The quality of description of the data by the models falls into three categories. In the aHM and aEMG model, one out of six parameters was significantly different from those of the data. In the HM, SBS, aSBS, EMG, and JBS models, two out of six parameters were significantly different from those of the data. Based on this analysis, the aHM and aEMG models are preferential to all other models.

We have examined for each of the models how apparent calcium affinities and apparent Hill slopes vary with the number of mutant monomers in the studied RyR variants (black, red, and green bars for $n_M = 0$–2). It is to be noted that the available experimental data (Li and Chen, 2001; Fig. 4 A, first set of bars) suggest that substitution of one monomer in the WT RyR by an M monomer results in only an ~10-fold decrease of calcium sensitivity, while substitution of the second monomer by an M monomer results in a further 20-fold de-

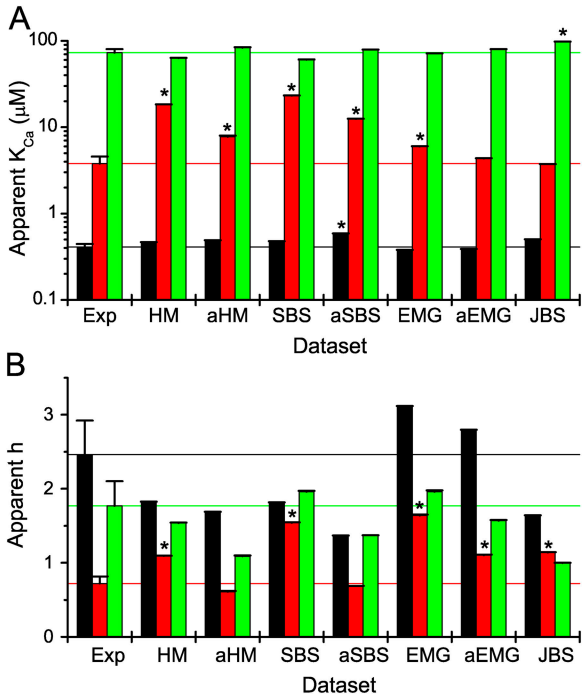

Figure 4. Hill parameters of individual models. Left axis, apparent calcium affinity (A) and apparent Hill slope (B) for the data (Li and Chen, 2001) and models. Black, red, and green columns represent values for n_M = 0, 1, and 2, respectively. Black, red, and green lines represent the values determined from the experimental data.

crease of Ca^{2+} sensitivity. The aHM, aSBS, EMG, aEMG, and JBS models (Fig. 4 A) were able to simulate the change of calcium sensitivity upon increase of the number of mutant monomers in the RyR tetramer, i.e., the logarithmic decrease in sensitivity of tetramer variants with increasing number of mutant monomers.

Furthermore, the experimental data (Li and Chen, 2001; Fig. 4 B, first set of bars) suggest that substitution of one monomer in the WT RyR by an M monomer results in a prominent decrease of the steepness of calcium dependence of activation, while substitution of the second monomer by an M monomer results in increase of the steepness. Differences between models were more prominent in the relationship between n_M and the apparent Hill slope for calcium dependence of channel activation (Fig. 4 B). The aEMG model, and to some extent also the models aHM, aSBS, and EMG, showed the experimentally observed nonmonotonous dependence of the apparent Hill slope on n_M. On the other hand, in the JBS model the value of the apparent Hill slope decreased monotonously with n_M, and in the HM and SBS models, it changed only slightly with n_M.

Evaluation of Models by Statistical Criteria. We have judged the goodness of fit of the above models by the χ^2 test. The following ranking of models was obtained (in the order of decreasing quality of fit): aEMG > aHM > JBS > aSBS > EMG > HM > SBS. The residuals of models aEMG, aHM, and JBS were normally distributed, and these models were not significantly different based on the F-test at P = 0.05. However, only the aEMG model could be accepted by the χ^2 test at P = 0.05 (see Table I).

DISCUSSION

This theoretical study was undertaken to explain the alterations of RyR channel Ca sensitivity observed upon substitution of individual monomers with a Ca-insensitive mutant within the tetrameric structure of the channel protein (Li and Chen, 2001). By analyzing the ability of several different RyR models to reproduce the available experimental data on Ca dependency of various WT/mutant hybrids, we provide evidence that in RyRs, calcium binding and channel opening are allosterically coupled. We introduce an RyR channel model, the aEMG model, that not only accounts for the calcium dependence of RyR activation but, for the first time, reflects the homotetrameric nature of the RyR channel.

The major finding of this work is that only models based on the MWC allosteric concept provided a satisfactory description of the calcium dependence for RyR channels that are composed of wild-type monomers and monomers deficient in calcium-dependent activation. There is a major conceptual difference between allosteric models (see Cox et al., 1997 for the Ca^{2+}-activated BK channel) and linear, Hodgkin-Huxley type fully cooperative models (Sitsapesan and Williams, 1994; Zahradníková et al., 1999; Dura et al., 2003). In linear models, the channel opens only when all the activation sites are occupied by Ca, that is, when the last calcium-dependent gate is unlocked. In allosteric models the channel may open with any number of calcium ions bound.

In the WT channel, the presence or absence of MWC states did not influence the calcium dependence of open probability. Indeed, as visually apparent from the black traces in Fig. 3, all models except aSBS and AG provided satisfactory fit to the wild-type data (χ^2 test, unpublished data). Only the presence of the mutant monomer exposed the existence of the MWC states, and therefore, for the whole dataset, all allosteric models provided a significantly better fit than their linear counterparts (see Table I).

Of the three tested allosteric models, only the aEMG model passed all tests. This model, in addition to allosteric coupling between calcium binding and channel opening, incorporates fully calcium-bound channel closed states of the L- and I-mode (Zahradníková and Zahradník, 1996) that limit its maximal open probability. In the absence of the L- and I-mode states, allosteric coupling was not sufficient to describe the experimental

observations (the aHM model, Table I). Similarly, in the absence of allosteric coupling, L- and I-mode states were not sufficient to describe the experimental observations (the EMG model, Table I). While in the aEMG model the MWC states are necessary for satisfactory description of mutant channel gating, occupancy of these states in the wild-type RyR was negligible (Fig. 3, third row right), much lower than in the aHM and aSBS models. This explains why previous kinetic experiments revealed that in the wild-type channel, binding of four calcium ions is required before RyR opening (Zahradníková et al., 1999).

It is noteworthy that several previously suggested RyR models share some characteristics with the aEMG model. Out of the three best-ranking gating schemes of the extensive analysis of RyR gating by Rosales et al. (2004), two of the models contained fully Ca-bound closed states following the open states, and the third model (McManus and Magleby, 1991) contained interconnected open states with not fully Ca-liganded channel. A gating scheme sharing both features was proposed for explaining kinetic properties of RyR activation by Ca^{2+} (Fill et al., 2000). Neither of these models, however, attributed particular Ca^{2+}-dependent gating transitions to individual channel monomers and therefore could not be tested against the data of Li and Chen (2001).

Allosteric gating models have not been previously proposed for activation of the ryanodine receptor by Ca^{2+}. Nevertheless, analogous models were useful for description of RyR regulation by the DHPR voltage sensors (Rios et al., 1993) and for regulation of the inositol trisphosphate receptor (IP3R) by ATP (Bezprozvanny and Ehrlich, 1993) as well as by IP_3 and calcium (Mak et al., 2003). In this work, we have applied a similar approach and extended it by (a) considering the effect of nonequivalent, i.e., wild-type and mutant monomers, (b) examining the tested models in the whole parameter space by nonlinear fitting, and (c) applying statistical methods to analyze the quality of models.

The Model and the Identity of the Binding Site

The parameters of the aEMG model relate to four functional elements of the channel: the calcium binding site on the RyR monomer (K_{Ca}), the resting-active equilibrium (K_{O0}), coupling between calcium binding and channel opening (f), and L- and I-mode equilibria (K_{OL}, K_{CL}, and K_{CI}). In the optimized model, the mutation of E3987 selectively decreased the calcium affinity of the binding site by three orders of magnitude, without affecting the remaining equilibria.

Niu and Magleby (2002) showed that a binding site created by a single amino acid would result in mutant subunits having no calcium affinity, which would lead to a progressive decrease of the Hill coefficient with increasing number of mutant monomers, a feature not observed experimentally (Li and Chen, 2001). Therefore, it seems safe to conclude that either the Ca^{2+} binding pocket of the RyR monomer is created by several amino acids, one of which is the glutamate E3987, or that this amino acid allosterically affects the calcium dissociation constant of the binding site while affecting neither the transitions between the closed and open conformations of the channel, nor coupling between calcium binding and channel opening. In this context it is noteworthy that the E2100 residue of the IP3R1 receptor, homologous with E3987 of the RyR2 channel, was proposed to play a direct role in Ca^{2+} binding (Tu et al., 2003).

Implications of the Allosteric Gating Principle

The allosteric nature of RyR regulation by Ca^{2+} has profound implications for our understanding of RyR2 gating at resting calcium concentrations. In contrast to previously proposed mechanisms, where the RyR channel could open only upon Ca^{2+} binding, the aEMG model predicts nonzero ($P_O^{min} = 1 \times 10^{-4}$) open probability in the absence of Ca^{2+}. This might explain the discrepancy between the substantial resting calcium spark frequency (100 pl^{-1} s^{-1} at 10 nM Ca^{2+}; Li et al., 2002) and the low calcium sensitivity (high apparent K_{Ca}) of the RyR2 under physiological conditions ($\sim$10 μM; Cannell et al., 1994; Lukyanenko and Györke, 1999). The proposed concept of RyR activation provides a unifying picture that can help to understand several phenomena, such as nonzero open probabilities observed in planar lipid bilayers at very low Ca^{2+} concentrations in the skeletal RyR (Lee et al., 2002), activation of RyRs by agonists such as sulmazole (Williams and Holmberg, 1990), caffeine (Sitsapesan and Williams, 1990), and ATP (Kermode et al., 1998) in the absence of Ca^{2+}, or the observed increase in resting open probability caused by an RyR2 mutation implicated in hereditary arrhythmia (Jiang et al., 2002).

Implications of Monomer-dependent RyR Regulation

Allosteric regulation of the RyR, discussed in the previous paragraph, operates at the level of the whole tetramer, i.e., at the concerted closed-to-open transition of the RyR (parameters K_{O0} and f). On the other hand, agents that affect the calcium affinity of individual monomers would be expected to modulate channel activity by acting on each monomer independently, i.e., in a manner analogous to that of the E3987A mutation. Similarly, RyR monomers differing in their phosphorylation status (for reviews see Meissner 2004; Wehrens et al., 2005), FKBP binding (Kaftan et al., 1996, Marx and Marks, 2002), and/or calmodulin binding (for reviews see Meissner 2002, 2004) might be independently modulated as well. These and similar effects might contribute to the often-observed heterogeneity in the behavior of single RyR channels (Ma, 1995; Zahradníková and Zahradník, 1995; Zahradníková et al., 1995; Copello et al., 1997).

Further Directions

Several important questions pertaining to steady-state activity of the RyR are not addressed by this study. Most importantly, the inactivation mechanisms, that is, the molecular mechanisms that are responsible for the additional gating states of the L- and I-mode (Zahradníková and Zahradník, 1995) and for the nature of channel inhibition by Ca^{2+} and Mg^{2+} (Laver et al., 1997; Zahradníková et al., 2003) still remain unresolved. It is also not clear whether the calcium affinity decrease in the E3987A mutation is caused by differences in the calcium off-rate, on-rate, or both. In other channel types (Zhou et al., 1996; Reimann et al., 2001; Powe et al., 2002), as well as in the E-F hand calcium binding protein calmodulin (Black et al., 2000; Tikunova et al., 2001), variations in the on- as well in the off-rate of binding were observed upon mutation of binding sites. The exact mechanism of the affinity change in RyR2 mutants could be determined by kinetic studies using caged calcium (Zahradníková et al., 1999, 2003, 2004) or caged calcium buffers (Velez et al., 1997).

Conclusions

We have found that the calcium dependence of activation in RyR channels containing subunits deficient in calcium binding can be best explained by the existence of allosteric coupling between calcium binding and channel opening. These results open new insights into the molecular mechanisms of RyR activation by calcium and provide important clues for understanding RyR regulation in health and disease. Taken together, the modeling approach described in this work may prove to be valuable for analysis and description of RyR modulation based on monomer-dependent phenomena.

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