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REGULATION OF MAMMALIAN RYANODINE RECEPTORS

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1. ABSTRACT

Ryanodine receptors (RyRs) are large, high conductance Ca^{2+} channels that control the level of intracellular Ca^{2+} by releasing Ca^{2+} from an intracellular compartment, the sarco/endoplasmic reticulum. Mammalian tissues express 3 closely related ryanodine receptors (RyRs) known as skeletal muscle (RyR1), cardiac muscle (RyR2) and brain (RyR3) . The RyRs are isolated as 30S protein complexes comprised of four 560 kDa RyR2 subunits and four 12.6 kDa FK506 binding protein (FKBP12.6) subunits. Multiple endogenous effector molecules and posttranslational modifications regulate the RyRs. This chapter reviews the regulation of the mammalian RyRs by endogenous effector molecules.

2. INTRODUCTION

Ryanodine receptors (RyRs) are large, high conductance Ca^{2+} channels that control the level of intracellular Ca^{2+} by releasing Ca^{2+} from an intracellular compartment, the sarco/endoplasmic reticulum. They are known as ryanodine receptors because they bind the plant alkaloid ryanodine with high affinity and specificity and to distinguish them from another intracellular Ca^{2+} release channel family, the inositol 1,4,5-tris phosphate receptors (IP_3R). There are three widely expressed mammalian RyR isoforms: RyR1 is the dominant isoform in skeletal muscle, RyR2 is found in high levels in cardiac muscle, and RyR3 is expressed in many tissues at low levels including diaphragm and brain. They are also known as skeletal muscle (RyR1), cardiac muscle (RyR2) and brain (RyR3) RyRs because they were first isolated from these tissues. All three have been purified as 30 S protein complexes composed of four 560 kDa subunits and four small 12 kDa FK506 binding proteins (FKBP) with a total molecular weight of ~2,200 kDa (Table 1). They are cation-selective channels that have high conductance for mono- and divalent cations and are regulated by a large number of endogenous and exogenous effectors. Ca^{2+} ions are the principal activators of the cardiac and brain isoforms. In cardiac muscle during an action potential, dihydropyridine- and voltage-sensitive (L-type) Ca^{2+} channels (DHPRs) located in the surface membrane and tubular infoldings of the surface membrane (T-tubule) mediate the influx of Ca^{2+} that open SR Ca^{2+} release channels. Regulation of the skeletal isoform differs significantly from that of the two other isoforms. A unique property of the mammalian skeletal muscle RyR is that its activity is regulated via direct protein-protein interactions with the voltage-sensing T-tubule L-type Ca^{2+} channel/dihydropyridine receptor. Morphological evidence indicates the presence of a second population of RyR1s in mammalian skeletal muscle that are not directly linked to L-type Ca^{2+} channels, which raises the question how unlinked RyRs are activated. One suggestion is that Ca^{2+} ions released by DHPR-linked

Table 1. Properties of mammalian Ca^{2+} release channels/ RyRs

Type	RyR1	RyR2	RyR3
Major tissue	Skeletal muscle	Cardiac muscle	Diaphragm, Brain
Sedimentation coefficient	30 S	30 S	30 S
Subunit composition, RyR peptide + FKBP	4 + 4	4 + 4	4 + 4
Amino acids/RyR peptide	5035	4970	4870
Activation	DHPR (Voltage sensor)/ Ca^{2+}	Ca^{2+}	Ca^{2+}

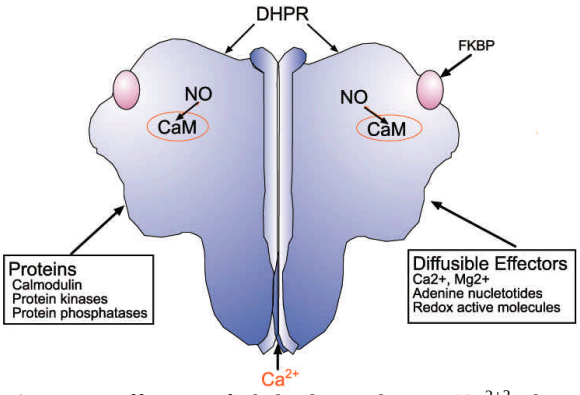

Figure 1. Effectors of skeletal muscle RyR/$Ca^{2+?}$ release channel.

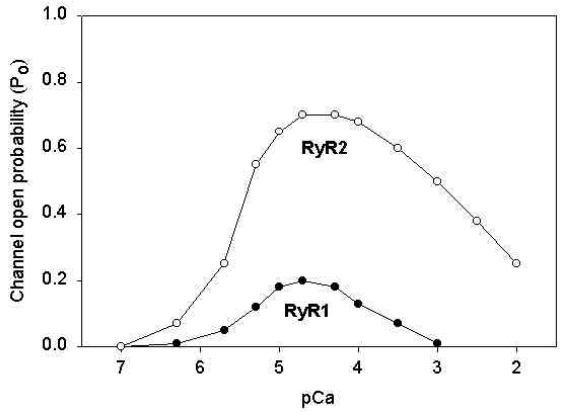

Figure 2. $Ca^{2+?}$ dependence of single skeletal muscle (RyR1) and cardiac muscle (RyR2) Ca^{2+} release channels.

RyR1s activate DHPR-unlinked RyRs by a Ca^{2+}-induced mechanism resembling that in cardiac muscle (1). In an alternative mechanism, neighboring RyR1s are physically linked leading to simultaneous opening and closing, termed coupled gating (2).

In both skeletal and cardiac muscle, Ca^{2+} release through the RyR ion channels is highly regulated by ligands and protein-protein interactions including inhibitors such as Mg^{2+} and monovalent cations, anions such as Cl^- and adenine nucleotides that activate the channels, and proteins such as calmodulin (CaM) **(**Figure 1). RyRs also contain phosphorylation sites and a large number of free sulfhydryls suggesting that protein kinases, and reactive nitrogen and oxygen species have a role in regulating channel activity *in vivo.* This chapter focuses on the regulation of the mammalian RyRs by the above endogenous effectors. RyRs interact with other proteins including the voltage-sensing L-type Ca^{2+} channel/dihydropyridine receptor and various sarcoplasmic reticulum proteins. These areas of research are beyond the scope of this review.

3. REGULATION OF RYRS BY CA^{2+}

Regulation of RyR ion channel activity has been studied *in vitro* by three complementary methods. The Ca^{2+} efflux behavior of passively or actively (via the Ca^{2+} pump) loaded, junctionally-derived T-tubule-attached (triads) or -detached ("heavy") SR vesicles has been examined using rapid mixing and filtration methods. Second, [^{3}H]ryanodine binding studies suggest that ryanodine is a suitable ligand for probing the functional states of the release channelRegulation of RyR ion channel activity has been studied *in vitro* by three complementary methods. TheCa^{2+} efflux behavior of passively or actively (via the Ca^{2+} pump) loaded, junctionally-derived T-tubule-attached (triads) or -detached ("heavy"). Third, regulation of the channel is studied in single channel recordings following the incorporation of SR vesicles or purified RyRs into planar lipid bilayers. Although single channel recordings provide direct information, an advantage of the vesicle flux and [^{3}H]ryanodine binding measurements is that they yield data averaging the kinetic behavior of a large number of channels.

RyR1s are activated by Ca^{2+} binding to high-affinity, Ca^{2+} specific sites and inhibited by Ca^{2+} binding to low-affinity, less selective sites, giving rise to the characteristic bimodal Ca^{2+} dependence of channel activity shown in Figure 2. The binding sites are accessible from the cytosolic side in the large cytosolic foot region of RyRs. A bimodal Ca^{2+} activation/inactivation curve is also obtained in Ca^{2+} efflux and [^{3}H]ryanodine binding studies with skeletal muscle SR vesicles, with RyR activities being maximal at µM Ca^{2+} (3-5). RyR2 (Figure 2) and RyR3 (6-8) are activated by Ca^{2+} to a greater extent and require higher Ca^{2+} concentrations for inactivation than RyR1. Single channel measurements indicate that SR lumenal Ca^{2+} regulates the RyRs by binding to lumenal channel sites (9, 10) or by accessing cytosolic Ca^{2+} activation and inactivation sites following their passage to the cytosolic receptor side (11, 12). The reasons for the different results are not clear but may reflect a predominance of one of the two mechanisms, depending on the experimental conditions.

It is likely that the bimodal Ca^{2+}-dependence of the RyRs is a consequence of at least two classes of Ca^{2+} binding sites, a high affinity activation site and a low affinity inactivation site. Several lines of evidence suggest that the carboxyl-terminal one-third of RyR1 has a critical role in activation by Ca^{2+}. Antibodies raised against a

negatively charged sequence PEPEPEPEPEPE at aa 4489-4499 blocked Ca^{2+}-dependent activation of the channel (13). A single site mutation E4032A in RyR1, equivalent to E3987A in RyR2 and E3885A in RyR3, forms a functional channel with normal conductance but with greatly reduced Ca^{2+} sensitivity (14). A deletion mutant encoding the carboxyl-terminal 1377 amino acids of RyR1 (residues 3661-5037) was activated by μM Ca^{2+} (15). Less is known about the location of Ca^{2+} inactivation site(s). Unlike the full-length RyR1, truncated RyR1 (Δ1-3660) failed to close at high Ca^{2+}, suggesting that the N-terminal foot structure has a role in Ca^{2+} regulation (15). On the other hand, RyR1/RyR2 chimeras containing the RyR2 carboxyl-terminal domain showed reduced inhibition at elevated Ca^{2+} (16, 17), similar to RyR2 (see Figure 2), suggesting that the carboxyl-terminal region (3720-5037) has a role in Ca^{2+} inactivation. The mutation studies are complicated because mutations may induce long-range conformational changes that distort the assembly of the large multimeric RyR complexes.

4. MODULATION OF CA^{2+}-DEPENDENT RYR ACTIVITIES BY MONOVALENT IONS

Ca^{2+}-activated RyR ion channels are modulated by other molecules such as pH, ionic strength, and cation and anion composition. RyR1 and RyR2 activities were reduced by decreasing the pH from ~7.5 to 6 (18, 19). Typically, an increase in salt concentration increases RyR activity, despite the fact that monovalent cations inhibit RyR activity by interacting with the Ca^{2+} activation and inactivation sites. When used as Cl^- salts, the order of effectiveness of monovalent ions in competing with Ca^{2+} at the RyR1 Ca^{2+} activation sites was $Li^+ > Na^+ > K^+ > Cs^+$ (21). Hill coefficients of greater than 1 indicate that monovalent cations inhibit RyR1 by a cooperative interaction involving at least two cations. An interesting exception is $choline^+$ that activated RyR1 at nM Ca^{2+} concentrations, thus behaving like a weak Ca^{2+} agonist. The action of $choline^+$ appears to be unique for RyR1 because $choline^+$ did not activate the cardiac RyR (20). Cl^- anions oppose the inhibitory effects of inorganic monovalent cations by binding to specific anion regulatory sites. An increase in RyR1 activity with salt concentration indicate that the activating effects of Cl^- predominate over the inhibitory effects conveyed by K^+ or Na^+ (21). Chaotropic anions (ClO_4^-, SCN^-, I^-, NO_3^-) and inorganic phosphate anions were more effective in raising RyR1 activity (22), whereas replacement of Cl^- by buffer anions (MES^-, $Pipes^-$) was inhibitory (21).

A distinguishing feature of mammalian RyRs is that at 0.25 –0.5 M KCl, Ca^{2+} ions activate RyR2 (Figure 2) and RyR3 (6-8) to a greater extent than RyR1. A lower binding affinity of Ca^{2+} to the Ca^{2+} inactivation sites of RyR2 and RyR3 appears to be the primary cause. As observed for RyR1, monovalent cations had an inhibitory effect on RyR2 by interacting with the Ca^{2+} activation and inactivation sites. Cl^- had an activating effect (20). Modulation of mammalian RyR3 activity by monovalent cations and anions has not been systematically studied.

5. MODULATION OF RYR ACTIVITY BY MG^{2+}?; SR^{2+} AND BA^{2+} IN THE ABSENCE OF ADENINE NUCLEOTIDES

Mg^{2+}, Sr^{2+} and Ba^{2+} compete with Ca^{2+} for the high- and low-affinity Ca^{2+} binding sites of RyR1 and RyR2, but with different effects (5) (23) (20). Besides Ca^{2+}, cytoplasmic Sr^{2+} activated RyR1 and RyR2 by binding with more than 100 times lower affinity to the channel activation sites. In contrast, Mg^{2+} and Ba^{2+} had an inhibitory effect. The order of mono- and divalent cations in competing with Ca^{2+} at the RyR1 and RyR2 Ca^{2+} activation sites was $Mg^{2+} > Ba^{2+} > Li^+ > K^+$ (20).

Elevated levels of divalent cations typically inhibit the RyRs. Under conditions that eliminate binding to the high affinity Ca^{2+} activation sites, mM Mg^{2+}, Sr^{2+}, Ba^{2+} inhibited RyR1 and RyR2 with an efficacy essentially identical to Ca^{2+}, suggesting that the low-affinity inhibitory site has a broad divalent cation specificity. On the other hand, ionic strength affected the efficacy of divalent cations in inhibiting RyR1, as indicated by an inactivation constant of ~0.1 and ~1.35 mM in 0.1 mM and 0.5 mM KCl media, respectively (21).

6. REGULATION OF RYR ACTIVITY IN THE PRESENCE OF ADENINE NUCLEOTIDES

In the absence of Mg^{2+} and ATP at neutral pH, rabbit skeletal muscle SR vesicles release their Ca^{2+} stores with a first-order rate constant of 1-2 s^{-1} (3-5). In vivo SR Ca^{2+} release occurs on a millisecond time scale. An early key observation therefore was that Ca^{2+}-induced Ca^{2+} release was potentiated by mM ATP (3, 24, 25). The presence of μM Ca^{2+} and mM ATP or the nonhydrolyzable ATP analog AMP-PCP yielded maximal release rates with a first-order rate constant of 20 - 100 s^{-1} (3, 5, 26). Ca^{2+} release from cardiac SR vesicles was maximally activated by μM Ca^{2+} and mM AMP-PCP, yielding a release rate with a first order rate constant of ~100 s^{-1}. In this case the potentiating effects of the nonhydrolyzable ATP analog were less pronounced because Ca^{2+} alone yielded a release rate with a first order constant of ~50 s^{-1}. In single channel measurements, μM Ca^{2+} and mM ATP fully activated RyR1 (27) and RyR2 (28) with an increase in the channel open time (P_o) to ~1. ATP itself is a poor activator, because RyRs were only minimally activated by adenine nucleotide in [^{3}H]ryanodine binding and single channel measurements at [Ca^{2+}] <50 nM (6, 7, 29).

Various other adenine nucleotides (AMP-PCP, ADP, AMP, cAMP, adenosine, adenine) potentiate SR Ca^{2+} release, suggesting that activation occurs by effector binding rather than by RyR phosphorylation (24). Cyclic ADPribose has a role in regulating intracellular Ca^{2+} release in a variety of cells (30). However, most studies using isolated membrane fractions or purified receptors did not show a direct, physiologically relevant interaction of the RyRs with cADPribose. Cyclic ADP ribose appears to have a more indirect role by increasing SR Ca^{2+} uptake in heart, which was suggested to lead to RyR2 activation (31).

Other trinucleotides (CTP, GTP, ITP, UTP) had no substantial effect on the release of SR Ca^{2+} (24, 32).

Most ATP in cells is bound to Mg^{2+}. It is therefore likely that MgATP, rather than free ATP, is a major physiological effector of the RyR ion channels. However, determining the regulation of the channel complexes by MgATP is complicated by the presence of uncomplexed Mg^{2+} ions that inhibit RyRs by binding to the high-affinity and low-affinity Ca^{2+} binding sites. Additionally, CaATP complexes may form near the release site during SR Ca^{2+} release. Ca^{2+} release studies with skeletal muscle SR vesicles showed that at µM [Ca^{2+}], Mg^{2+} and adenine nucleotide strongly potentiated Ca^{2+} release when present at concentrations approximating those in muscle (5 mM each, 0.7 mM free Mg^{2+} and ATP each) (5). An increase in free Mg^{2+} from 0.1 to 4 mM inhibited SR Ca^{2+} release, which supports that free Mg^{2+} in muscle is an important determinant of RyR1 activity. One interesting suggestion is that voltage-activation of the DHPR lowers the affinity of RyR1 for Mg^{2+}, thus overcoming the inhibitory effect exerted by cytosolic Mg^{2+} (33).

The effects of MgATP on the RyR2 are more modest. MgATP antagonized channel inactivation by mM Ca^{2+}, without substantially affecting the maximal level of channel activity (18, 20). Further, > 2 mM free Mg^{2+} was required to detect a significant decrease in channel activity in the presence of ATP or the nonhydrolyzable analog AMP-PCP. Other Ca^{2+}-dependent mechanisms include regulation by calsequestrin and calmodulin, SR lumenal and cytosolic Ca^{2+} binding proteins, respectively (see below).

7. MODULATION OF RYRS BY CALMODULIN

Calmodulin (CaM) is a small 16.7 kDa cytosolic protein that influences SR Ca^{2+} release through a direct interaction with RyRs as well as other proteins that regulate SR Ca^{2+} release such as the sarcolemmal voltage dependent Ca^{2+} channel (DHPR), calmodulin dependent protein kinase (CaMKII), and calmodulin stimulated protein phosphatase (calcineurin) (see below).

Early studies indicated that the Ca^{2+}-bound form of calmodulin (CaCaM) inhibits the RyR1 and RyR2 ion channels in the absence of ATP, which suggested inhibition via a direct interaction rather than through phosphorylation (34-36). More recent [^{3}H]ryanodine and single channel measurements confirmed CaM inhibition at free [Ca^{2+}] > 1 µM but have also indicated significant differences in the regulation of RyRs by the Ca^{2+}-free form of CaM (apoCaM). At low free Ca^{2+} concentrations (<1 µM), apoCaM had a stimulatory effect on RyR1 (29, 37) and RyR3 (6) channel activities, whereas RyR2 was unaffected (38) or inhibited (39) by CaM at [Ca^{2+}] < 1 µM.

Studies investigating the structural basis of the functional effects of CaM on the RyRs have focused on RyR1. Skeletal muscle SR vesicles (39, 40) and the purified channel complex (39) bind a single metabolically labeled [^{35}S]CaM per RyR1 subunit with nM affinity, independent of cytosolic Ca^{2+}. ApoCaM and CaCaM share a single CaM binding domain in RyR1 (aa residues 3616-3643), with CaM apparently shifting its points of interaction N-terminally upon binding Ca^{2+}, as shown in binding studies with RyR1 derived peptides (41) and by site directed mutagenesis (42). Cryo-electron microscopy revealed that the position of calmodulin binding in RyR1 is shifted approximately 32 Å after binding Ca^{2+}, suggesting that binding and release of Ca^{2+} to CaM produces major conformational changes in the large RyR1 channel complex (43). ApoCaM and CaCaM bind to and dissociate from RyR1 and RyR2 on a time scale of seconds to minutes (39). It is therefore likely that CaM is constitutively bound to the receptor.

An unresolved question is why apoCaM affects the RyRs differently, because the CaM binding domain identified in RyR1 is highly conserved among the RyRs. Direct binding studies suggest that cardiac SR membranes bind 1 CaCaM and 0.25 ApoCaM per RyR2 subunit (38) or 2 CaCaM and 1 ApoCaM (39) per subunit. Purification of RyR2 decreased the stoichiometry of CaCaM binding to 1 CaM/RyR2 subunit and eliminated CaCaM-dependent inhibition of [^{3}H]ryanodine binding, effects not observed for RyR1. A conformational change in the purified receptor rather than removal of a necessary cofactor may be responsible for the lack of function. Single channel measurements showed that application of a transmembrane potential restored channel inhibition by CaM (39). Direct binding of CaM to the native RyR3 was not reported since no tissue expresses RyR3 alone.

8. OXIDATION OF RYRS

Working skeletal muscle produces reactive oxygen intermediates (44). Reactive oxygen intermediates are also extensively formed during reoxygenation of ischemic tissue and include superoxide, hydroxyl radicals and hydrogen peroxide. In support of a functional role of these compounds, redox-active compounds and antioxidant enzymes modulate excitation-contraction coupling and force production in striated muscle.

RyR1 is an excellent target for reactive oxygen intermediates formed in muscle because it contains a large number of free sulfhydryls. The tetrameric mammalian RyR1 has 100 cysteines per 560 kDa RyR1 peptide and 1 per 12 kDa FK505 binding protein (404 per complex). Up to 50 cysteines per RyR1 subunit (200 per complex) are free (45). Accordingly in skeletal muscle, a large number of RyR1 thiols are likely in a reduced state because cells maintain a reducing environment through thiol-reducing compounds, the most abundant being glutathione.

No unifying picture has yet evolved to explain the complex redox modulation of the RyRs. RyR1 redox state and function are dependent on O_2 tension, glutathione transmembrane redox potential, and Ca^{2+}, Mg^{2+} and calmodulin that control RyR1 channel activity. Altering O_2 tension alone modulated RyR1 activity by oxidizing and reducing up to 8 thiols per RyR1 subunit (45) by a mechanism that remains to be determined. RyR1 thiols

sense the activity state of the receptor. [^{3}H]Ryanodine rate binding studies revealed that at highly oxidizing redox potentials (high GSSG/GSH ratio), RyR1 was more sensitive to activation by Ca^{2+} (46). Channel closing at <µM Ca^{2+} or mM Ca^{2+} or Mg^{2+} caused the redox potential to become less negative, favoring the formation of free thiols. Highly reactive (hyperreactive) thiols help to confer Ca^{2+} sensitivity because a redox response was no longer observed when the closed channel was pretreated with 7-diethylamino-3- (4'-maleimidylphenyl)-4-methylcoumarin (CPM), an alkylating reagent, that specifically labels RyR1 at nM concentrations. In single channel measurements, RyR1 responded to redox potentials produced by SR lumenal and cytoplasmic glutathione, indicating the presence of a unique transmembrane redox sensor in RyR1 (47). An SR transporter selective for glutathione was identified and the involvement of hyperreactive thiols in the function of the transmembrane redox sensor in RyR1 was described (47).

Studies have attempted to identify the cysteines involved in redox modulation of the RyRs. NEN alkylation and diamide oxidation of skeletal muscle SR membranes and trypsin digestion suggested that C3635 is involved in redox and calmodulin modulation of RyR1 activity (48). On the other hand, while a C3635 to alanine substitution resulted in loss of S-nitrosylation by NO (see below), modulation by Ca^{2+}, calmodulin, oxygen tension or glutathione was not altered (49). A likely explanation for these results is that alkylation of C3635 introduces major steric effects, whereas a C3635 to alanine substitution does not.

Dependence of RyR1 ion channel activity on free thiol content was determined by exploring the effects of three physiological determinants of cellular redox state - oxygen tension, reduced (GSH) or oxidized (GSSG) glutathione, and NO/O_2^- (released by SIN-1). Nearly half of the 404 cysteines within the tetrameric RyR1 channel complex were reduced (free thiol) in the presence of 5 mM GSH at pO_2~10 mm Hg, i.e. under conditions comparable to resting muscle (50). Oxidation of ~10 RyR1 thiols (~48 to ~38 free thiols per RyR1 subunit) had little effect on channel activity. Channel activity increased reversibly as the number of free thiols was reduced to ~23 per subunit, whereas more extensive oxidation (~13 free thiols per subunit) inactivated the channel irreversibly. Thus, RyR1 has at least three functional classes of thiols: 1) a large group of functionally inert thiols that may protect RyR1 from oxidation under conditions of low oxidative stress in normal working muscle, 2) another large group of redox active thiols that controls channel response to conditions of moderate oxidative stress during extensive exercise, and 3) a group of thiols that may be susceptible to oxidative injury under extreme conditions.

Redox active species affected the activity of RyR2, with the release of endogenous CaM causing release of Ca^{2+} from SR (51). Direct binding studies indicated that the affinity of apoCaM and CaCaM binding to RyR1 is lower under oxidizing conditions, as determined in the presence of reduced or oxidized glutathione (39). CaM-dependent RyR2 activity was also influenced by glutathione redox potential, although to a lesser extent than RyR1. Redox modulation of RyR3 activity by reactive oxgen species has not been systematically studied.

9. S-NITROSYLATION OF RYRS

Nitric oxide (NO) is a ubiquitous regulator of cell function. Mammalian tissues express three isoforms of nitric oxide synthase, known as endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) nitric oxide synthases. In normal skeletal muscle the predominant isoform is nNOS, whereas in cardiac muscle, the major isoform is eNOS (52). Both isoforms are targeted to sarcolemmal caveolea by caveolae structural protein caveolin. Immunolocalization of nNOS to isolated cardiac but not not skeletal muscle SR vesicles has been reported, suggesting a unique localization of nNOS to cardiac SR (53). iNOS is absent or very low in normal skeletal muscle and heart but may increase, depending on disease state (52).

The mechanism of NO action in muscle is not well understood. NO activates guanylate cyclase, which accounts for some of its physiological effects. However, NO is also known to affect cellular functions involving S-nitrosylation and oxidation of free thiols. Both RyR1 (45) and RyR2 (54) are endogenously S-nitrosylated, supporting that NO is a physiological modulator of skeletal and cardiac muscle excitation-contraction coupling (55-58).

Modulation of RyR1 activity by NO and NO-related molecules was demonstrated in vesicle-Ca^{2+} flux, single channel and [^{3}H]ryanodine binding measurements. Activating (59, 60) and inhibitory (61) effects were reported, suggesting that NO and NO-related molecules modify the channel in multiple ways. In two studies, NO-generating agents both activated and inhibited the RyR1 in lipid bilayers, depending on donor concentration, membrane potential, and the presence of channel agonists and other sufhydryl modifying reagents (62, 63).

In vitro S-nitrosylation of RyR1 depends on O_2 tension and on whether NO or NO-generating molecules are used. NO S-nitrosylated RyR1 at physiologically relevant oxygen tension (pO_2 ~ 10 mm Hg) but not in ambient air (pO_2~150 mm Hg) (45), whereas the NO-generating molecule NOC-12 S-nitrosylated RyR1 in an oxygen-independent manner (64). Changes in oxygen tension oxidize/reduce as many as 6-8 thiols in each RyR1 subunit (45), which may explain the responsiveness of RyR1 to NO at tissue pO_2 but not ambient air. Site-directed mutagenesis studies demonstrated that at physiological O_2 concentrations, NO specifically S-nitrosylates Cys3635 out of ~50 free cysteines per RyR1 subunit (49). C3635 is in the CaM binding domain of RyR1, which may explain why NO transduces its functional effect only in the presence of calmodulin (45). Thus, different cysteines within the channel appear to be responsible for the nitrosative and oxidative regulation of RyR1.

NO and NO-generating molecules were also reported to activate (59) and inactivate (65) RyR2. NO-

related molecules S-nitrosylate and oxidize the cardiac RyR in ambient O_2 tension. S- nitrosylation and oxidation (2-3 and ~3 sites/RyR subunit, respectively) led to activation of single RyR2s that was reversed by the sulfhydryl reducing agent dithiothreitol, whereas oxidation of a greater number of thiols was not reversed by dithiothreitol (54). The level of S-nitrosylation depended on channel conformation because it was reduced by the RyR inhibitor Mg^{2+}. Thus, NO-related molecules affect the cardiac RyR via covalent modifications of thiol groups, leading to reversible or irreversible alteration of RyR2 ion channel activity. S-Nitrosylation of RyR2 was suggested to be physiologically significant in the normal heart (57, 58), whereas excess oxidation during periods of oxidative stress can lead to deleterious loss of control. NO may have a role in ischemia-reperfusion injury, however, its function is controversial, as both protective (66) and deleterious (67) effects were described.

Taken together, current evidence suggests that the effects of NO and NO-related molecules on the RyRs depend on the experimental conditions, including redox state, the presence of allosteric effectors of the RyRs and the identity of NO-related molecules. Future work needs to address the isoform and tissue specificity of interaction of the RyRs with NO and NO-related molecules, the molecular basis of this specificity, and as it relates to the role of NO in overall cellular function.

10. PHOSPHORYLATION OF RYRS

Endogenous kinases and phosphatases that phosphorylate and/or modulate RyR1 include cAMP-dependent protein kinase A (PKA), calmodulin-dependent kinase II (CaMKII), and protein phosphatase 1 (68-73). Phosphorylation of Ser2843 by endogogenous kinases (74) and *in vitro* phosphorylation of Ser2843 by cAMP-, cGMP- and CaM-dependent protein kinases (75) have been reported. However, the presence of additional phosphorylation sites is likely, as CaMKII also phosphorylated threonine residue(s). Functional studies comparing the effects of endogenous and exogenous kinases also support the presence of more than one phosphorylation site (69).

RyR2 forms a large multi-protein complex that includes PKA, protein phosphatases 1 and 2A (PP1 and PP2A), and anchoring proteins for PKA and PP1 and PPA2 that bind to RyR2 via leucine/isoleucine zipper motifs (73, 76). Regulation of channel activity by additional protein kinases and phosphatases has been reported. These include CaMKII (77-79), protein kinase C (PKC) isoforms α and β (79), and calcineurin (80). Guse et al. (81) described the transient tyrosine phosphorylation of a ryanodine receptor upon T cell stimulation.

In vitro phosphorylation of Ser2809 (corresponding to Ser2843 in RyR1) by CaMK activated the calmodulin-inhibited RyR ion channel isolated from cardiac muscle (77). On the other hand, Takasago et al. (82) found that an endogenous CaMK decreased [^{3}H]ryanodine binding , while exogenous addition of PKA, cGMP-dependent protein kinase (PKG) and PKC increased [^{3}H]ryanodine binding. Peptide mapping indicated the predominant phosphorylation of one peptide by PKA, PKC and PKG, whereas endogeneous CaMK phosphorylated another peptide. Valdivia et al. (83) observed that PKA regulated the RyR2 dynamically by increasing the responsiveness of RyR to photoreleased Ca^{2+}, which was followed by a lower steady state open channel level. Hain et al. (78) found that phosphorylation removed channel blockade by Mg^{2+} when either applying PKA or CaMK II. Furthermore, calmodulin was shown to block the channel in the dephosphorylated state, which was overcome by treatment with CaMK but not PKA. More recent studies indicate that PKA phosphorylation destabilizes the RyR2 in failing hearts. PKA dissociated FKBP12.6 from RyR2 and increased the appearance of subconductance states (76). In failing hearts, reduced levels of PP1 and PPA2 in the RyR2 macromolecular complex rather than an increased PKA activity appeared to be responsible for RyR2 hyperphosphorylation and formation of “leaky” channels.

Protein kinases also influence SR Ca^{2+} release through interactions with proteins that influence SR Ca^{2+} release. The sarcolemmal voltage-dependent L-type Ca^{2+} channel (DHPR), like RyR2, is phosphorylated by PKA, PKC and CaMK II. Phosphorylation of the SR Ca^{2+} pump regulatory protein phospholamban indirectly increases RyR2 activity by elevating the SR Ca^{2+} load and thereby RyR activity. Indeed in permeabilized cardiomyocytes, PKA activation increased RyR2 activity (measured as sparks) in wild-type but not mutant cells lacking phospholamban (84). PKA activation caused no change in spark frequency in phospholamban-deficient cells, even though ^{32}P-phosphorylation was increased. These data challenge the results of Marks et al. (76) that extensive PKA-mediated phosphorylation of RyR2 leads to the formation of leaky RyR2 channels. A caveat in the study of Li et al. (84) is that the studies were done with cardiomyocytes isolated from normal hearts and the stoichiometry of RyR2 phosphorylation was not determined. Other potential targets are RyR associated proteins. Phosphorylation of skeletal muscle calsequestrin (85), triadin (86) and sarcolumenin (87) has been reported.

11. CONCLUSION

Ryanodine receptors are Ca^{2+} release channels that control the levels of intracellular Ca^{2+} by releasing Ca^{2+} from an intracellular Ca^{2+} storing compartment, the endo/sarcoplasmic reticulum. They form large macromolecular protein complexes of four 560-kDa receptor peptides and various associated proteins. Ca^{2+} ions are the primary activators of the mammalian cardiac muscle (RyR2) and brain (RyR3) isoforms. Regulation of the skeletal muscle isoform (RyR1) differs significantly from that of the two other isoforms. A unique property of the mammalian RyR1 is that its activity is regulated by a direct interaction with the voltage sensing L-type Ca^{2+} channel/dihydropyridine receptor. RyRs are modulated by multiple endogenous effectors including Mg^{2+}, H^+, ATP, calmodulin, protein kinases and phosphatases. Modulation by NO and other redox active molecules suggests a critical

role for cysteine residues in RyR activity. Taken together, RyRs are subject to regulation by multiple effector molecules. Exactly, how RyR regulation influences cellular functions remains to be established.

12. ACKNOWLEDGEMENT

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13. REFERENCES

1. Rios, E. & G. Pizarro: Voltage sensor of excitation-contraction coupling in skeletal muscle. *Physiol Rev* 71, 849-908 (1991)
2. Marx, S. O., K. Ondrias & A. R. Marks: Coupled gating between individual skeletal muscle Ca^{2+} release channels (ryanodine receptors). *Science* 281, 818-821 (1998)
3. Nagasaki, K. & M. Kasai: Fast release of calcium from sarcoplasmic reticulum vesicles monitored by chlortetracycline fluorescence. *J Biochem* 94, 1101-1109 (1983)
4. Kirino, Y., M. Osakabe, and H. Shimizu: Ca^{2+}-induced Ca^{2+} release from fragmented sarcoplasmic reticulum: Ca^{2+}-dependent passive Ca^{2+} efflux. *J Biochem* 94, 1111-1118 (1983)
5. Meissner, G., E. Darling & J. Eveleth: Kinetics of rapid Ca^{2+} release by sarcoplasmic reticulum. Effects of Ca^{2+}, Mg^{2+}, and adenine nucleotides. *Biochemistry* 25, 236-244 (1986)
6. Chen, S. R. W., X. Li, K. Ebisawa & L. Zhang: Functional characterization of the recombinant type 3 Ca^{2+} release channel (ryanodine receptor) expressed in HEK293 cells. *J Biol Chem* 272, 24234-24246 (1997)
7. Jeyakumar, L. H., J. A. Copello, A .M. O'Malley, G.. M. Wu, R. Grassucci, T. Wagenknecht & S. Fleischer: Purification and characterization of ryanodine receptor 3 from mammalian tissue. *J Biol Chem*. 273, 16011-16020 (1998)
8. Murayama, T., T. Oba, E. Katayama, H. Oyamada, K. Oguchi, M. Kobayashi, K. Otsuka & Y. Ogawa: Further characterization of the type 3 ryanodine receptor (RyR3) purified from rabbit diaphragm. *J Biol Chem* 274, 17297-17308 (1999)
9. Gyorke, I. & S. Gyorke: Regulation of the cardiac ryanodine receptor channel by luminal Ca^{2+} involves luminal Ca^{2+} sensing sites. *Biophys J* 75, 2801-2810 (1998)
10. Sitsapesan, R. & A. .J. Williams: Regulation of current flow through ryanodine receptors by lumenal Ca^{2+}. *J Membrane Biol* 159, 179-185 (1997)
11. Tripathy, A. & G. Meissner: Sarcoplasmic reticulum lumenal Ca^{2+} has access to cytosolic activation and inactivation sites of skeletal muscle Ca^{2+} release channel. *Biophys J* 70, 2600-2615 (1996)
12. Xu, L., and G. Meissner: Regulation of cardiac muscle Ca^{2+} release channel by sarcoplasmic reticulum lumenal Ca^{2+}. *Biophys J* 75, 2302-2312 (1998)
13. Chen, S. R., L. Zhang & D. H. MacLennan: Antibodies as probes for Ca^{2+} activation sites in the Ca^{2+} release channel (ryanodine receptor) of rabbit skeletal muscle sarcoplasmic reticulum. *J Biol Chem* 268, 13414-13421 (1993)
14. Li, P. & S. R. Chen: Molecular basis of Ca^{2+} activation of the mouse cardiac Ca^{2+} release channel (ryanodine receptor). *J Gen Physiol* 118, 33-44 (2001)
15. Bhat, M. B., J. Y. Zhao, H. Takeshima & J. J. Ma: Functional Calcium Release Channel Formed By the Carboxyl-Terminal Portion Of Ryanodine Receptor. *Biophys J* 73, 1329-1336 (1997)
16. Du, G. G. & D. H. MacLennan: Ca^{2+} inactivation sites are located in the COOH-terminal quarter of recombinant rabbit skeletal muscle Ca^{2+} release channels (ryanodine receptors). *J Biol Chem* 274, 26120-26126 (1999)
17. Nakai, J., L. Gao, L. Xu, C. Xin, D. A. Pasek & G. Meissner: Evidence for a role of C-terminus in Ca^{2+} inactivation of skeletal muscle Ca^{2+} release channel (ryanodine receptor). *FEBS Letters* 459, 154-158 (1999)
18. Xu, L., G. Mann & G. Meissner: Regulation of cardiac Ca^{2+} release channel (ryanodine receptor) by Ca^{2+}, H+, Mg^{2+}, and adenine nucleotides under normal and simulated ischemic conditions. *Circ Res* 79, 1100-1109 (1996)
19. Ma, J. & J. Zhao: Highly cooperative and hysteretic response of the skeletal muscle ryanodine receptor to changes in proton concentrations. *Biophys J* 67, 626-633 (1994)
20. Liu, W., D. A. Pasek & G. Meissner: Modulation of Ca^{2+}-gated cardiac muscle Ca^{2+}-release channel (ryanodine receptor) by mono- and divalent ions. *Am J Physiol* 274, C120-C128 (1998)
21. Meissner, G., E. Rios, A. Tripathy & D. A. Pasek: Regulation of skeletal muscle Ca^{2+} release channel (ryanodine receptor) by Ca^{2+} and monovalent cations and anions. *J Biol Chem* 272, 1628-1638 (1997)
22. Fruen, B. R., J. R. Mickelson, T. .J. Roghair, H. L. Cheng & C. F. Louis: Anions that potentiate excitation-contraction coupling may mimic effect of phosphate on Ca^{2+} release channel. *Am J Physiol* 266, C1729-C1735 (1994)
23. Laver, D. R., T. M. Baynes & A. F. Dulhunty: Magnesium inhibition of ryanodine-receptor calcium channels: evidence for two independent mechanisms. *J Membrane Biol* 156, 213-229 (1997)
24. Meissner, G: Adenine nucleotide stimulation of Ca^{2+}-induced release in sarcoplasmic reticulum. *J Biol Chem* 259, 2365-2374 (1984)
25. Ogawa, Y. & S. Ebashi: Ca-releasing action of β,γ-methylene adenosine triphosphate on fragmented sarcoplasmic reticulum. *J Biochem* 80, 1149-1157 (1976)
26. Moutin, M. J. & Y. Dupont: Rapid filtration of Ca^{2+}-induced Ca^{2+} release from skeletal sarcoplasmic reticulum. *J Biol Chem* 263, 4228-4235 (1988)
27. Smith, J. S., R. Coronado & G. Meissner: Single channel measurements of the calcium release channel from skeletal muscle sarcoplasmic reticulum. Activation by Ca^{2+} and ATP and modulation by Mg^{2+}. *J Gen Physiol* 88, 573-588 (1986)
28. Rousseau, E., J. S. Smith, J. S. Henderson & G. Meissner: Single channel and $^{45}Ca^{2+}$ flux measurements of the cardiac sarcoplasmic reticulum calcium channel. *Biophys J* 50, 1009-1014 (1986)
29. Tripathy, A., L. Xu, G. Mann & G. Meissner: Calmodulin activation and inhibition of skeletal muscle Ca^{2+} release channel (ryanodine receptor). *Biophys J* 69, 106-119 (1995)

30. Galione, A. & G. C. Churchill: Cyclic ADP ribose as a calcium-mobilizing messenger. *Science's Signal Transduction Knowledge Environment* E1 (2000)
31. Lukyanenko, V., I. Gyorke, T. F. Wiesner & S. Gyorke: Potentiation of Ca^{2+} release by cADP-ribose in the heart is mediated by enhanced SR Ca^{2+} uptake into the sarcoplasmic reticulum. *Circ Res* 89, 614-622 (2001)
32. Morii, H. & Tonomura, Y: The gating behavior of a channel for Ca^{2+}-induced Ca^{2+} release in fragmented sarcoplasmic reticulum. *J. Biochem (Tokyo)* 93, 1271-1285 (1983)
33. Lamb, G. D: Excitation-contraction coupling in skeletal muscle: comparisons with cardiac muscle. *Clin Exp Pharm Physiol* 27, 216-224 (2000)
34. Meissner, G: Evidence of a role for calmodulin in the regulation of calcium release from skeletal muscle sarcoplasmic reticulum. *Biochemistry* 25, 244-251 (1986)
35. Meissner, G. & J. S. Henderson: Rapid calcium release from cardiac sarcoplasmic reticulum vesicles is dependent on Ca^{2+} and is modulated by Mg^{2+}, adenine nucleotide, and calmodulin. *J Biol Chem* 262, 3065-3073 (1987)
36. Smith, J. S., E. Rousseau & G. Meissner: Calmodulin modulation of single sarcoplasmic reticulum Ca^{2+}-release channels from cardiac and skeletal muscle. *Circ Res* 64:352-359 (1989)
37. Buratti, R., G. Prestipino, P. Menegazzi, S. Treves & F. Zorzato: Calcium dependent activation of skeletal muscle Ca^{2+} release channel (ryanodine receptor) by calmodulin. *Biochem Biophys Res Commun* 213, 1082-1090 (1995)
38. Fruen, B. R., J. M. Bardy, T. M. Byrem, G. M. Strasburg & C. F. Louis: Differential Ca^{2+} sensitivity of skeletal and cardiac muscle ryanodine receptors in the presence of calmodulin. *Am J Physiol* 279, C724-C733 (2000)
39. Balshaw, D. M., L. Xu, N. Yamaguchi, D. A. Pasek & G. Meissner: Calmodulin binding and inhibition of cardiac muscle calcium release channel (ryanodine receptor). *J Biol Chem* 276, 20144-20153 (2001)
40. Moore, C. P., G. Rodney, J. Z. Zhang, L. Santacruz-Toloza, G. Strasburg & S. L. Hamilton: Apocalmodulin and Ca^{2+} calmodulin bind to the same region on the skeletal muscle Ca^{2+} release channel. *Biochemistry* 38, 8532-8537 (1999)
41. Rodney, G. G., C. P. Moore, B. Y. Williams, J. Z. Zhang, J. Krol, S. E. Pedersen & S. L. Hamilton: Calcium binding to calmodulin leads to an N-terminal shift in its binding site on the ryanodine receptor. *J Biol Chem* 276, 2069-2074 (2001)
42. Yamaguchi, N., C. Xin & G. Meissner: Identification of apocalmodulin and Ca^{2+}-calmodulin regulatory domain in skeletal muscle Ca^{2+} release channel, ryanodine receptor. *J Biol Chem* 276, 22579-22585 (2001)
43. Samso, M., and T. Wagenknecht: Apocalmodulin and Ca^{2+}-calmodulin bind to neighboring locations on the ryanodine receptor. *J Biol Chem* 277, 1349-1353 (2002)
44. Reid, M. B: reactive oxygen and nitric oxide in skletal muscle. *News Physiol Sci* 11, 114-121 (1996)
45. Eu, J. P., J. Sun, L. Xu, J. S. Stamler & G. Meissner: The skeletal muscle calcium release channel: coupled O2 sensor and NO signaling functions. *Cell* 102, 499-509 (2000)
46. Xia, R., T. Stangler & J. J. Abramson: Skeletal muscle ryanodine receptor is a redox sensor with a well defined redox potential that is sensitive to channel modulators. *J Biol Chem* 275, 36556-36561 (2000)
47. Feng, W., G. Liu, P. D. Allen & I. N. Pessah: Transmembrane redox sensor of ryanodine receptor complex. *J Biol Chem* 275, 35902-35907 (2000)
48. Moore, P. C., J. Z. Zhang & S. L. Hamilton: A role for cysteine 3635 of RYR1 in redox modulation and calmodulin binding. *J Biol Chem* 274, 36831-36834 (1999)
49. Sun, J., C. Xin, J. P. Eu, J. S. Stamler & G. Meissner: Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. *Proc Natl Acad Sci USA* 98, 11158-11162 (2001)
50. Sun, J., L. Xu, J. P. Eu, J. S. Stamler & G. Meissner: Classes of thiols that influence the activity of the skeletal muscle calcium release channel. *J Biol Chem*, 276, 15625-15630 (2001)
51. Kawakami, M. & E. Okabe: Superoxide anion radical-triggered Ca^{2+} release from cardiac sarcoplasmic reticulum through ryanodine receptor Ca^{2+} channel. *Mol Pharm* 53, 497-503 (1998)
52. Stamler, J. S. & G. Meissner: Physiology of nitric oxide in skeletal muscle. *Physiol Rev* 81, 209-237 (2001)
53. Xu, K. Y., D. L. Huso, T. M. Dawson, D. S. Bredt & L. C. Becker: Nitric oxide synthase in cardiac sarcoplasmic reticulum. *Proc Natl Acad Sci USA* 96, 657-662 (1999
54. Xu, L., J. P. Eu, G. Meissner & J. S. Stamler: Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* 279, 234-237 (1998)
55. Kelly, R. A., J. Balligand & T. W. Smith: Nitric oxide and cardiac function. *Circ Res* 79, 363-380 (1996)
56. Reid, M. B: Role of nitric oxide in skeletal muscle: synthesis, distribution and functional importance. *Acta Physiol Scand* 162, 401-409 (1998)
57. Petroff, M. G., S. H. Kim, S. Pepe, C. Dessy, E. Marban, J. L. Balligand & S. J. Sollott: Endogenous nitric oxide mechanisms mediate the stretch dependence of Ca^{2+} release in cardiomyocytes. *Nature Cell Biol* 3, 867-873 (2001)
58. Ziolo, M. T., H. Katoh & D. M. Bers: Positive and negative effects of nitric oxide on $Ca(^{2+})$ sparks: influence of beta-adrenergic stimulation. *Am J Physiol* 281, H2295-H2303 (2001)
59. Stoyanovsky, D., T. Murphy, P. R. Anno, Y. M. Kim & G. Salama: Nitric oxide activates skeletal and cardiac ryanodine receptors. *Cell Calcium* 21, 19-29 (1997)
60. Aghdasi, B., M. B. Reid & S. L. Hamilton: Nitric oxide protects the skeletal muscle Ca^{2+} release channel from oxidation induced activation. *J Biol Chem* 272, 25462-25467 (1997)
61. Meszaros, L. G., I. Minarovic & A. Zahradnikova: Inhibition of the skeletal muscle ryanodine receptor calcium release channel by nitric oxide. *FEBS Letters* 380, 49-52 (1996)
62. Suko, J., H. Drobny & G. Hellmann: Activation and inhibition of purified skeletal muscle calcium release channel by NO donors in single channel current recordings. *Biochim Biophys Acta* 1451, 271-287 (1999)
63. Hart, J. D. & A. F. Dulhunty: Nitric oxide activates or inhibits skeletal muscle ryanodine receptors depending on

its concentration, membrane potential and ligand binding. *J Membrane Biol* 173, 227-236 (2000)
64. Sun, J., L. Xu, J. P. Eu, J. S. Stamler & G. Meissner: Modulation of skeletal muscle calcium release channel/ryanodine receptor (RyR1) by NOC-12 occurs independent of oxygen tension. *Biophys J* 82, 624a. (2002)
65. Zahradnikova, A., I. Minarovic, R. C. Venema & L.G. Meszaros: Inactivation of the cardiac ryanodine receptor calcium release channel by nitric oxide. *Cell Calcium* 22, 447-454 (1997)
66. Lefer, D. J., K. Nakanishi, W. Johnston & J. Vinten-Johansen: Antineutrophil and myocardial protecting actions of a novel nitric oxide donor after acute ischemia and reperfusion. *Circulation* 88, 2337-2350 (1993)
67. Woolfson, R. G., V. C. Patel, G. H. Neild & D. M. Yellon: Inhibition of nitric acid synthesis reduces infarct size by an adenosine-dependent mechanism. *Circulation* 91, 1545-1551 (1995)
68. Leddy, J. J., B. J. Murphy, Y. Qu, J. P. Doucet, C. Pratt & B. S. Tuana: A 60 kDa polypeptide of skeletal-muscle sarcoplasmic reticulum is a calmodulin-dependent protein kinase that associates with and phosphorylates several membrane proteins. *Biochem J* 295, 849-856 (1993)
69. Hain, J., S. Nath, M. Mayrleitner, S. Fleischer & H. Schindler: Phosphorylation modulates the function of the calcium release channel of sarcoplasmic reticulum from skeletal muscle. *Biophys J* 67, 1823-1833 (1994)
70. Damiani, E., G. Tobaldin, E. Bortoloso & A. Margreth: Functional behaviour of the ryanodine receptor/Ca($^{2+}$)-release channel in vesiculated derivatives of the junctional membrane of terminal cisternae of rabbit fast muscle sarcoplasmic reticulum. *Cell Calcium* 22, 129-150 (1997)
71. Zhao, S. M., N. R. Brandt, A. H. Caswell & E. Y. C. Lee: Binding of the catalytic subunit of protein phosphatase-1 to the ryanodine-sensitive calcium release channel protein. *Biochemistry* 37, 18102-18109 (1998)
72. Dulhunty, A. F., D. Laver, S. M. Curtis, S. Pace, C. Haarmann & E. M. Gallant: Characteristics of irreversible ATP activation suggest that native skeletal ryanodine receptors can be phosphorylated via an endogenous CaMKII. *Biophys J* 81, 3240-3252 (2001)
73. Marx, S. O., S. Reiken, Y. Hisamatsu, M. Gaburjakova, J. Gaburjakova, Y. M. Yang, N. Rosemblit & A. R. Marks: Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. *J Cell Biol* 153, 699-708 (2001)
74. Varsanyi, M. & H E. Meyer: Sarcoplasmic reticular Ca^{2+} release channel is phosphorylated at serine 2843 in intact rabbit skeletal muscle. *Biol Chem Hoppe-Seyler* 376, 45-49 (1995)
75. Suko, J., I. Maurer-Fogy, B. Plank, O. Bertel, W. Wyskovsky, M. Hohenegger & G. Hellmann: Phosphorylation of serine 2843 in ryanodine receptor-calcium release channel of skeletal muscle by cAMP-, cGMP- and CaM-dependent protein kinase. *Biochim Biophys Acta* 1175, 193-206 (1993)
76. Marx, S. O., S. Reiken, Y. Hisamatsu, T. Jayaraman, D. Burkhoff, N. Rosemblit & A. R. Marks: PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 101, 365-376 (2000)
77. Witcher, D. R., R. J. Kovacs, H. Schulman, D. C. Cefali & L. R. Jones: Unique phosphorylation site on the cardiac ryanodine receptor regulates calcium channel activity. *J Biol Chem* 266, 11144-11152 (1991)
78. Hain, J., H. Onoue, M. Mayrleitner, S. Fleischer & H. Schindler: Phosphorylation modulates the function of the calcium release channel of sarcoplasmic reticulum from cardiac muscle. *J Biol Chem* 270, 2074-2081 (1995)
79. Allen, B. G. & S. Katz: Phosphorylation of cardiac junctional and free sarcoplasmic reticulum by PKCalpha, PKCbeta, PKA and the Ca^{2+}/calmodulin-dependent protein kinase. *Mol Cell Biochem* 155, 91-103 (1996)
80. Bandyopadhyay, A., D. W. Shin, J.O. Ahn & D. H. Kim: Calcineurin regulates ryanodine receptor/Ca^{2+}-release channels in rat heart. *Biochem J* 352, 61-70 (2000)
81. Guse, A. H., A. Y. Tsygankov, K. Weber & G. W. Mayr: Transient tyrosine phosphorylation of human ryanodine receptor upon T cell stimulation. *J Biol Chem* 276, 34722-34727 (2001)
82. Takasago, T., T. Imagawa, K. Furukawa, T. Ogurusu & M. Shigekawa: Regulation of the cardiac ryanodine receptor by protein kinase-dependent phosphorylation. *J Biochem (Tokyo)* 109, 163-170 (1991)
83. Valdivia, H. H., J. H. Kaplan, G. C. Ellis-Davies & W. J. Lederer: Rapid adaptation of cardiac ryanodine receptors: modulation by Mg^{2+} and phosphorylation. *Science* 267, 1997-2000 (1995)
84. Li, Y., E. G. Kranias, G. A. Mignery & D. .M. Bers: Protein kinase A phosphorylation of the ryanodine receptor does not affect calcium sparks in mouse ventricular myocytes. *Circ Res* 90, 309-316 (2002)
85. Szegedi, C., S. Sarkozi, A. Herzog, I. Jona & M. Varsanyi: Calsequestrin: more than 'only' a luminal Ca^{2+} buffer inside the sarcoplasmic reticulum. *Biochem J* 337, 19-22 (1999)
86. Damiani, E., E. Picello, L. Saggin & A. Margreth: Identification of triadin and of histidine-rich Ca($^{2+}$)-binding protein as substrates of 60 kDa calmodulin-dependent protein kinase in junctional terminal cisternae of sarcoplasmic reticulum of rabbit fast muscle. *Biochem Biophys Res Comm* 209, 457-465 (1995)
87. Orr, I. & V. Shoshan-Barmatz: Modulation of the skeletal muscle ryanodine receptor by endogenous phosphorylation of 160/150-kDa proteins of the sarcoplasmic reticulum. *Biochim Biophys Acta* 1283, 80-88 (1996).

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