A Postulated Role of the Near Amino-terminal Domain of the Ryanodine Receptor in the Regulation of the Sarcoplasmic Reticulum Ca$^{2+}$ Channel*

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To test the hypothesis that interactions among several putative domains of the ryanodine receptor (RyR) are involved in the regulation of its Ca$^{2+}$ release channel, we synthesized several peptides corresponding to selected NH$_2$-terminal regions of the RyR. We then examined their effects on ryanodine binding and Ca$^{2+}$ release activities of the sarcoplasmic reticulum isolated from skeletal and cardiac muscle. Peptides 1–2s, 1–2c, and 1 enhanced ryanodine binding to cardiac RyR and induced a rapid Ca$^{2+}$ release from cardiac SR in a dose-dependent manner. The order of the potency for the activation of the Ca$^{2+}$ release channel was 1–2c > 1 > 1–2s. Interestingly, these peptides produced significant activation of the cardiac RyR at near zero or subactivating [Ca$^{2+}$], indicating that the peptides enhanced the Ca$^{2+}$ sensitivity of the channel. Peptides 1–2e, 1–2s, and 1 had virtually no effect on skeletal RyR, although occasional and variable extents of activation were observed in ryanodine binding assays performed at 36 °C. Peptide 3 affected neither cardiac nor skeletal RyR. We propose that domains 1 and 1–2 of the RyR, to which these activating peptides correspond, would interact with one or more other domains within the RyR (including presumably the Ca$^{2+}$-binding domain) to regulate the Ca$^{2+}$ channel.

The ryanodine receptor (RyR)$^1$ is a large homotetrameric molecule that contains the calcium release channel of the sarcoplasmic reticulum (SR) (1–4). The carboxyl-terminal region of the RyR is composed of 4–12 (5, 6) transmembrane helices, which form the calcium release channel. The major portion of the RyR molecule protrudes into the cytoplasm, spanning the gap between the plasma membrane and the SR (7). This bulky cytoplasmic domain of the RyR, often called the junctional foot, presumably serves as a receptor for various types of stimuli, effectors (8–13), and regulatory proteins such as calmodulin (14–16) and FK506-binding protein (17, 18). Thus, there must be an intricate communication network within the receptor molecule to send the signal from various cytoplasmic effector/regulator binding domains to the Ca$^{2+}$ release channel domain.

However, only very limited information is available regarding the actual mechanism for channel regulation.

Several pieces of information suggest the hypothesis that the near NH$_2$-terminal region of the RyR may be involved in its channel regulation. First, this is one of the two regions where most mutations have been found in patients with malignant hyperthermia and central core disease (19–26), and experimental models of these mutations in fact produced abnormalities in Ca$^{2+}$ release channel regulation (27–31). An antibody raised against this NH$_2$-terminal region altered the Ca$^{2+}$-dependence of channel gating (32). Chemical modification of the NH$_2$-terminal region of the RyR affected intramolecular crosslinking under conditions that would produce oxidation-induced activation of the Ca$^{2+}$ channel (33, 34). Furthermore, this region has a primary structure that is highly homologous to the IP$_3$ binding region of the IP$_3$ receptor, which is clearly involved in the regulation of its channel gating (35–38).

The goal of the present study is to test the above hypothesis. For this purpose, we synthesized peptides corresponding to two selected domains of the near NH$_2$-terminal region, designated as domain 1–2 and 3 (see Table I). We then investigated the effects of these synthetic peptides on ryanodine binding and SR Ca$^{2+}$ release using SR vesicles isolated from both skeletal and cardiac muscles. The peptides corresponding to domain 1–2 activated the RyR2 and induced a rapid Ca$^{2+}$ release from cardiac SR. These peptides produced no activation of the RyR1 as long as the temperature during the assay does not exceed a certain level. The other peptide corresponding to domain 3 affected neither RyR2 nor RyR1. As shown in the [Ca$^{2+}$]-dependence of activation, the peptides corresponding to domain 1–2 activate the RyR2 in zero or near zero [Ca$^{2+}$]. Furthermore, they serve as an amplifier of Ca$^{2+}$-dependent activation of the RyR2. The present results suggest that the domain corresponding to these peptides, namely domain 1–2, is involved in the mechanism of activating the SR Ca$^{2+}$ channel by mediation of intramolecular domain-domain interaction.

EXPERIMENTAL PROCEDURES

Background and Terminology of the Domain Peptides Used

The use of synthetic peptides corresponding to key domains of the RyR to examine their effects on RyR function would be a useful approach to characterize putative functions of these domains. Since several pieces of evidence suggest that the NH$_2$-terminal region of the RyR may serve as an important regulatory domain of channel function (see Introduction), we selected some subdomains from this region of the RyR1 and RyR2 as illustrated in Table I. Domain 1–2s and its cardiac counterpart, domain 1–2c, consist of two subdomains: domain 1, which is composed of an identical sequence for both RyR1 and RyR2 as illustrated in Table I. Domain 1–2s and its cardiac counterpart, domain 1–2c, consist of two subdomains: domain 1, which is composed of an identical sequence for both RyR1 and RyR2, and domain 2, where there is some difference between both isoforms (cf. underlined residues). The synthesized peptides will be identified by names identical to their corresponding in situ domains just defined.
Preparation
Cardiac Microsomes—Dog ventricular cardiac muscle was homogenized in four volumes of 0.3 M sucrose, 40 mM imidazole, pH 6.8, 5 μg/ml leupeptin, 0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 0.1 M potassium gluconate, proteolytic enzyme inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 0.8 μg/ml antipain, 2.0 μg/ml soybean trypsin inhibitor), 20 mM MES, pH 6.8 (PI buffer), with a glass/glass homogenizer, and centrifuged again at 143,000 × g for 45 min. The resultant pellet was resuspended in PI buffer to a final concentration of 20–30 mg/ml, frozen immediately in liquid N₂, and stored at −78 °C.

Skeletal Microsomes—Triad-enriched microsomal fractions were prepared from the rabbit back paraspинарис and hind leg skeletal muscle by a method of differential centrifugation as described previously (39). Microsomes from the final centrifugation were homogenized in PI buffer to a final concentration of 20–30 mg/ml, frozen immediately in liquid N₂, and stored at −78 °C.

Synthesis of Peptides
Peptides were synthesized on an Applied Biosystems model 431 A synthesizer employing Fmoc (N-9-fluorenylmethoxycarbonyl) as the α-amino protecting group. The peptides were cleaved and deprotected with 95% trifluoroacetic acid and purified by reverse-phase high pressure liquid chromatography.

[3H]Ryanodine Binding Assay
Dog cardiac (1.5 mg/ml) or rabbit skeletal (0.5 mg/ml) microsomes were incubated in 0.1 ml of a reaction solution containing 10 mM (dog cardiac) or 8 mM (rabbit skeletal) [3H]Ryanodine (68.4 Ci/ml, NEN Life Science Products), 0.3 μM KCl, 1 mM EGTA, and various amounts of CaCl₂ to create various levels of Ca²⁺. The homogenate was then centrifuged at 5,500 × g, in a Warning buffer to a final concentration of 20–30 mg/ml, frozen immediately in liquid N₂, and stored at −78 °C.

Effects of Domain Peptides on Ryanodine Binding—We examined two groups of synthetic peptides corresponding to the two domains of the RyR1, domain 1–2 and domain 3 (cf. Table I) for their effects on ryanodine binding to the RyR1 and RyR2. Fig. 1 depicts the results of the ryanodine binding assay carried out at 36 °C. As seen in Fig. 1, peptide 1–2s produced a significant enhancement of ryanodine binding to the RyR2 in a concentration-dependent manner. In many experiments, peptide 1–2s produced no appreciable enhancement of ryanodine binding to the RyR1 in the same concentration range. In some other experiments carried out at 36 °C, however, peptide 1–2s produced various extents of enhancement of ryanodine binding in RyR1. Fig. 2A is a histogram illustrating the relationship between the frequency of occurrence and the extent of enhancement of ryanodine binding to the RyR2 and RyR1 produced by the maximally activating concentration of peptide 1–2s (200 μM) at 36 °C. As shown, a significant amount of enhancement (151–210%) occurred in all experiments in case of the RyR2. In case of the RyR1, however, the level of enhancement varied over a wide range, from no effect to levels comparable to the RyR2. We carried out the same type of ryanodine binding assay at 22 °C (the temperature at which all stopped-flow experiments described below were carried out), and the effects on the RyR2 and the RyR1 were compared. As shown, peptide 1–2s produced a significant and consistent activation of the RyR2 (Fig. 2B). Interestingly, the peptide produced no appreciable effect on the RyR1 in all experiments. Furthermore, peptide 1–2c produced significant activation of ryanodine binding to RyR2, even in ryanodine binding experiments performed at 4 °C assay (data not shown). These results indicate that the domain peptide 1–2s activates the RyR in a RyR2-specific manner. However, RyR1 also becomes responsive to peptide activation above a transitional temperature, which appears to be in the vicinity of 36 °C. Another domain peptide, peptide 3s, did not produce any appreciable effect on either the RyR1 or the RyR2, as determined by the ryanodine binding assay at 36 °C (Fig. 3), providing a negative control for the above experiments. The amino acid sequence of the region of the RyR1 corresponding to peptide 1–2s (i.e. domain 1–2s) and that of the corresponding region of the RyR2 (domain 1–2c) are essentially identical, but are different in four residues located in their subdomain 2 (cf. Table I). We compared the effects of both isoforms of peptide 1–2 on ryanodine binding to the RyR2 at 36 °C and at different Ca²⁺ concentrations in the assay solution. The synthetic peptide corresponding to the domain 1–2c, namely peptide 1–2c, enhanced ryanodine binding to the RyR2 (Fig. 4). The concentration dependence of activation was indistinguishable between peptides 1–2s and 1–2c at 10 μM Ca²⁺ (Fig. 4) This suggests that the activating function of these peptides may be localized within their subdomain 1 where both peptides have a common amino acid sequence. As shown in Fig. 4, peptide 1 corresponding to subdomain 1 in fact activated RyR2 with the same concentration dependence of activation as the longer peptides 1–2s and 1–2c.

We have carried out the same type of experiment at lower concentrations of Ca²⁺; at 0.2 μM and nominal zero Ca²⁺. Table
We incubated with 10 nM [3H]ryanodine in a solution containing 0.3 M diac SR vesicles (RyR2). Dog cardiac muscle microsomes (1.5 mg/ml) were incubated with 10 nM [3H]ryanodine in a solution containing 0.3 M KCl, 10 μM CaCl₂, 20 mM MOPS, pH 7.2, and various concentrations of peptide 1–2s as indicated. Ryanodine binding in the absence of peptides (control) was 0.91 ± 0.13 pmol/μg. Each datum point represents the mean ± S.D. of four or more experiments carried out in duplicate.

II depicts the AC₅₀ values for each peptide at three different [Ca²⁺] values including 10 μM described above. Interestingly, at lower Ca²⁺ concentrations, some differences in the potency of activation among these domain peptides became visible, i.e. the extent of activation by peptide 1–2s is much smaller than the extent of activation by peptide 1–2c and peptide 1. This indicates that, at this submaximally activating concentration of Ca²⁺, the apparent affinity of peptide 1–2s to the RyR2 is somewhat lower than peptides 1–2c and peptide 1. It also appears that peptide 1–2c produces a slightly larger rate of activation than peptide 1 at high peptide concentrations (data not shown). Thus, the order of the strength of activation of these peptides at 0.2 μM and nominal zero Ca²⁺ is 1–2c ≥ 1 > 1–2s. It should be noted that, at nominal zero Ca²⁺, all of these three peptides produced a small but significant increase in the ryanodine binding to the RyR2. This suggests that there is a unique feature of Ca²⁺-independent activation, which is not usually seen with many known activating reagents. As seen from the comparison of the AC₅₀ values at three different [Ca²⁺] values (Table II), the AC₅₀ values are significantly larger for all peptides at lower [Ca²⁺] values. These results indicate that the domain peptides bind preferentially to the calcium-bound RyR2, although the presence of Ca²⁺ is not an essential requirement for the activation by these peptides.

Domain Peptides Induce Ca²⁺ Release from Cardiac SR—Fig. 5 depicts the time courses of Ca²⁺ release from cardiac SR induced by various concentrations of peptides 1–2s, 1–2c, and 1. In these experiments, a solution containing cardiac microsomes that had been loaded with calcium by MgATP-dependent Ca²⁺ transport (Solution A) was mixed with a solution containing various concentrations of peptides (Solution B). Then, the time course of the changes in the Ca²⁺ concentration was monitored using fluo-3 at a starting [Ca²⁺] of 0.25 μM. As seen, these peptides produced a rapid Ca²⁺ release in a dose-dependent manner mirroring the effect of these peptides on ryanodine binding experiments with the RyR2. In Fig. 6, the initial rates of Ca²⁺ release are plotted as a function of the concentration of these three peptides added. The order of the strength of inducing Ca²⁺ release estimated from this plot is 1–2c > 1 > 1–2s, consistent with ryanodine binding assays carried out at equivalent [Ca²⁺].

An interesting feature revealed in these Ca²⁺ release experiments is the biphasic change in the Ca²⁺ signal. As seen in Fig. 5, the rapid Ca²⁺ release produced by higher concentrations of peptide 1–2c (>40 μM) is followed by a decrease of the fluo-3 signal. Both the magnitude and the rate of this descending phase increased with the increase in peptide concentration. As seen from the comparison among the three peptides at the equivalent concentration (e.g. see 160 μM), the order of the ability of producing the descending phase is 1–2c > 1 > 1–2s. Interestingly, this is the same as the order of the ability of inducing Ca²⁺ release (see above). The descending phase presumably represents re-uptake of the released Ca²⁺ via the SR Ca²⁺ pump. Thus, it appears that a faster and larger Ca²⁺ release results in a faster and larger uptake of the released Ca²⁺ in a coordinated manner. As seen in the release/uptake curve produced by 160 μM peptide 1–2c (Fig. 5), the Ca²⁺ level often became even lower than the starting level. This indicates that the Ca²⁺ pumping rate became considerably larger than the steady-state pumping rate (the rate prior to the induction of Ca²⁺ release) after peptide-induced Ca²⁺ release.

None of these peptides induced Ca²⁺ release from skeletal muscle SR even at high concentrations, in agreement with the ryanodine binding data obtained at the equivalent temperature, 22 °C. Furthermore, peptide 3s, which had no effect on ryanodine binding to the RyR1 or the RyR2, did not produce any Ca²⁺ release from either cardiac or skeletal muscle SR.

**Ca²⁺ Dependence of Domain Peptide Activation of the RyR2—**Fig. 7 shows the Ca²⁺ dependence of ryanodine binding to the RyR2 in the absence or presence of 100 μM peptide 1. As seen, peptide 1 produces significant enhancement of ryanodine binding to RyR2 not only in the activating [Ca²⁺] range, but also in the zero to subactivating [Ca²⁺] range. The extent of activation by the peptide is about 200% of control at maximally activating [Ca²⁺], while it increases considerably at submaximally activating [Ca²⁺]. This indicates that these domain peptides not only serve as an amplifier of the Ca²⁺-dependent activation of the RyR2, but also as a Ca²⁺-independent activator.

As shown in Fig. 8, at zero [Ca²⁺] peptide 1 produced a significant enhancement of ryanodine binding to the RyR2 (p < 0.001), but had no effect on the RyR1. Conversely, calmodulin, which is known to activate the RyR1 at a very low [Ca²⁺] (15), produced a significant activation of the RyR1 (p = 0.001), but had no effect on the RyR2. Thus, it appears that these domain peptides produce a calmodulin-like function for the RyR2.

**DISCUSSION**

One of the important unsolved questions in the E-C coupling is how various stimuli received in the cytoplasmic domains of the RyR are transmitted to the trans-membrane Ca²⁺ channel domain to regulate the channel functions. Several pieces of indirect evidence in the literature suggest the hypothesis that the NH₂-terminal ~700-amino acid region of the RyR may represent one of the putative regulatory domains of channel function. This is the region with frequent occurrence of human malignant hyperthermia mutations and in the porcine animal model of this disease (19–26). These mutations consistently produce an abnormal regulation of the RyR (27–31, 42). A monoclonal antibody raised against the region containing Gly¹⁸⁴² in fact produced a significant activation of the RyR1 and a Ca²⁺-hypersensitization of the RyR1 (32). Thiokal alkaloids of the 170 kDa NH₂-terminal region affected intramolecular S-S formation, suggesting that this region may be involved in intramolecular domain-domain interactions (33, 34). Furthermore, the NH₂-terminal region of the RyR is highly homologous to the corresponding region of the IP₃ receptor, which contains the IP₃ binding site and which plays an important role in the regulation of the IP₃ receptor channel function (35–38).

We initiated this study to identify the putative regulatory domain(s) located in the NH₂-terminal region of the RyR and to characterize its (their) function utilizing synthetic peptides as
probes. This strategy is based upon the following principle. If any subdomain of this region interacts with the target domain of functional importance, the extrinsically added peptide corresponding to that NH₂ domain would bind to its target domain. Then, this peptide-to-target domain interaction would produce some effects on the RyR function that are similar to those obtained by the in vivo NH₂ domain-to-target domain interaction. This would not only permit us to identify the putative regulatory domain, but would also allow us to characterize its mode of regulation.

One of the most important findings of the present study is that the synthetic peptides (peptide 1–2s and 1–2c), which correspond to the domain 1–2 encompassing the Leu⁵⁹⁰–Gly⁶²⁸ (RyR1) and Leu⁶⁰¹–Gly⁶³⁹ (RyR2) regions, respectively (see Table I), produced significant activation of the RyR2 and induced Ca²⁺ release from cardiac SR. Domain 1–2 of the RyR1 and its counterpart of the RyR2 share the same sequence in the region of subdomain 1. The corresponding peptide 1 produced about the same effects as peptide 1–2c, indicating that the essential activating function is localized in subdomain 1. Interestingly, these peptides were virtually incapable of inducing Ca²⁺ release from skeletal SR. Generally, they produced no effect on ryanodine binding to the RyR1 either, as long as the assay temperature was kept below 36 °C. This indicates that the activation is specific to the cardiac isoform of the RyR under such limited conditions. At 36 °C, however, the domain peptide 1–2 became capable of activating the RyR1. The extents of activation varied over a broad range, from no activation to a full activation comparable with that of the RyR2. This would indicate that there is a common mechanism between both RyR isoforms by which the RyR channel is regulated by mediation of an intramolecular domain-domain interaction, but in the RyR1 the peptide probe revealed such a mechanism only at a high temperature. A large variation in the extent of activation seen

FIG. 2. Effects of peptide 1–2s on [³H]ryanodine binding to the RyR2 and the RyR1 at two assay temperatures: A, 36 °C; B, 22 °C. Histograms show the frequency of occurrences of different extents of enhancement of ryanodine binding to the RyR2 and the RyR1 induced by 200 μM peptide 1–2s. The assay solutions consisted of 0.3 M KCl, 10 mM CaCl₂, 20 mM MOPS, pH 7.2, with 10 nM or 8 nM [³H]ryanodine for dog cardiac muscle microsomes and rabbit skeletal muscle microsomes, respectively.

FIG. 3. Lack of effect of peptide 3s on [³H]ryanodine binding to cardiac SR vesicles (RyR2) and skeletal triads (RyR1). Ryanodine binding assays were carried out at 36 °C in the presence of various concentrations of peptide 3s as described under "Experimental Procedures." Each datum point represents the mean ± S.D. of at least three experiments carried out in duplicate.

FIG. 4. Concentration dependence of activation of RyR2 by peptides 1–2s, 1–2c, and 1 at 10 μM Ca²⁺. Ryanodine binding assays were performed at 36 °C (see "Experimental Procedures"). Each datum point represents the mean ± S.D. of at least three experiments carried out in duplicate.

TABLE II
The concentrations for half-maximal activation (AC₅₀) of ryanodine binding to the RyR2 by three isoforms of domain peptide 1–2
Data represent the mean ± S.D. of three or more experiments carried out in duplicate. *, p ≤ 0.05 (1–2c versus 1–2s). **, p ≤ 0.05 (1–2s versus 1).

| [Ca²⁺] | 1–2s | 1–2c | 1 |
|--------|------|------|---|
| 10     | 23.0 ± 7.2 | 25.3 ± 7.8 | 22.7 ± 7.0 |
| 0.2    | 251.7 ± 57* | 134.0 ± 47.6 | 176.9 ± 28.9 |
| ~0     | 179.3 ± 30** | 117.8 ± 26.5 | 102.9 ± 19.5 |
at 36 °C suggests that in the vicinity of this temperature there is a conformational transition from a “tighter” domain-domain interaction to a somewhat “loose” interaction, permitting the added peptides to exert an additional or complimentary activation. Another important aspect of this study is the finding that peptide 3 corresponding to the other domain encompassing the Asp324–Val351 segment affected neither the RyR1 nor the RyR2. Thus, it appears that the ability to activate the RyR2 is specifically localized in the domain 1, although further screening studies might reveal more regulatory regions/domains with similar or different functions.

As shown in the present ryanodine binding assays, these peptides are capable of activating the RyR2 at zero or nearly zero [Ca\(^{2+}\)]. It is particularly interesting to note that the mode of activation by these domain peptides at nominal zero Ca\(^{2+}\) is similar to that of the effect of calmodulin on the RyR1. Since calmodulin has no effect on the RyR2 and domain peptides have no effects on the RyR1, it is tempting to speculate that the domain peptides (hence, the corresponding \textit{in situ} domain, domain 1–2) have a calmodulin-like function on the RyR2 at very low Ca\(^{2+}\) concentrations. As shown in this study, at a threshold level of [Ca\(^{2+}\)] for the activation of cardiac muscle, \textit{i.e.} 0.2 μM (43, 44), these peptides produced a significant activation of the RyR2 and induced a rapid Ca\(^{2+}\) release from cardiac SR. This suggests that the corresponding domain (\textit{i.e.} domain 1–2) may be involved in the enhancement of cardiac contractility in the vicinity of the threshold [Ca\(^{2+}\)] required for activation. At maximally activating or even higher [Ca\(^{2+}\)] values, these peptides still produced a significant enhancement of the Ca\(^{2+}\)-dependent activation of the RyR2. Thus, the domain corresponding to these peptides (\textit{i.e.} domain 1–2) seems to play several important functions, \textit{e.g.} (a) activation of the RyR2 at nearly zero Ca\(^{2+}\), and (b) amplification of the Ca\(^{2+}\)-dependent activation of the RyR2 and opening of the Ca\(^{2+}\) channel at near threshold or subthreshold [Ca\(^{2+}\)] values.

The amino acid sequence of peptide 1–2s represents the common sequence of domain 1–2s of the RyR1 of several animal species. Similarly, peptide 1–2c represents the common sequence of the RyR2 of several animal species. Thus, the difference in four amino acid residues between the two peptides (\textit{cf.} underlined residues in Table I) represents definitive characteristics distinguishing between the RyR1 and the RyR2. As
shown in the present studies, the $A_C_{50}$ of peptide 1–2c was significantly lower than that of peptide 1–2s. This indicates that the affinity of binding to the putative regulatory domain described above is higher for the cardiac sequence than the skeletal sequence. The extent of activation by peptide 1–2c appears to be significantly larger than that of peptide 1. These results suggest that the skeletal-type sequence that is present in subdomain 2 may function as a suppressor of the activating function localized in subdomain 1, whereas the cardiac-type sequence of subdomain 2 may function as an enhancer. Interestingly, the differences in the apparent affinity among these peptides became evident only at lower $[Ca^{2+}]$ values (0 or 0.2 mM), and such differences were abolished at higher $[Ca^{2+}]$ values (10 mM), suggesting that the affinity of these peptides increases with $[Ca^{2+}]$. Thus, the cardiac sequence of domain 1–2 may be important for the regulation at low $[Ca^{2+}]$ values, but the common sequence in domain 1 may be sufficient at higher $[Ca^{2+}]$ values.

The remaining important questions concern the general mechanism by which the RyR is regulated in a $Ca^{2+}$-dependent manner, and more specifically the mechanism by which the $Ca^{2+}$-dependent activation of the RyR2 is amplified by domain peptide 1–2. Since this domain peptide is a copy of domain 1–2 and it has discrete effects on the RyR, it is conceivable that within the RyR molecule this domain is interacting with its target domain to regulate the $Ca^{2+}$ channel. We tentatively locate the target domain in the postulated modulatory domain containing the putative high affinity $Ca^{2+}$ binding site (45). Regarding the mode of channel regulation, there are at least two alternative possibilities as follows. First, the interdomain interaction may facilitate the $Ca^{2+}$-dependent channel opening. Conversely, it is also possible that the interdomain interaction serves as a channel blocking mechanism. Then, why did the added domain peptide activate the RyR channel in a RyR2-specific manner? In the case of the first possibility (namely the interdomain interaction produces channel activation), the interaction between domain 1–2 and the $Ca^{2+}$-modulatory domain (see above) would be much tighter in the RyR1 than in the RyR2. In other words, the domain-mediated regulatory mechanism is fully functional in the RyR1, but its function is only partial in the RyR2. Therefore, the interaction of the domain peptide with the target domain will exert additional or supplementary effects in the RyR2. At higher temperatures, the interdomain interactions would become less tight, and the activating effects of peptides would become pronounced even in the RyR1. In the case of the second possibility (i.e., the interdomain interaction produces channel closing), the isoform-dependent differences in the tightness of interdomain interaction and its temperature dependence would be entirely opposite to the above. For instance, the interdomain interaction would be much tighter in the RyR2 than in the RyR1, producing more prominent de-blocking effects of the added peptides in the RyR2. Clearly more work is required to elucidate the detailed mechanism(s) of the channel regulation mediated by domain-domain and peptide-domain interactions.

Our recent study on polylysine-induced $Ca^{2+}$ release from the skeletal muscle SR (46) suggested the presence of a tight coordination between $Ca^{2+}$ release and the subsequent uptake of the released $Ca^{2+}$. As shown in the present study, there appears to be a similar release/uptake coordination mechanism also in the cardiac SR. Assuming that the domain peptides used here are simulating the exact features of the in vivo channel regulation mechanism, we would propose that the mechanism discussed above is involved not only in the potentiation of channel opening, but also in the acceleration of $Ca^{2+}$ re-uptake.

In conclusion, the synthetic peptides corresponding to one of the conserved NH$_2$-terminal domains of the RyR1 and the RyR2 (designated as domain 1–2) activated the RyR2. These peptides activated the RyR2 at nearly zero $[Ca^{2+}]$, and also amplified $Ca^{2+}$-dependent activation of the RyR2. They also induced a rapid $Ca^{2+}$ release from the cardiac SR. Thus, it appears that the domain corresponding to these peptides, namely domain 1–2, is capable of performing these functions in situ, by interacting with the putative regulatory domain(s).

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