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Depletion of FKBP does not affect the interaction between isolated ryanodine receptors ☆

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Abstract

The ryanodine receptors/calcium release channels (RyRs) usually form two dimensional regular lattices in the endoplasmic/sarcoplasmic reticulum membranes. The native RyR is associated with many auxiliary proteins, including FKBP. It has been indicated that FKBP may play a role in the intermolecular interaction and coupled gating of neighboring RyRs. However, a more recent study shows that FKBP12 is not involved in the physical linkage between neighboring RyR1s. In the present work, the effect of FKBP12 on the interaction between RyR1s isolated from rabbit skeletal muscle was investigated in an aqueous medium with photon correlation spectroscopy. We found that the depletion of FKBP12 did not affect the oligomerization of RyR1s in the medium containing different [KCl] or under different channel functional states. No evidence is obtained for the involvement of FKBP12 in the intermolecular interaction between RyR1s.

Keywords: Ryanodine receptor/calcium release channel; RyR array; Coupled gating; Intermolecular interaction; FKBP12; Modulation; Photon correlation spectroscopy

Ryanodine receptor (RyR) is a prototypical member of the Ca^{2+} release channel superfamily located in the endoplasmic/sarcoplasmic reticulum (SR) and plays a pivotal role in intracellular Ca^{2+} signaling processes, such as excitation–contraction coupling [1–4]. Three isoforms of RyRs have been identified in mammals, designated as skeletal (RyR1), cardiac (RyR2), and brain (RyR3) [1]. Intriguingly, RyR1s and RyR2s in intact cells are usually found to assemble into 2-D paracrystalline arrays in SR membrane [5–7]. This pattern of RyR organization seems to be highly conserved from crustaceans to vertebrates, suggesting that the array formation is critical to the RyR-mediated Ca^{2+} signaling in vivo [5–8]. However, the detailed function and operating mechanism of RyR arrays are still unclear.

A number of auxiliary proteins, such as FKBP, are associated with native RyR and modulate the channel function [1,3]. FKBP, a *cis–trans* prolyl isomerase, associates with RyR with a stoichiometry of one FKBP per subunit of RyR tetramer [9,10]. RyR1 binds FKBP12 and RyR2 binds FKBP12.6 [9,10]. FKBP has been shown to stabilize the intramolecular interaction among RyR subunits and enhance the coordination of RyR gating [11,12]. In a recent electrophysiological study, Marx et al. [13,14] have found that FKBP mediates

☆ *Abbreviations:* RyR, ryanodine receptor/calcium release channel; SR, sarcoplasmic reticulum; EM, electron microscopy; PCS, photon correlation spectroscopy; *Z*-average, average hydrodynamic diameter; DTT, dithiothreitol; PC, phosphatidylcholine; Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

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the simultaneous opening and closing of neighboring reconstituted RyRs isolated from both skeletal and cardiac muscle cells. Thus, FKBP may also play an important role in the intermolecular interaction and coupled gating of neighboring RyRs [13–15]. However, more recently, an electron microscopy (EM) observation of RyR1 arrays formed on artificial lipid bilayer indicates that FKBP is not involved in the physical linkage between neighboring RyR1s [16]. Obviously, the role of FKBP plays in the intermolecular interaction between RyR1s needs to be investigated.

It is well known that photon correlation spectroscopy (PCS) is highly sensitive to the change in the aggregation state of proteins in solution [19]. In our previous works, we have successfully applied this technique to quantitatively study the interaction between isolated RyR1s within an aqueous system [17,18]. It is found that RyR1–RyR1 interaction is modulated by the concentration of monovalent cations, Na^+ and K^+ [17]. More recently, we have observed that the intermolecular interaction of RyR1s is also affected by their functional states [18]. It is proposed that these modulations, especially the latter one, are of importance for the regulation of the function of RyR1 arrays.

In the present work, PCS was used to study the effect of FKBP12 on the interaction between RyR1s. The oligomerization of isolated RyR1s in the presence/absence of FKBP12 was examined and compared. The FKBP12 modulation of RyR1 oligomerization was investigated both in the medium containing different [KCl] and under different channel functional states. We could not obtain any evidence for the effect of FKBP12 on the interaction between isolated RyR1s.

Materials and methods

Isolation and purification of RyR1. The isolation and purification of RyR1 from rabbit skeletal muscle were as described previously [17,18]. In brief, the heavy sarcoplasmic reticulum (HSR) vesicles were prepared by sucrose step gradient centrifugation (20%/35%/40%, w/w). Then, the HSR was solubilized with Chaps. The solubilized proteins were fractionated by centrifugation on a 6–20% linear sucrose gradient. The protein composition of 1 ml fractions was monitored by SDS–polyacrylamide gel electrophoresis and identified by Western blot analysis. The fraction containing highly purified RyR1 was used for this study. The buffer for storing the isolated RyR1s contained 1 M KCl, 20 mM K–Pipes, 100 μM EGTA, 1 mM DTT, 1 mM diisopropylfluorophosphate, pH 7.1, with 10 mM Chaps, 3 mg/ml PC, and ~17% sucrose.

To prepare RyR1s depleted of FKBP12, HSR vesicles (4 mg protein/ml) were preincubated with 5 μg/ml rapamycin (Sigma) at 37 °C for 30 min. Then, FKBP12 depleted HSR vesicles were collected by centrifugation and used for the preparation of FKBP12-depleted RyR1 as described by Barg et al. [20].

Treatment of purified RyR1 with FKBP12 or rapamycin. Purified RyR1s depleted of FKBP12 (40 μg/ml) were incubated at 25 °C for 45 min in the presence of 10 μg/ml FKBP12 (human recombinant FKBP12, Sigma). Normally purified RyR1s (40–100 μg/ml) were incubated at 25 °C for 45 min in the presence of 10 μg/ml FKBP12 or 1 μg/ml rapamycin.

Rapamycin (1 mM) was stored at −70 °C in ethanol:Tween (9:1, V/V) and diluted before use in 130 mM KCl–20 mM NaCl, 20 mM Na–Pipes, pH 7.1. FKBP12 was diluted with the same buffer and stored at 4 °C.

Photon correlation spectroscopy. PCS experiments were performed on a Zetasizer $3000HS_A$ (Malvern Instruments, UK) with a He–Ne laser, operating at 633 nm wavelength. The scattering angle for size analysis was fixed at 90°. All measurements were carried out at 20 °C. Solvent and particle refractive indexes were set to 1.330 and 1.520, respectively. Solvent viscosity was set to 1.00 for analysis at 20 °C. CONTIN was chosen as analysis method due to its suitability for describing smooth distributions.

To prepare samples for PCS measurement, the samples were diluted to a final solution containing different concentration of K^+ or/and Na^+. The diluted RyR1 samples also contained 0.3 mg/ml PC, 1 mM Chaps, 0.1 mM DTT, 100 μM EGTA, 20 mM Pipes, and 4–10 μg/ml RyR1, and the pH was 7.1. After dilution, all of the RyR1 samples were immediately mixed (300 rpm) on Thermomixer (Eppendorf, USA) at 20 °C for 30 min before PCS measurement. A control sample, which contained 1 mM Chaps and 0.3 mg/ml PC in the absence of RyR1, was also examined [17]. It was found that PC and Chaps formed ~26 nm homogeneous particles independent of the presence of FKBP12 and rapamycin used in this work.

Results

Identification of purified RyR1 with SDS–PAGE

First, the purified RyR1 was identified with 3–20% SDS–PAGE (Fig. 1A). Two bands, indicated by arrows, were identified as of RyR1 origin by Western blotting with anti-RyR antibody 34C. The first band is consistent with the intact RyR1 monomer, while the second band represents a cleaved RyR1 fragment [21]. Obviously, only a small amount of RyR1 underwent proteolysis during purification. Besides these two bands, only one contaminating band between 97 and 116 kDa, generated by Ca^{2+}-ATPase [21], was observed. The purity of intact RyR1 was about 80–90%.

FKBP12 associates tightly with RyR1, with sufficient affinity to co-purify with receptor [22,23]. The immunosuppressive drugs rapamycin or FK506 induce the dissociation of FKBP from RyR [24,25]. As described in Materials and methods, in order to purify RyR1 depleted of FKBP12, the HSR was preincubated with rapamycin at 37 °C for 30 min. To see if this preincubation could completely dissociate FKBP12 from RyR1, such purified RyR1 was examined by 15% SDS–PAGE (Fig. 1B). For comparison, the purified RyR1 without the preincubation of rapamycin was also examined (Fig. 1B). An apparent band near 14 kDa was observed for purified RyR1 without the preincubation step, indicating that FKBP12 still bound to RyR1. This band disappeared for purified RyR1 with the preincubation step, indicating that FKBP12 was depleted from purified RyR1. In our paper, the purified RyR1 associated with FKBP12 is designated as RyR1 (FKBP+), and the

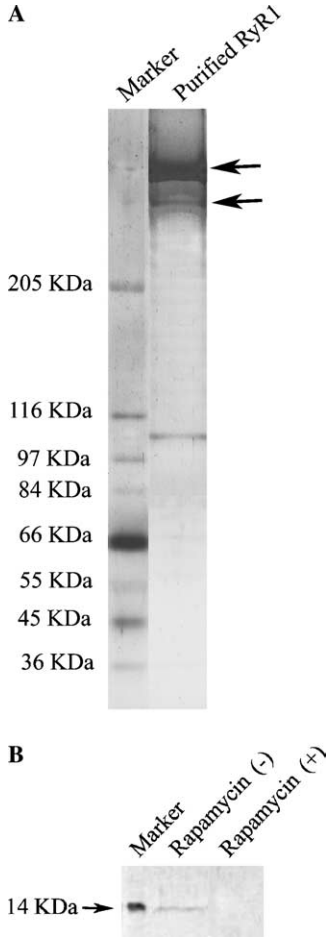

Fig. 1. Identification of purified RyR1 with SDS–PAGE. (A) Silver-stained polyacrylamide gel (3–20%) of purified RyR1 preparations (1 μg) showing the high purity of intact RyR1. (B) Representative silver-stained polyacrylamide gel showing the effect of rapamycin treatment of HSR on the dissociation of FKBP12 from purified RyR1. Both the results of SDS–PAGE (15%) of purified RyR1 preparations (1 μg) with or without the preincubation of HSR with rapamycin during purification are shown.

purified RyR1 depleted of FKBP12 is designated as RyR1 (FKBP−).

FKBP12 does not affect [KCl]-dependent oligomerization of RyR1s

In our previous studies, we found that the interaction/oligomerization of purified RyR1s is dependent on the concentration of KCl in the medium [17]. RyR1s are mono-disperse at high salt concentration and RyR1s oligomerize readily at low salt concentration. To test if the removal of FKBP12 affects the oligomerization behavior of RyR1s at high/low KCl media, the size distributions of RyR1 (FKBP−) in the absence or presence of saturated FKBP12 were detected by PCS at 0.35 M KCl and 0.14 M KCl, respectively. At 0.35 M KCl, similar monomodal distributions with the peak at ~30 nm were observed for RyR1s, in spite of the dissociation or association of FKBP12 (Fig. 2A). As revealed by cryo-EM and three-dimensional reconstruction, RyR1 presents an overall shape of a square prism (28 nm × 28 nm × 12 nm) [26]. Such monomodal size distributions indicate that the depletion of FKBP12 does not affect the single-protein state of RyR1s in 0.35 M KCl medium. At 0.14 M KCl, similar bimodal distributions of RyR1s in the absence/presence of

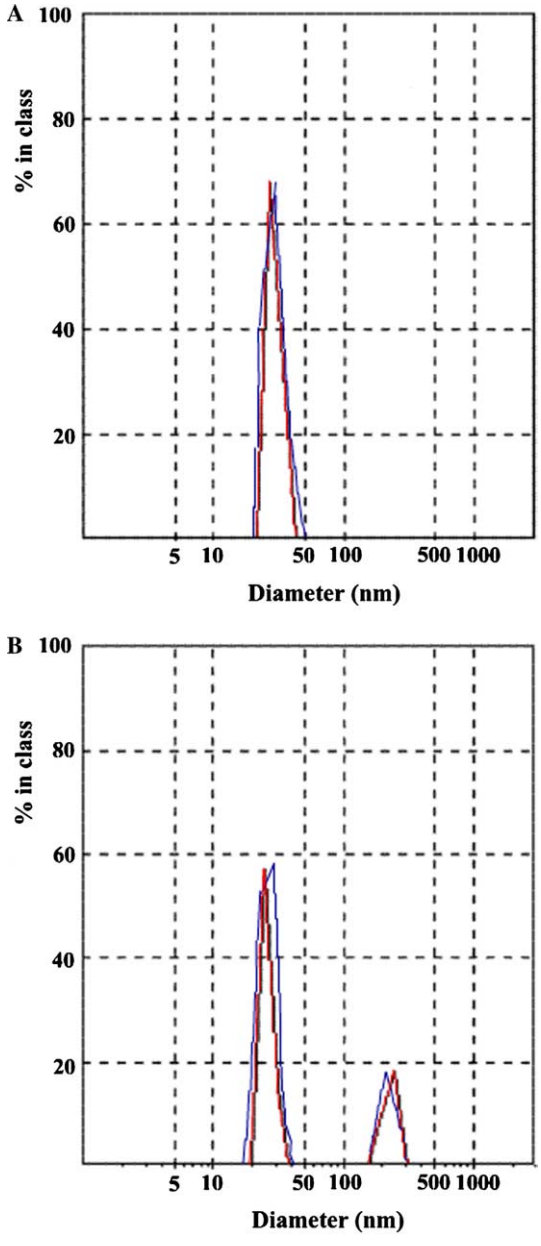

Fig. 2. PCS determination of FKBP12 modulation of the size distribution of purified RyR1s in the medium containing 0.35 M KCl (A) and 0.14 M KCl (B), respectively. Red line: RyR1 (FKBP−); blue line: RyR1 (FKBP−) saturated with FKBP12. All samples contained 4 μg/ml RyR1s. Similar results were obtained in other five experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

FKBP12 were observed (Fig. 2B). Besides the peak at ~30 nm, a second peak with a larger diameter could be observed, indicating some RyR1 oligomers were formed in solution. This result indicates that the depletion of FKBP12 does not affect the oligomerization of RyR1s in 0.14 M KCl medium.

In order to quantitatively evaluate the effect of FKBP12 on the [KCl]-dependence of the oligomerization of RyR1s, we investigated the average hydrodynamic diameter (*Z*-average) of RyR1 (FKBP−) at different [KCl] in the absence or presence of saturated FKBP12. Although *Z*-average obtained from PCS measurements does not represent the actual dimensions of RyR1 oligomers, it provides a way to semi-quantify oligomerization state of RyR1 samples. As shown in Fig. 3A, similar [KCl]-dependence of *Z*-average curves were obtained for 4 μg/ml RyR1s with or without the association of FKBP12. In both cases, the *Z*-average of RyR1s was kept at ~30 nm in the medium containing [KCl] more than 0.18 M, indicating the mono-disperse nature of RyR1s at high [KCl]. With decreasing [KCl], the *Z*-average of RyR1s increased, representing the increased oligomerization of RyR1s. Similarity of the two curves shows clearly that the depletion of FKBP12 does not affect the [KCl]-dependent oligomerization of RyR1s.

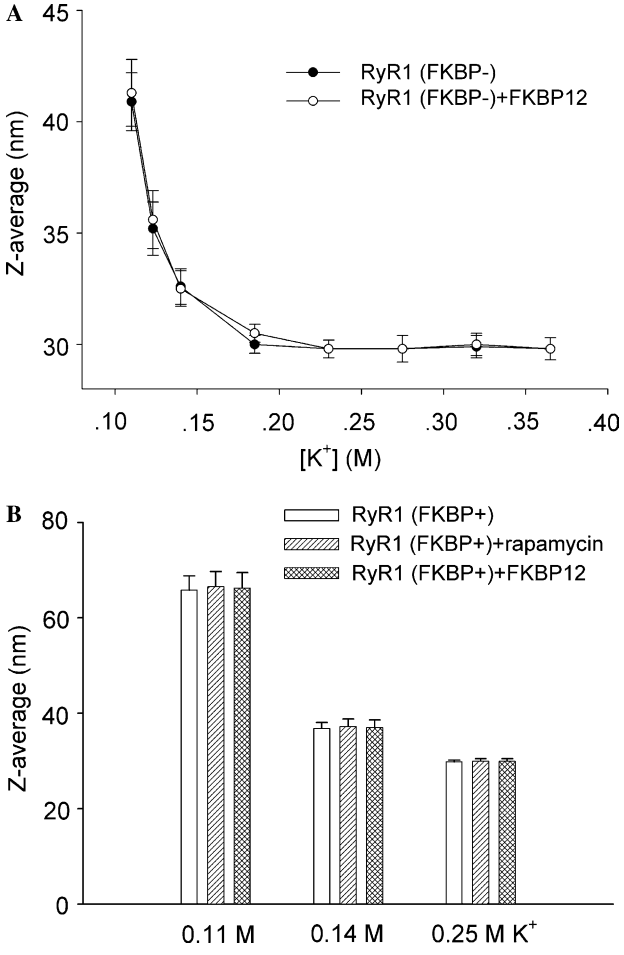

Fig. 3. The effect of FKBP12 on the [KCl]-dependence of *Z*-average of purified RyR1s. (A) Results obtained for 4 μg/ml RyR1 (FKBP−) and RyR1 (FKBP−) saturated with FKBP12. (B) *Z*-averages determined at different [KCl] for three groups of 8.5 μg/ml RyR1 (FKBP+) samples: RyR1 (FKBP+), RyR1 (FKBP+) treated with rapamycin, and RyR1 (FKBP+) treated with FKBP12. The results are presented as means of 3–5 independent experiments. The error bars represent the standard deviation.

We also examined the FKBP12 modulation of RyR1 oligomerization with RyR1 (FKBP+) at different [KCl]. To dissociate FKBP12 from RyR1 (FKBP+), the purified RyR1 samples were treated with rapamycin at 25 °C for 45 min before the determination of PCS. To avoid the possible dissociation of small amount of FKBP12 from RyR1 (FKBP+) during purification, the purified RyR1 samples were incubated with saturated FKBP12. The results showed that the similar [KCl]-dependence of *Z*-average curves as shown in Fig. 3A were obtained for the three groups of 4 μg/ml RyR1 (FKBP+) samples: RyR1 (FKBP+), RyR1 (FKBP+) treated with rapamycin, and RyR1 (FKBP+) treated with saturated FKBP12 (data not shown). In our previous work, we found that the oligomerization level of RyR1 sample is related to RyR1 concentration [17]. RyR1s with high concentration tend to form large oligomers in the same low salt medium. Then, the modulation of RyR1–RyR1 interaction would be more sensitively detected for the samples containing a high concentration of RyR1s. Thus, we increased the concentration of RyR1 (FKBP+) from 4 to 8.5 μg/ml and detected the FKBP12 modulation of *Z*-average at different [KCl]. As shown in Fig. 3B, similar *Z*-averages were also obtained for three groups of 8.5 μg/ml RyR1 (FKBP+) samples at 0.11, 0.14, and 0.25 M KCl, respectively. These results further indicate that FKBP12 does not affect the interaction between isolated RyR1s at different [KCl].

FKBP12 does not affect functional state-dependent oligomerization of RyR1s

In our previous work, we found that the interaction between RyR1s is potently modulated by their functional states [18]. Thus, we also examined the FKBP12 modulation of the oligomerization of RyR1s at closed or open functional state. As shown in Fig. 4, when RyR1s were at their closed state in the presence of 0 Ca^{2+}, similar *Z*-averages (~65 nm) were obtained for three groups of 8.5 μg/ml RyR1 (FKBP+) samples: RyR1 (FKBP+), RyR1 (FKBP+) treated with rapamycin, and RyR1 (FKBP+) treated with saturated FKBP12. When RyR1s were activated to their full open state in the co-presence of 50 μM Ca^{2+} and 5 mM AMP, the *Z*-averages of three groups of RyR1 (FKBP+) samples all decreased to a

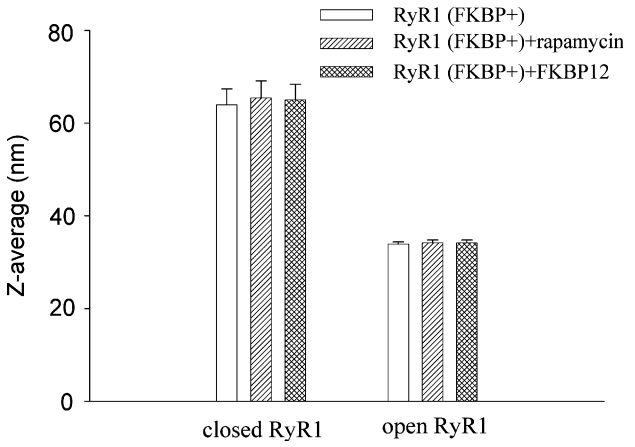

Fig. 4. The effect of FKBP12 on the oligomerization of purified RyR1 at different functional states. The medium containing 0 Ca^{2+} was used to stabilize RyR1s at their closed state, and the co-presence of 50 μM Ca^{2+} and 5 mM AMP was used to induce the full open state of the channels. At each functional state, *Z*-averages were examined for three groups of RyR1 (FKBP+) samples: RyR1 (FKBP+), RyR1 (FKBP+) treated with rapamycin, and RyR1 (FKBP+) treated with FKBP12. All experiments were performed in solution containing 130 mM K^+–20 mM Na^+. All samples contained 8.5 μg/ml RyR1s. Data are presented as means ± SD for 3–5 independent experiments.

similarly low level (~34 nm). Obviously, the interaction between RyR1s with or without the association of FKBP12 is similarly modulated by the channel functional states.

Discussion

Considering the difficulty in carrying out the in vivo study of RyR1–RyR1 interaction with the present techniques, we have established an in vitro aqueous research system in our previous work [17,18]. The application of PCS provides a sensitive and quantitative way to study the effect of multiple factors on the interaction between purified RyR1s. The modulations of RyR1–RyR1 interaction by Na^+/K^+ and by channel functional states have been successfully investigated [17,18]. In the present work, such aqueous research system and research method were further applied to study the FKBP12 modulation of RyR1–RyR1 interaction. Our results show that the depletion of FKBP12 has no effect on the RyR1–RyR1 interaction, both in the medium containing different [KCl] and for RyR1s at different channel functional states.

Recently, by successfully crystallizing RyR1 into a 2-D large array on positively charged lipid membranes, Yin et al. [16] have analyzed the organization and disposition of individual RyR1s within the array by image processing. The domain participating in physical coupling of adjacent RyR1s is identified as subdomain 6. However, from a single-particle image analysis of the RyR1–FKBP12 complex, the binding site for FKBP12 on RyR1 is located between sub-domain 3 and 9, which are on the opposite site of subdomain 6 [27]. Thus, FKBP12 is not a direct intermediate protein for the linkage of neighboring RyR1s [16]. Obviously, our finding is consistent with this structural evidence.

By examining the interaction/aggregation of proteins, the conformational changes of proteins could be efficiently and sensitively detected by PCS [28,29]. In our previous work, we have also found that PCS is very sensitive to the conformational changes of RyR1s, such as those induced by the activation of RyR1s by Ca^{2+} or AMP [18]. For example, only the activation of ~10% RyR1 by 0.8 μM Ca^{2+} in 10 μg/ml RyR1 sample could induce the *Z*-average to decrease from 68.0 ± 2.8 to 64.6 ± 2.5 nm ($P < 0.01$). However, in the present work PCS did not detect any effect of FKBP12 on the intermolecular interaction between RyR1s. Our results suggest that conformational changes of RyR1s accompanying the dissociation of FKBP12 are very little or the induced conformational changes do not efficiently affect the interaction between RyR1s.

Our results are in apparent disagreement with those in the study by Marx et al. [13,14]. In their studies, it was found that FKBP12 mediates the synchronized gating of neighboring RyR1s. Thus, FKBP12 is assumed to be involved in the RyR1–RyR1 intermolecular interaction. It should be noted that the study of Marx et al. and ours have both used the purified RyR1. The major difference between their study and our study is that they investigated the gating of RyR1s which are reconstituted into lipid bilayer whereas we examined the RyR1–RyR1 interaction in an aqueous medium. It is still unknown if the difference in research system, such as the existence of lipid bilayer, leads to the different results.

The native RyR is bound to many auxiliary proteins [1,3]. The demonstration of the effect of these auxiliary proteins on the functional and oligomeric state of RyR channels is critical to elucidate the function and operating mechanism of RyR arrays. In the present work, we take the first step to investigate the FKBP12 modulation of RyR1–RyR1 interaction. Our experimental paradigm opens up the possibility of looking at the effect of other RyR1 auxiliary proteins on the interaction between RyR1s.

Acknowledgments

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References

[1] G. Meissner, Ryanodine receptor/Ca^{2+} release channels and their regulation by endogenous effectors, Annu. Rev. Physiol. 56 (1994) 485–508.

[2] G.D. Lamb, Excitation–contraction coupling in skeletal muscle: comparisons with cardiac muscle, Clin. Exp. Pharmacol. Physiol. 27 (3) (2000) 216–224.

[3] M. Fill, J.A. Copello, Ryanodine receptor calcium release channels, Physiol. Rev. 82 (2002) 893–922.

[4] D.M. Bers, Cardiac excitation–contraction coupling, Nature 415 (2002) 198–205.

[5] C. Franzini-Armstrong, J. Kish, Alternate disposition of tetrads in peripheral couplings of skeletal muscle, J. Mus. Res. Cell Motil. 16 (1995) 319–324.

[6] C. Franzini-Armstrong, The sarcoplasmic reticulum and the control of muscle contraction, FASEB J. 13 (1999) S266–S270.

[7] C. Franzini-Armstrong, F. Protasi, V. Ramesh, Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles, Biophys. J. 77 (3) (1999) 1528–1539.

[8] K.E. Loesser, L. Castellani, C. Franzini-Armstrong, Dispositions of junctional feet in muscles of invertebrates, J. Muscle Res. Cell Motil. 13 (1992) 161–173.

[9] A.R. Marks, P. Tempst, K.S. Hwang, M.B. Taubman, M. Inui, C. Chadwick, S. Fleischer, B. Nadal-Ginard, Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum, Proc. Natl. Acad. Sci. USA 86 (1989) 8683–8687.

[10] A.P. Timerman, T. Jayaraman, G. Wiederrecht, H. Onoue, A.R. Marks, S. Fleischer, The ryanodine receptor from canine heart sarcoplasmic reticulum is associated with a novel FK-506 binding protein, Biochem. Biophys. Res. Commun. 198 (1994) 701–706.

[11] A.B. Brillantes, K. Ondrias, A. Scott, E. Kobrinsky, E. Ondriasova, M.C. Moschella, T. Jayaraman, M. Landers, B.E. Ehrlich, A.R. Marks, Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein, Cell 77 (1994) 513–523.

[12] E. Kaftan, A.R. Marks, B.E. Ehrlich, Effects of rapamycin on ryanodine receptor/Ca^{2+}-release channels from cardiac muscle, Circ. Res. 78 (1996) 990–997.

[13] S.O. Marx, K. Ondrias, A.R. Marks, Coupled gating between individual skeletal muscle Ca^{2+} release channels (ryanodine receptors), Science 281 (1998) 818–821.

[14] S.O. Marx, J. Gaburjakova, M. Gaburjakova, C. Henrikson, K. Ondrias, A.R. Marks, Coupled gating between cardiac calcium release channels (ryanodine receptors), Circ. Res. 88 (2001) 1151–1158.

[15] D.M. Bers, M. Fill, Muscle contraction: coordinated feet and the dance of ryanodine receptors, Science 281 (1998) 790–791.

[16] C.C. Yin, L.M. Blayney, F.A. Lai, Physical coupling between ryanodine receptor-calcium release channels, J. Mol. Biol. 349 (3) (2005) 538–546.

[17] X.F. Hu, K.Y. Chen, R. Xia, Y. Xu, J.L. Sun, J. Hu, P.H. Zhu, Modulation of the interactions of isolated ryanodine receptors of rabbit skeletal muscle by Na^{+} and K^{+}, Biochemistry 42 (18) (2003) 5515–5521.

[18] X.F. Hu, X. Liang, K.Y. Chen, H. Xie, Y. Xu, P.H. Zhu, J. Hu, Modulation of the oligomerization of isolated ryanodine receptors by their functional states, Biophys. J 89 (3) (2005), in press.

[19] R. Pecora, Dynamic Light Scattering: Application of Photon Correlation Spectroscopy, Plenum Press, New York, 1985.

[20] S. Barg, J.A. Copello, S. Fleischer, Different interactions of cardiac and skeletal muscle ryanodine receptors with FK-506 binding protein isoforms, Am. J. Physiol. 272 (1997) C1726–C1733.

[21] F.A. Lai, G. Meissner, Purification and reconstitution of the ryanodine-sensitive Ca^{2+} release channel complex from muscle sarcoplasmic reticulum, Methods Mol. Biol. 13 (1992) 287–305.

[22] T. Jayaraman, A.M. Brillantes, A.P. Timerman, S. Fleischer, H. Erdjument-Bromage, P. Tempst, A.R. Marks, FK506 binding protein associated with the calcium release channel (ryanodine receptor), J. Biol. Chem. 267 (1992) 9474–9477.

[23] Y. Qi, E.M. Ogunbunmi, E.A. Freund, A.P. Timerman, S. Fleischer, FK-binding protein is associated with the ryanodine receptor of skeletal muscle in vertebrate animals, J. Biol. Chem. 273 (1998) 34813–34819.

[24] S. Schreiber, Chemistry and biology of the immunophilins and their immunosuppressive ligands, Science 251 (1991) 283–287.

[25] A.R. Marks, Cellular functions of immunophilins, Physiol. Rev. 76 (1996) 631–649.

[26] T. Wagenknecht, M. Samso, Three-dimensional reconstruction of ryanodine receptors, Front. Biosci. 7 (2002) d1464–d1474.

[27] T. Wagenknecht, M. Radermacher, R. Grassucci, J. Berkowitz, H.B. Xin, S. Fleischer, Locations of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor, J. Biol. Chem. 272 (1997) 32463–32471.

[28] D.N. Petsev, B.R. Thomas, S.-T. Yau, P.G. Vekilov, Interactions and aggregation of apoferritin molecules in solution: effects of added electrolytes, Biophys. J. 78 (2000) 2060–2069.

[29] A.L. Papish, L.W. Tari, H.J. Vogel, Dynamic light scattering study of calmodulin-target peptide complexes, Biophys. J. 83 (2002) 1455–1464.