FK-binding Protein Is Associated with the Ryanodine Receptor of Skeletal Muscle in Vertebrate Animals*

(Received for publication, September 23, 1998)

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The ryanodine receptor/calcium release channel (RyR1) of sarcoplasmic reticulum from rabbit skeletal muscle terminal cisternae (TC) contains four tightly associated FK506-binding proteins (FKBP12). Dissociation and reconstitution studies have shown that RyR1 can be modulated by FKBP12, which helps to maintain the channel in the quiescent state. In this study, we found that the association of FKBP with RyR1 of skeletal muscle is common to each of the five classes of vertebrates. TC from skeletal muscle representing animals from different vertebrates, i.e. mammals (rabbit), birds (chicken), reptiles (turtle), fish (salmon and rainbow trout), and amphibians (frog), were isolated. For each, we find the following: 1) FKBP12 is localized to the TC (there are four FKBP binding sites/ryanodine receptor); 2) soluble FKBP exchanges with the bound form on RyR1 of TC; 3) release of FKBP from terminal cisternae by drug (FK590) treatment leads to a significant reduction in the net calcium loading rate, consistent with channel activation (the calcium loading rate is restored to the control value by reconstitution with FKBP12); and 4) RyR1 of skeletal muscle TC can bind to and exchange with either FKBP12 or FKBP12.6 (FKBP12.6 is the novel FKBP isoform found selectively associated with RyR2 of dog cardiac sarcoplasmic reticulum). We conclude that FKBP is an integral part of the RyR1 of skeletal muscle in each of the classes of vertebrate animals. The studies are consistent with a role for FKBP in skeletal muscle excitation-contraction coupling.

FK506 is a powerful immunosuppressive drug that prevents T-cell activation and is thereby used to prevent allograft rejection following transplant surgery. FK506-binding protein (FKBP12), a cytosolic receptor for FK506, has a molecular mass of 11.8 kDa and is widely expressed in eukaryotic cells and tissues, predominantly in the cytosol. The sequence is highly conserved throughout eukaryotic phylogeny (1, 2).

In rabbit skeletal muscle, FK-binding protein is bound to the ryanodine receptor of terminal cisternae of SR (3–10), in a stoichiometry of four FKBP/ryanodine receptor (4, 8); i.e. for mammalian skeletal muscle, the ryanodine receptor is a hetero-oligomer with a structural formula of (RyR1 protomer)4 (FKBP12)4. Although FKBP is tightly bound to the RyR of terminal cisternae of SR, soluble FKBP readily exchanges with FKBP12 on the RyR in TC (6). The ryanodine receptor of heart SR is also associated with FKBP (7) in a stoichiometry of 4 (8), albeit with a novel isoform, i.e. FKBP12.6. The latter differs from FKBP12 by 18 of 108 amino acids (8). Recently, we found that the ryanodine receptor from skeletal muscle binds to and exchanges with both FKBP12 and FKBP12.6, whereas the RyR2 of heart binds to and exchanges only with FKBP12.6 (9). In vitro reconstitution studies indicate that FKBP12 modulates the channel function of the ryanodine receptor of skeletal muscle (RyR1) (4, 5, 10). In this regard, FKBP stabilizes the closed conformation of the skeletal muscle ryanodine receptor. Image enhancement analysis of cryo-electron micrographs of RyR1 shows that four FKBP bind to RyR1 in 4-fold symmetry and pinpoint the binding sites (11). FKBP binds near the transverse tubule face of RyR1. This observation is consistent with FKBP being involved in the coupling of the dihydropyridine receptor to the ryanodine receptor in excitation-contraction coupling in skeletal muscle. If FKBP is essential for excitation-contraction coupling in skeletal muscle, it would be expected to be generally associated with and modulate the function of ryanodine receptors from diverse animals. In this study, we find that FKBP12 is associated with and modulates the skeletal muscle RyR1 calcium release channel in each of the five classes of vertebrates.

EXPERIMENTAL PROCEDURES

Materials—Both L-683,590 and [3H]FK816 were from Merck. L-683,590 is a structural and functional analogue of FK506, referred to as FK590, [3H]FK816 is a dihydropropyl derivative of FK506. 35S-protein labeling mix (l-[35S]methionine) and [H]hryanodine were purchased from NEN Life Science Products. Goat anti-rabbit alkaline phosphatase conjugate and nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate color development reagents for immunoblotting were obtained from Promega. Immobilon-P membrane was purchased from Millipore Corp. CHAPS, antipyrylazo III, and ruthenium red were obtained from Sigma. Sodium dodecyl sulfate, acrylamide, methylene bisacrylamide, and SDS-PAGE molecular weight standards were obtained from Bio-Rad. TSK GEL G3000SW column was purchased from Tosohaas.

General Methods—The protein concentration of the TC membrane fractions was determined by the Folin reaction (13) using bovine serum albumin as a standard. SDS-PAGE was performed with a mini-slab gel apparatus (Hoeffer Scientific) using the buffer system described by Laemmli (14). The protein content of recombinant 35S-labeled FKBP was estimated by quantitative densitometry of Coomassie Blue-stained SDS-PAGE gels using an image processing system (Technology Resource Inc., Nashville, TN). Bovine serum albumin was used as the protein standard.

Western Blot Analysis of TC Fractions—TC (20 μg of protein) was subjected to SDS-gel electrophoresis and transferred to 0.45 μm Immobilon-P transfer membranes (Millipore) using a semidyed transfer appa-
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Characteristics of TC from animals of different vertebrate classes

| Vertebrate classes | Terminal cisternae fractions | [3H]Ryanodine binding | Ca2+ loading rate | Ca2+ loading rate ratio (+RR/RR) |
|-------------------|-----------------------------|-----------------------|-----------------|-------------------------------|
|   | pmol/mg protein | µmol Ca2+ /min/mg protein | | |
| Mammal (rabbit)   | I4mid | 18.2 | 0.507 | 1.245 | 2.64 |
|                   | R4mid | 21.0 | 0.093 | 0.880 | 9.46 |
| Bird (chicken)    | I3   | 3.45 | 0.367 | 0.655 | 1.78 |
|                   | I4   | 11.3 | 0.071 | 0.110 | 1.55 |
|                   | R3   | 6.9 | 0.604 | 1.316 | 2.18 |
|                   | R4   | 21.0 | 0.158 | 0.502 | 3.17 |
| Fish (rainbow trout) | I3 | 2.7 | 0.206 | 0.955 | 4.63 |
|                   | I4   | 5.8 | 0.143 | 0.223 | 1.53 |
|                   | R3   | 3.9 | 0.379 | 0.731 | 1.94 |
|                   | R4   | 6.4 | 0.110 | 0.375 | 3.41 |
| Amphibian (frog)  | I3   | 2.1 | 0.088 | 0.204 | 2.32 |
|                   | I4   | 6.6 | 0.052 | 0.174 | 3.35 |
|                   | R3   | 3.17 | 0.013 | 0.017 | 1.31 |
|                   | R4   | 6.3 | 0.004 | 0.023 | 5.75 |
| Reptile (turtle)  | I3   | 12.6 | 0.364 | 0.905 | 2.49 |
|                   | I4   | 16.3 | 0.044 | 0.225 | 5.10 |
|                   | R3   | 8.6 | 0.103 | 0.202 | 1.96 |
|                   | R4   | 15.8 | 0.051 | 0.193 | 3.79 |

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RESULTS

Isolation and Characterization of Skeletal Muscle SR Fractions from Different Vertebrate Species—Terminal cisternae enriched fractions were prepared from skeletal muscle from the different vertebrate animals by the procedure developed for rabbit skeletal muscle (12). Muscle microsomes were prepared and subfractionated on a sucrose density gradient. As found previously for rabbit, the highest density fractions (3, 4, R3, and R4) were most enriched in terminal cisternae of SR as indicated by ryanodine binding (Table I). This was confirmed by SDS-PAGE. In other words, the terminal cisternae fractions of rabbit, chicken, turtle, fish, and frog, representing the five classes of vertebrates, were enriched in bands referable to ryanodine receptor, calcium pump protein, and calsequestrin. The calcium pump protein and calsequestrin are quantitatively the major proteins of terminal cisternae as observed by Coomassie Blue staining (Fig. 1A) and staining with Stains-all for calsequestrin (Fig. 1B). The calsequestrin bands appear dark blue except chicken (red-purple). The positions of molecular size markers (Bio-Rad), the top (T), and dye front (D) are indicated to the left in A and to the right in B. The experiment was repeated twice with essentially similar results. In these studies only rainbow trout is shown. Results obtained with salmon were similar to those obtained with rainbow trout.

Ryanodine binding to the terminal cisternae enriched fractions is the highest in R4 and next highest in I4 (see Table I). The lowest density fractions (1, 2, R1, and R2) had little ryanodine binding, as observed for the rabbit skeletal muscle terminal cisternae preparations (data not shown) (12). Another diagnostic of terminal cisternae is the enhanced net Ca$^{2+}$ loading rate in the presence of ruthenium red (20). The net calcium loading rate of terminal cisternae is determined predominantly by the Ca$^{2+}$ loading rate referable to the Ca$^{2+}$ pump protein, minus the Ca$^{2+}$ leak rate via the calcium release channel. A decrease in net Ca$^{2+}$ loading rate reflects channel activation. In the presence of ruthenium red, which closes the RyR channel, this enhanced Ca$^{2+}$ loading rate in terminal cisternae from rabbit skeletal muscle is approximately 5–10-fold. The enhanced Ca$^{2+}$ loading rate in the terminal cisternae from other species varied from approximately 3 to 6. The longitudinal tubule fractions (R2 and I2), which were essentially devoid of ryanodine receptor, had a higher calcium loading rate compared with R4 and I4, which was not enhanced with ruthenium red (data was shown, see Ref. 20 for rabbit).

FKBP Is Associated with Skeletal Muscle Terminal Cisternae in Each of the Classes of Vertebrates—Terminal cisternae fractions from rabbit, chicken, turtle, fish, and frog skeletal muscle
were analyzed by Western blotting using the FKBP antibody (3) that recognizes the N-terminal sequences of both FKBP12 and FKBP12.6 (Fig. 3). The immunoreactive bands in the terminal cisternae fractions from the different species of vertebrates were observed with mobility in the range of ~12 and 12.6 kDa. The mobility of the FKBP bands from the different animals varied somewhat. Rabbit has the same mobility as human recombinant FKBP12, and chicken is only slightly slower. The fish had the slowest mobility, just slightly faster than human recombinant FKBP12.6. The turtle and frog were between chicken and fish.

Quantitation of FKBP Binding Sites in the RyR of Terminal Cisternae—The stoichiometry of FKBP/ryanodine receptor in the terminal cisternae fractions from mammal, bird, reptile, and fish was measured by drug binding. The stoichiometry was 4 for rabbit and frog, but was <4 for fish (1.7), reptile (1.5), and bird (2.5) (Table II). This lower stoichiometry reflects unfilled FKBP binding sites on the ryanodine receptor (Table II), which were measured using FKBP exchange methodology (6). We find approximately four FKBP binding sites on the RyR1 from skeletal muscle in each of the classes of vertebrates. The binding sites could be filled by either FKBP12 or FKBP12.6 (Table III).

The FKBP exchange methodology (6) previously developed for rabbit terminal cisternae had to be modified for some of the vertebrate classes, since the skeletal muscle terminal cisternae of turtle, fish, and frog were found to be sensitive to temperature inactivation. This was manifest by the inability to obtain good FKBP exchange as well as the loss of ryanodine binding. The terminal cisternae from fish skeletal muscle lost nearly all ryanodine binding activity at 25 °C incubation for 30 min. Terminal cisternae from turtle and frog lost the ryanodine binding activity when incubated at 37 °C for 30 min. For this reason, the exchange was performed at different temperatures.

The conditions for exchange are summarized in Table III. The stoichiometry of FKBP12 or FKBP12.6 binding sites/ryanodine receptor for the different animals is approximately 4 as determined from the $B_{\text{max}}$ for FKBP, obtained from exchange isotherms (Fig. 4), and the measurement of high affinity ryanodine binding.

The combined studies in Tables II and III indicate that there are four FKBP binding sites/ryanodine receptor in RyR1 from skeletal muscle in each of the classes of vertebrates, although the terminal cisternae of fish, reptile, and bird were isolated with less than a full quota of FKBP (stoichiometry of less than 4).

**RyR1 Contains Four FKBP Binding Sites, Which Can Be Filled by FKBP12 or FKBP12.6**—We previously reported that the ryanodine receptor of rabbit skeletal muscle TC can bind approximately four equivalents of FKBP12 or FKBP12.6. This could mean that there are a total of eight FKBP binding sites, four each for FKBP12 and FKBP12.6; alternatively, there may be four sites that can bind either FKBP12 and/or FKBP12.6. To answer this question, exchange was carried out with a combination of both FKBP12 and 12.6, each at nearly saturating concentration (6, 9). We find that there are only four FKBP binding sites, which can be occupied by either isomor or the combination of FKBP isomers (Table IV).

**FKBP Modulates the Net Calcium Loading Rate of TC in Different Vertebrate Animals**—We previously found that removal of FKBP from rabbit terminal cisternae activated the RyR by making the RyR more sensitive to Ca$^{2+}$ (5, 21) as studied by single channel measurements in planar lipid bilayers. This was supported by the macroscopic assay of net Ca$^{2+}$ loading in the terminal cisternae (4, 20). The Ca$^{2+}$ loading rates of terminal cisternae from different species were differentially sensitive to inactivation. The time and temperature of
The FKBP binding sites of the RyRs from TC of different animal species were obtained from the apparent $B_{\text{max}}$ values of $[^{35}\text{S}]$FKBP12, and $[^{35}\text{S}]$FKBP12.6 were obtained from exchange isotherms (see Fig 3) (6). $[^{3}H]$Ryanodine binding was performed at 60 nM $[^{3}H]$ryanodine. The binding data for TC fractions are expressed as the mean ± S.E. for two preparations. The exchange studies on rabbit, fish, and frog were carried out on two different preparations with quantitatively similar results. The studies on chicken and turtle were carried out twice on the same preparation with quantitatively similar results. Fraction 3 of chicken skeletal muscle TC, which has lower ryanodine binding, more comparable with fraction 4 of fish and frog, was used for the exchange isotherms. The exchange isotherms were performed for 30 min at different temperatures (37 °C for rabbit and chicken, room temperature for turtle and frog, and 0 °C for fish).

**TABLE III**

| Vertebrate classes | Species | $[^{35}\text{S}]$FKBP12.0 | $[^{35}\text{S}]$FKBP12.6 |
|--------------------|---------|--------------------------|--------------------------|
|                    | $K_d$ μM | $B_{\text{max}}$ pmol/mg protein | $K_d$ μM | $B_{\text{max}}$ pmol/mg protein |
| Mammal             | Rabbit (R4mid) | 0.24 ± 0.03 | 79.4 ± 4.2 | 0.12 ± 0.04 | 71.1 ± 7.0 |
| Bird               | Chicken (R3) | 0.31 ± 0.05 | 28.2 ± 1.7 | 0.38 ± 0.10 | 28.5 ± 3.4 |
| Amphibian          | Frog (I4) | 0.49 ± 0.09 | 21.3 ± 2.9 | 0.12 ± 0.03 | 34.0 ± 2.6 |
| Fish               | Rainbow trout (R4) | 0.54 ± 0.18 | 33.7 ± 5.5 | 0.18 ± 0.05 | 25.3 ± 2.3 |

**TABLE IV**

The FKBP binding sites/calcium release channel in terminal cisternae of SR from diverse species (from exchange studies)

The FKBP binding sites of the RyRs from TC of different animal species were obtained from the apparent $B_{\text{max}}$ values of $[^{35}\text{S}]$FKBP12, and $[^{35}\text{S}]$FKBP12.6 were obtained from exchange isotherms (see Fig 3) (6). $[^{3}H]$Ryanodine binding was performed at 60 nM $[^{3}H]$ryanodine. The binding data for TC fractions are expressed as the mean ± S.E. for two preparations. The exchange studies on rabbit, fish, and frog were carried out on two different preparations with quantitatively similar results. The studies on chicken and turtle were carried out twice on the same preparation with quantitatively similar results. Fraction 3 of chicken skeletal muscle TC, which has lower ryanodine binding, more comparable with fraction 4 of fish and frog, was used for the exchange isotherms. The exchange isotherms were performed for 30 min at different temperatures (37 °C for rabbit and chicken, room temperature for turtle and frog, and 0 °C for fish).

**FIG. 4.** $[^{35}\text{S}]$FKBP exchange isotherms with the TC from different classes of vertebrates. Exchange isotherms were performed by incubation of TC at 2.0 mg/ml in IHM buffer containing 0.05–1.2 μM $[^{35}\text{S}]$FKBP12.0 for 30 min at 37 °C (for rabbit (V) and chicken (●), 0 °C (for fish (○)), and room temperature (for turtle (▲) and frog (▲)). Nonspecific binding was obtained by adding a 50-fold excess of unlabeled FKBP at each concentration of $[^{35}\text{S}]$FKBP. Specific binding is calculated from (total – nonspecific) binding. $K_d$ and $B_{\text{max}}$ obtained from this data are summarized in Table III. Exchange studies of chicken SkM TC were carried out with fraction 3 of chicken TC, which has lower ryanodine binding, more comparable with fraction 4 of fish and frog.

**DISCUSSION**

We find that FK-binding protein is associated with the ryanodine receptor of skeletal muscle terminal cisternae in animals representing each of the five classes of vertebrates. Until now, studies of FKBP association with RyR1 were limited to mammalian skeletal muscle. In this study, we find that each of the five classes of vertebrates shares a similarity of key char-

**TABLE IV**

The generality of FKBP associated with RyR in vertebrates

| Vertebrate classes | Species | $[^{35}\text{S}]$FKBP12.0 | $[^{35}\text{S}]$FKBP12.6 |
|--------------------|---------|--------------------------|--------------------------|
| Mammal             | Rabbit (R4mid) | 0.24 ± 0.03 | 79.4 ± 4.2 | 0.12 ± 0.04 | 71.1 ± 7.0 |
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| Amphibian          | Frog (I4) | 0.49 ± 0.09 | 21.3 ± 2.9 | 0.12 ± 0.03 | 34.0 ± 2.6 |
| Fish               | Rainbow trout (R4) | 0.54 ± 0.18 | 33.7 ± 5.5 | 0.18 ± 0.05 | 25.3 ± 2.3 |

**FIG. 4.** $[^{35}\text{S}]$FKBP exchange isotherms with the TC from different classes of vertebrates. Exchange isotherms were performed by incubation of TC at 2.0 mg/ml in IHM buffer containing 0.05–1.2 μM $[^{35}\text{S}]$FKBP12.0 for 30 min at 37 °C (for rabbit (V) and chicken (●), 0 °C (for fish (○)), and room temperature (for turtle (▲) and frog (▲)). Nonspecific binding was obtained by adding a 50-fold excess of unlabeled FKBP at each concentration of $[^{35}\text{S}]$FKBP. Specific binding is calculated from (total − nonspecific) binding. $K_d$ and $B_{\text{max}}$ obtained from this data are summarized in Table III. Exchange studies of chicken SkM TC were carried out with fraction 3 of chicken TC, which has lower ryanodine binding, more comparable with fraction 4 of fish and frog.

**TABLE IV**

The ryanodine receptor of mammalian skeletal muscle TC fraction has four binding sites for FKBP, which can be filled with either FKBP isoform

Rabbit skeletal muscle TC (100 μg) in IHM buffer were exchanged with nearly saturating amounts (3 μM) each of both $[^{35}\text{S}]$FKBP12 and $[^{35}\text{S}]$FKBP12.6 for 30 min at 37 °C (6,9), and the amount of $[^{35}\text{S}]$FKBP in the pellet was determined following sedimentation and washing of the TC in a Beckman TL100.1 rotor as described under “Experimental Procedures.” Both specific activity of $[^{35}\text{S}]$FKBP12.0 and $[^{35}\text{S}]$FKBP12.6 was adjusted to 350 cpm/pmol. Nonspecific binding was estimated in the presence of a 50-fold excess of each FKBP unlabeled isoform. Specific binding was obtained as the difference between total binding and nonspecific binding. The data for this experiment represent the mean ± S.E. of two different preparations each. The TC/triad fractions were prepared as described by Ikemoto et al. (23) and Saito et al. (12).

| Rabbit SkM TC/triad preparation | $[^{3}H]$Ryanodine binding | Exchange with both $[^{35}\text{S}]$FKBP12 and $[^{35}\text{S}]$FKBP12.6 | Stoichiometry of FKBP/RyR |
|---------------------------------|---------------------------|-------------------------------------------------------------|--------------------------|
| Ikemoto et al. (23)             | 10.4 ± 0.78               | 41.6 ± 2.2                                                  | 4.04 ± 0.25              |
| Saito et al. (12)               | 19.9 ± 0.85               | 70.4 ± 5.6                                                  | 3.62 ± 0.74              |

**DRUG TREATMENT**

Drug treatment had to be optimized as a compromise for efficient extraction of FKBP and the ability to restore Ca$^{2+}$ loading activity with FKBP reconstitution. Table V summarizes drug ($[^{3}H]$FK816) and $[^{3}H]$ryanodine binding values of terminal cisternae from the different species treated using the optimized conditions for FKBP release. Compared with the control, $[^{3}H]$FK816 binding for each incubated control was essentially unchanged (not shown). Likewise, there was no difference between control and drug-treated terminal cisternae for $[^{3}H]$ryanodine binding. Most of the FKBP was released by the drug treatment. The percentage of FKBP remaining in the pellet after extraction of FKBP with FK590 was 30% (chicken), 29% (turtle), 18% (frog), and 6% (fish), respectively. Release of FKBP from the ryanodine receptor of terminal cisternae by drug treatment reduced the net Ca$^{2+}$ loading rate, consistent with the increased Ca$^{2+}$ leak from the RyR referable to the drug release. Thus, FKBP12 and FKBP12.6 modulate the calcium release channel in the skeletal muscle terminal cisternae from the different species.

**DISCUSSION**

We find that FK-binding protein is associated with the ryanodine receptor of skeletal muscle terminal cisternae in animals representing each of the five classes of vertebrates. Until now, studies of FKBP association with RyR1 were limited to mammalian skeletal muscle. In this study, we find that each of the five classes of vertebrates shares a similarity of key char-
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Table V

Drug treatment conditions to extract FKBP

| Vertebrate classes | Species | Treatment | \[^{[3}H\]Ryranodine binding | \[^{[3}H\]FK816 binding | FKBP remaining in the TC |
|--------------------|---------|-----------|-----------------------------|-----------------------------|-------------------------|
| Mammal             | Rabbit (R4mid) | Control | 14.8 | 63.0 | % |
|                    |          | Drug-treated | 15.4 | 5.7 | 9 |
| Bird               | Chicken (R4) | Control | 15.7 | 21.1 | 30 |
|                    |          | Drug-treated | 16.1 | 6.5 | 30 |
| Reptile            | Turtle (R4) | Control | 13.8 | 21.2 | 29 |
|                    |          | Drug-treated | 11.2 | 6.2 | 29 |
| Amphibian          | Frog (I4) | Control | 5.0 | 31.6 | 18 |
|                    |          | Drug-treated | 5.5 | 5.8 | 18 |
| Fish               | Rainbow trout (RA) | Control | 3.8 | 11.4 | 6 |
|                    |          | Drug-treated | 3.3 | 0.7 | 6 |

FIG. 5. FKBP modulates channel function of skeletal muscle RyR from diverse species of vertebrate animals. The terminal cisternae fraction (75 μg) was treated with FK590 (5 μM for fish) to remove FKBP under different conditions (30 min at 37 °C for mammal, 30 min at room temperature for bird and reptile, and 15 min at room temperature for amphibian and fish). FKBP release from terminal cisternae was measured with an \[^{[3}H\]FK816 binding assay. This study was repeated twice or more with quantitatively similar results (±20%).

\[Ca^{2+}\] loading of terminal cisternae, a macroscopic measure of channel activity (20). Thus, FKBP modulates the RyR. The generality of FKBP association, exchange with RyR-bound FKBP, and modulation of RyR channel activity was found to be common for each of the vertebrate classes of animals.

Terminal cisternae fractions were prepared from animals representing each of the five classes of vertebrates by the procedure previously developed for rabbit skeletal muscle (12) (Table I). The terminal cisternae share characteristic features: 1) higher isopycnic density by equilibrium sedimentation; 2) the enrichment in SR proteins (i.e., calcium pump protein, calsequestrin, and ryanodine receptor); 3) net Ca\(^{2+}\) loading enhancement by the addition of ruthenium red, which closes the Ca\(^{2+}\) leak referable to the ryanodine receptor (Table I); 4) association of FKBP with the ryanodine receptor and FKBP modulation of channel function (Fig. 5).

To assess the role of FKBP in the modulation of the ryanodine receptor in terminal cisternae from the different classes of vertebrates, we had to optimize conditions for drug treatment in order to be able to restore function by reconstitution studies. The conditions developed are summarized in Table V and Fig. 5. Likewise, the conditions for exchange of soluble FKBP with RyR-bound FKBP in TC of SR (Table III) had to be optimized to retain ligand binding. The exchange condition of 37 °C previously worked out for mammalian TC was satisfactory also for bird. However, the treatment had to be modified for amphibian and reptile (to room temperature) and for fish (to 0 °C).

The skeletal muscle terminal cisternae from mammals contain mainly RyR1 with a small amount of RyR3 (<1%). However, for the other vertebrate classes, approximately equal amounts of RyR1 (α-isofrom) and RyR3 (β-isofrom) are present as major constituents (24–27). Even so, we measured four FKBP binding sites/ryanodine receptor in animals from each of the classes of vertebrates. The inference from these results is that RyR1 and RyR3 are similar with regard to the binding of FKBP; i.e., there are four FKBP binding sites/RyR3, and both RyR1 and RyR3 can bind either FKBP12 or FKBP12.6, unlike the cardiac RyR2, which selectively binds and exchanges only with FKBP12.6. Recent studies in our laboratory with the purified mammalian RyR3 receptor (28) confirm this inference.2

The presence of FKBP in the skeletal muscle fraction was confirmed by Western blot analysis (Fig. 3). The mobility of the FKBP from the different animals varied somewhat depending

\(^2\) Y. Qi, L. Jeyakumar, E. M. Ogunbunni, and S. Fleischer, manuscript in preparation.
on the species. Mobility alone is not sufficient to distinguish FKBP12 from FKBP12.6 or perhaps yet another isoform, since the difference in mobility of FKBP12 and FKBP12.6 is small and can be accounted for in part by a change in one or two amino acids. 3 RyR1 and RyR3 can bind both FKBP12 and FKBP12.6. FKBP12 is the predominant isoform in the cytosol of rabbit skeletal muscle and dog heart muscle. Since FKBP12 and FKBP12.6 bind comparably to RyR1 (9) and RyR3, we can infer that the skeletal muscle RyRs (RyR1 and RyR3) bind the FKBP isoform that predominates in the cytosol, i.e. FKBP12. The heart RyR2 isolates with FKBP12.6 and not FKBP12 due to its specificity for binding FKBP12.6 (9).

We had previously reported that RyR1 can bind four equivalents of FKBP12 or FKBP12.6. This could mean that RyR1 has eight binding sites (four for FKBP12 and four for FKBP12.6) or alternatively the RyR receptor could have four binding sites that can bind either FKBP isoform. This issue has been resolved. There are only four FKBP binding sites, which can bind either isoform (Table IV).

In summary, we find that the ryanodine receptors from skeletal muscle in animals from each of the vertebrate classes are similar with regard to their association with FK-binding protein: 1) there are four binding sites/ryanodine receptor; 2) the general properties of binding and exchange are similar; and 3) the ryanodine receptor channel is modulated by the presence of FKBP. The association of FKBP with the RyR is conserved throughout vertebrate evolution and is consistent with a role for FKBP in EC coupling in skeletal muscle.

Acknowledgments—We thank Yuqi Qiao and Dr. Loic Jeyakumar for help in the preparation of some of the terminal cisternae fractions and Dr. Julio Copello for advice on some of the experiments. Purified FKBP utilized in preliminary studies was kindly provided by Dr. Hong-Bo Xin. We also thank Dr. Greg Wiederrecht of Merck for continued interest and utilisation in preliminary studies was kindly provided by Dr. Hong-Bo Xin.

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*J. Biol. Chem.* 1998, 273:34813-34819.
doi: 10.1074/jbc.273.52.34813

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