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Comparison of Calcium Release from Sarcoplasmic Reticulum of Slow and Fast Twitch Muscles

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Summary. The mechanism of Ca^{2+} release from the sarcoplasmic reticulum (SR) of slow and fast twitch muscle was compared by examining biochemical characteristics, ryanodine binding, Ca^{2+} efflux, and single Ca^{2+} channel properties of SR vesicles. Although many features of the Ca^{2+} release channel were comparable, two functional assays revealed remarkable differences. The comparable properties include: a high molecular weight protein from both types of muscle was immunologically equivalent, and Scatchard analysis of [^{3}H]ryanodine binding to SR showed that the K_d was similar for slow and fast SR. In the flux assay the sensitivity to the agonists caffeine, doxorubicin, and Ca^{2+} and the antagonists Mg^{2+}, ruthenium red, and tetracaine differed only slightly. When SR vesicles were incorporated into lipid bilayers, the single-channel conductances of the Ca^{2-} release channels were indistinguishable. The distinguishing properties are: When Ca^{2-} release from passively $^{45}Ca^{2+}$-loaded SR were monitored by rapid filtration, the initial rates of Ca^{2+} release induced by Ca^{2+} and caffeine were three times lower in slow SR than in fast SR. Similarly, when Ca^{2+} release channels were incorporated into lipid bilayers, the open probability of the slow SR channel was markedly less, mainly due to a longer mean closed time. Our results indicate that slow and fast muscle have ryanodine receptors that are biochemically analogous, yet functional differences in the Ca^{2+} release channel may contribute to the different time to peak contraction observed in intact slow and fast muscles.

Key Words Ca^{2+} release channel · excitation-contraction coupling · slow twitch skeletal muscle · fast twitch skeletal muscle · planar lipid bilayers

Introduction

Mammalian skeletal muscles can be divided functionally into fast and slow twitch muscles [3, 5]. Fast muscles have shorter time to peak contraction (contraction time) and one-half relaxation time than slow twitch muscles [2, 3, 5, 9]. The difference in the relaxation times can be explained in part by a greater content of SR and higher rate of Ca^{2+} uptake per unit sarcoplasmic reticulum (SR) in fast SR [1, 8, 9, 17]. To extend the finding that the Ca^{2+} pumping rate differs in the two muscle types, MacLennan and his associates [22] recently showed that the structure of the Ca^{2+}-ATPase in slow SR differs from that in fast SR. All of these factors work together to reduce the intracellular Ca^{2+} concentration more rapidly, and hence, relax the fast twitch muscle more quickly than the slow twitch muscle.

The longer time to peak contraction in slow muscles of rabbit, rat, and guinea pig [2, 5, 9] may be due in part to differences in both myosin ATPase [5] and in the mobilization of intracellular Ca^{2+}. Using skinned fiber preparations, Salviati and Volpe [29] measured muscle contraction and hypothesized that differences in the time to peak concentration could also be related to the inherent properties of the Ca^{2+} release mechanisms. To test this hypothesis further, we compared Ca^{2+} release channels from slow twitch (slow) and fast twitch (fast) SR vesicles using biochemical (ryanodine binding, Ca^{2+} fluxes) and electrophysiological (single Ca^{2+} release channel) properties. Our results show that many properties of the Ca^{2+} release channel in slow and fast SR are similar; however, some parameters such as the initial rate of Ca^{2+} release and the mean closed time of single Ca^{2+} release channels differed significantly in a manner that may contribute to the functional differences observed between fast and slow twitch muscle.

Materials and Methods

Materials

[^{3}H]Ryanodine and $^{45}CaCl_2$ were obtained from New England Nuclear. Doxorubicin and caffeine were purchased from Sigma,

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ryanodine from Calbiochem, and lipids from Avanti Polar Lipids. All other reagents used were of analytical grade.

HSR Preparation

Slow and fast SR vesicles were prepared by differential centrifugation from slow twitch red muscles (e.g., soleus, semitendinosus, and intertransversarius) and fast twitch white muscles (e.g., vastus lateralis and adductor magnus) of rabbit [27, 29, 30, 32, 33] euthenized with T-61, as described previously [16]. The pellets were suspended in solutions containing 0.15 M KCl, 20 mM MOPS (pH 6.8), 0.3 M sucrose, and various protease inhibitors such as 0.1 mM phenylmethylsulfonyl fluoride, 10 mg/liter aprotinin, 0.8 mg/liter antipain, 2 mg/liter trypsin inhibitor and 0.3 M sucrose. The final protein concentration was adjusted to 10–30 mg/ml and stored at −70°C. To estimate mitochondrial contamination of slow and fast SR, cytochrome oxidase activity was measured as described previously [18]. Mitochondrial cytochrome *c* oxidase activity in slow and fast SR vesicles were 150 ± 5 and 55 ± 3 nmol/mg per min, respectively, suggesting 6 and 2% mitochondrial contaminations.

Electron Microscopy

The "heavy" SR preparations were examined by transmission electron microscopy. Five microliters of SR suspension (1–2 mg/ml) was applied to a Formvar-carbon coated specimen grid for 1 min, and the excess fluid was drawn off with filter paper. The grid was stained for 1 min with five μl of 1% aqueous phosphotungstic acid, pH 6.5, blotted with filter paper and air dried. The negatively stained specimens were examined and photographed in a JEOL 100CX TEM operated at 80 kV.

[^{3}H]Ryanodine Binding Assay

SR vesicles (0.4 mg/ml) were incubated with 20 nM [^{3}H]ryanodine (54.7 Ci/mmol), unless otherwise specified, in a standard binding buffer containing 0.15 M KCl, 20 mM MOPS (pH 6.8), 6.25 mM EGTA, 5 mM $CaCl_2$ ($[Ca^{2+}]_{free}$ = 2 μM), for 2 hr at 37°C. The incubation mixtures were filtered (Millipore, 0.45 μm), rinsed with the same binding buffer (3 × 2.5 ml), dried, and counted. Specific binding of ryanodine is defined as the difference between total [^{3}H]ryanodine binding and nonspecific binding measured in the presence of 20 μM unlabeled ryanodine. Nonspecific binding was approximately 1–5% of the total binding.

Ca^{2+} Release Assay

For passive loading, SR vesicles were incubated overnight in a solution containing 0.15 M KCl, 20 mM MOPS (pH 6.8), and 5 mM $^{45}CaCl_2$ (10 μCi/ml) at 2–4°C in the presence of various protease inhibitors, as described above. To measure Ca^{2+} release, 20 μl of the loaded SR were diluted 100-fold into a "dilution" solution containing 2 μM Ca^{2+} and various concentrations of Ca^{2+} release activators or inhibitors. Ten sec after dilution, an aliquot (400 μl) of the diluted solution was filtered (Millipore, 0.45 μm), and washed two times with 2.5 ml of a solution containing (in mM): 150 KCl, 20 MOPS (pH 6.8), 10 $LaCl_3$ and 20 $MgCl_2$ [16].

The initial time course of Ca^{2+} release was measured using a rapid filtration system (Bio-Logic). The passively loaded SR vesicles were diluted with a solution containing (in mM): 20 MOPS (pH 6.8), 150 KCl, and 5 $CaCl_2$. Then, 0.3 ml of the diluted vesicles (approximately 150 μg of protein) were loaded onto a filter (Millipore, 0.45 μm). Forty sec after dilution, the reaction was started by passing various release solutions through the filter and was stopped by physical separation.

Planar Lipid Bilayer Methods

SR vesicles were incorporated into preformed planar lipid bilayers that were formed by painting a lipid/decane solution (dioleoyl phosphatidylethanolamine (DOPE) : dioleolyl phosphatidylserine, 3 : 1 or DOPE : dioleoyl phosphatidylglycerol, 3 : 1) across a hole in a Teflon partition which separated two Lucite compartments [24]. Channel incorporation was accomplished using the method outlined by Smith, Coronado and Meissner [31]. Briefly, vesicles were added to one compartment after the membrane was formed, usually the *cis* compartment (*cis* solution: 600 mM *n*-methyl *d*-glucamine Cl, 20 mM HEPES, 10 mM $CaCl_2$, 0.2 mM EGTA, pH 7.3; *trans* solution; 250 mM HEPES, 53 mM $Ca(OH)_2$, pH 7.3). The insertion of a chloride-permeable channel was the signal that a vesicle has fused with the bilayer. To monitor calcium release channels, the *cis* chamber was then perfused with a chloride-free solution: 250 mM HEPES-Tris, 1 mM EGTA, 0.5 mM $CaCl_2$, ($[Ca]_{free}$ = 0.1 μM) pH 7.3. In addition, the chloride-free solution was isosmotic to the *trans* solution to prevent incorporation of additional vesicles into the bilayer.

Channel insertion and subsequent experiments were monitored under voltage-clamp conditions with a pair of Ag/AgCl electrodes contacting the solutions via CsCl junctions. The channel currents were amplified using a patch-clamp amplifier (Yale model MK-5, Warner Instruments, Hamden, CT) and recorded on chart (General Scanning, Watertown, MA) and tape recorders (Dagan, Minneapolis, MN). Data were filtered to 300 Hz (Frequency Devices, MA) and transferred to a PDP 11/73 (Digitalized at 1 kHz, Indec Systems, Sunnyvale, CA). Channel kinetics were analyzed with software generously provided by Dr. Chris Miller (Brandeis University, Waltham, MA).

Miscellaneous

$[Ca^{2-}]_{free}$ in the Ca-EGTA solutions were calculated using a computer program using constants described previously [11]. Protein concentrations were determined by the Lowry method using bovine serum albumin as a standard [21].

Results

Characterization of Slow and Fast Twitch Skeletal SR

The biochemical properties of "heavy" SR fractions of slow and fast twitch muscles were compared (Table 1 and Fig. 1). The yield of the slow SR preparations was significantly lower than that of fast SR (0.57 ± 0.03, SE, n = 26 *vs.* 0.94 ± 0.07, n = 14, mg/g wet muscle) indicating that the density of the junctional SR in the slow twitch muscle is lower than that in fast SR. As found previously in unfractionated SR [34], the profiles of the two types of junc-

Table 1. Kinetic parameters of Ca^{2+} release from slow and fast SR

	SR vesicle diameter, nm	Ca^{2+} loading nmol/mg	Ca^{2+} release		
			Amount nmol/mg	% Release	Initial rate nmol/mg/sec
Slow	104 ± 3	35.2 ± 11.9	9.7 ± 3.1	28	74 ± 15
Fast	132 ± 3	75.6 ± 12.2	29.7 ± 10.0	39	251 ± 98

Ca^{2+} release experiments were carried out as described in the figure legend to Fig. 2. Values represent mean ± SE. For Ca^{2+} release experiments, five independent experiments were carried out for both slow and fast SR. SR vesicle sizes were measured by electron micrography as described in Materials and Methods, and diameters of 280 and 200 vesicles were measured for slow and fast SR, respectively.

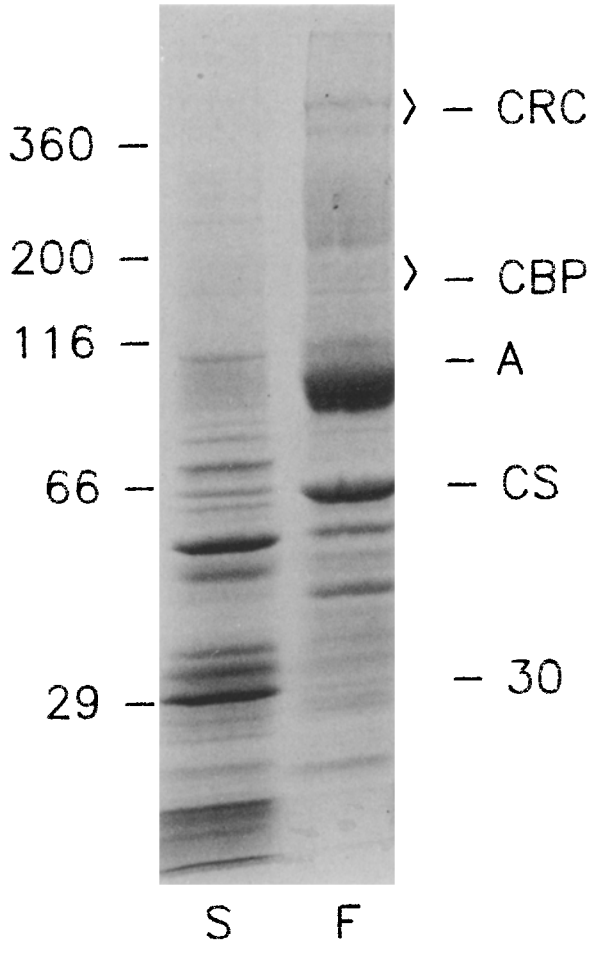

Fig. 1. Coomassie blue staining of slow (*S*) and fast (*F*) skeletal SR proteins. Junctional SR from slow and fast twitch skeletal muscles of rabbits were analyzed by SDS-polyacrylamide gel electrophoresis (7–15% gradient gels) as described under Materials and Methods. Each lane contained 100 μg of protein. *CRC*, Ca^{2+} release channel (M_r = 400–450 kDa); *CBP*, Ca^{2+} binding proteins (M_r = 160–180 kDa); *A*, (Ca^{2+} + Mg)-ATPase (M_r = 114 kDa for slow SR and 100 kDa for fast SR); *CS*, calsequestrin (M_r = 66 kDa); *30*, 30-kDa slow SR marker protein. The numbers on the left side represent molecular weight × 10^{-3} of molecular weight standards

tional SR proteins were also markedly different (Fig. 1). The relative amount of the 30-kDa protein, recently identified as a marker protein for slow SR [34], was approximately four times higher in slow SR than that in fast SR (Fig. 1). In addition, the ratios of ATPase/30-kDa protein, an index for muscle typing [34], were 0.5 and 8 for slow and fast SR, respectively, which is similar to previously published ratios, 0.7 and 12.5 for slow and fast SR. These results indicate that both the slow and fast SR preparations were derived from different muscle types, as found previously [27, 29, 30, 32, 33].

Calsequestrin, the major extrinsic Ca^{2+} binding protein in the lumen of the junctional SR, was identified by Stains-all [4] and quantified by densitometry scanning. The amount of calsequestrin in slow SR was 32% of that in fast SR. The high molecular weight Ca^{2+} release channel was identified by immunoblot (Western) analysis using polyclonal antibodies raised to fast skeletal Ca^{2+} release channel. The two bands in the high molecular weight regions (Fig. 1, labeled *CRC*) had positive immunoreactivities with the antibodies in both slow and fast SR (D.H. Kim & A. Marks, *unpublished data*), indicating that the Ca^{2+} release channels in the two types of SR are immunologically homologous. The amount of the Ca^{2+} release channel in slow SR vesicles was approximately 30% of that in fast SR. There was no detectable contamination of myofibrilar proteins in both types of SR.

Because a previous report showed that slow SR vesicles tend to form smaller vesicles than fast SR vesicles [32], we measured the diameter of the junctional SR vesicles by electron micrography. The diameter of the slow SR vesicles was 78% of that in fast SR (Table 1), which means that the average volume of a fast SR vesicle is approximately two times larger than a slow SR vesicle.

Ryanodine Binding to Slow and Fast SR

In light of evidence that ryanodine binds specifically to Ca^{2+} release channels [10, 20, 26], we compared ryanodine binding to slow and fast SR. The experi-

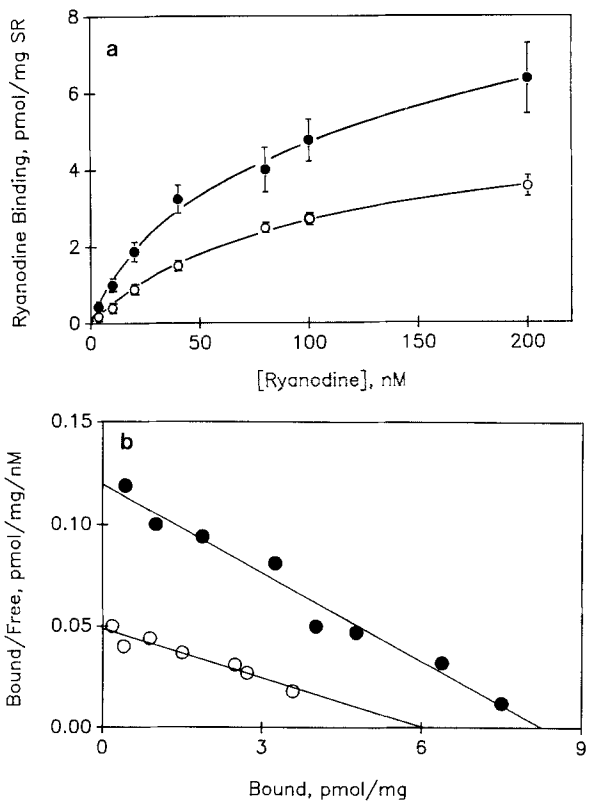

Fig. 2. Specific binding of [^{3}H]ryanodine to slow (○) and fast (●) skeletal muscle SR vesicles. (*a*) SR vesicles (0.4 mg/ml) were incubated with various concentrations of [^{3}H]ryanodine in a solution containing 0.15 M KCl, 20 mM MOPS (pH 6.8), 6.25 mM EGTA, and 5 mM $CaCl_2$($[Ca^{2+}]_{free}$ = 2 μM) for 2 hr at 37°C. Specific binding of ryanodine is defined as the difference between the total binding and the nonspecific binding measured in the presence of 20 μM unlabeled ryanodine. Nonspecific binding was approximately 1–5% of the total binding. Points represent the means of six independent experiments. By Student's *t* test the values are not significantly different at the $P < 0.05$ level. (*b*) Scatchard analysis of bound/free (pmol/mg/nM) *versus* bound (pmol/mg) for slow (○) and fast SR (●) yields apparent B_{max} and K_d values of 5.5 pmol/mg and 104 nM, respectively, for slow SR, and 8.1 pmol/mg and 70 nM, respectively, for fast SR

mental conditions used for ^{3}H-ryanodine binding were the same as those used to measure Ca^{2+} efflux from vesicles except that incubation times were longer (2 hr rather than 10 sec), and the temperature for binding studies was higher (37 rather than 22°C). The binding of [^{3}H]ryanodine to slow and fast SR (Fig. 2*A*) exhibited saturation kinetics that, by Scatchard analysis (Fig. 2*B*) defined a single class of high-affinity ryanodine binding sites. The K_d and B_{max} of slow and fast SR were as follows: k_d 104 ± 36 *vs.* 70 ± 19 nM; B_{max} 5.5 ± 1.2 *vs.* 8.1 ± 1.3 pmol/mg.

The effects of the Ca^{2+} release activators, caffeine, doxorubicin, ATP, and Ca^{2+} and the inhibitors ruthenium red, Mg^{2+}, and calmodulin on ryanodine binding differed slightly (Table 2). While 2 mM caffeine and 10 μM doxorubicin enhanced ryanodine binding in both slow and fast SR, there was less enhancement in slow SR than in fast SR (Table 2). In both types of muscle, ryanodine binding was maximally activated at approximately 5 μM Ca^{2+}, the concentration that maximally activated Ca^{2+} release (Fig. 3). The effects of the various Ca^{2+} release inhibitors were similar in slow and fast SR (Table 2). Consistent with other reports for fast SR [23], ryanodine binding to fast SR increased as pH of the binding medium increased (Table 3). However, activation of ryanodine binding by increasing pH (6.8 to 8.4) was greater in slow SR (fivefold) than in fast SR (twofold).

Kinetics of Ca^{2+} Release from Slow and Fast Twitch Skeletal SR

When slow and fast SR were loaded with 5 mM $^{45}Ca^{2+}$ and Ca^{2+} release from the passively $^{45}Ca^{2+}$-loaded SR was triggered by 2 mM caffeine and 2 μM Ca^{2+} [7], three major differences were observed (Fig. 4 and Table 1). *First*, the extent of Ca^{2+} loading into slow SR was approximately half that taken up by fast SR. The lower level of Ca^{2+} loading into slow SR vesicles could not readily be explained by slower influx or proteolytic membrane damage, because the passive Ca^{2+} loading was carried out by incubation of the SR vesicles overnight at 0–2°C in the presence of 5 mM $^{45}Ca^{2+}$ and various protease inhibitors. The difference in loading most likely is due to the tendency of slow SR preparations to form smaller vesicles than fast SR preparations (Table 1, ref. [32]) and the lower amount of calsequestrin in slow SR (Fig. 1). *Second*, the percent of the Ca^{2+} released from the vesicles from slow SR was 28% less than that from fast SR (Table 1). This difference may reflect the lower number of SR vesicles having Ca^{2+} release channels in slow SR (Fig. 1). *Third*, the initial rates of Ca^{2+} release from slow SR were 29% of those from fast SR. We hypothesize that the observed difference in the rate of Ca^{2+} release is related to the difference in the time to peak contraction in the two muscle types.

To compare the concentration dependence of several agonists and antagonists of Ca^{2+}-induced Ca^{2+} release, vesicles loaded with $^{45}Ca^{2+}$ were studied. Caffeine and doxorubicin increased Ca^{2+} release from both types of SR with comparable sensitivities (Table 4), but the amount of Ca^{2+} released was approximately three times greater in fast SR, consistent with the results obtained for Ca^{2+}-induced Ca^{2+} release (Table 1). When testing the inhibitors, vesicles were diluted with various concentrations of these inhibitors in the presence of 2 μM

Table 2. Effect of various compounds on ryanodine binding to slow and fast SR[a]

	n	Slow SR pmol/mg	*n*	Fast SR pmol/mg
Control	7	1.10(100) ± 0.18	14	1.62(100) ± 0.27
2 mM Caffeine	8	1.41(128) ± 0.30	9	2.69(166) ± 0.59
10 μM Doxorubicin	5	1.64(149) ± 0.39	7	4.10(253) ± 1.02
1 mM ATP	5	2.21(201) ± 0.20	7	3.05(188) ± 0.41
1 μM Ruthenium red	5	0.00(0) ± 0.00	4	0.02(1) ± 0.02
0.5 mM Mg^{2+}	2	0.25(23) ± 0.01	2	0.46(28) ± 0.04
1 μM Calmodulin	2	0.79(72) ± 0.01	4	0.63(39) ± 0.07

[a] SR vesicles (0.4 mg/ml) were incubated with 20 nM [^{3}H]ryanodine in a standard binding solution containing various calcium release activators and inhibitors. The numbers in parentheses are % of control binding. Values represent means ± SE. *n* represents number of experiments.

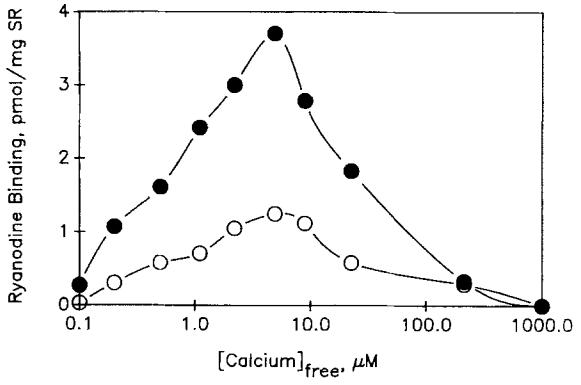

Fig. 3. Ca^{2+} dependence of [^{3}H]ryanodine binding to slow (○) and fast (●) SR. [^{3}H]ryanodine binding was performed as described in the legend to Fig. 2 at 20 nM [^{3}H]ryanodine and various $[Ca^{2+}]_{free}$. Values are averages of four independent experiments

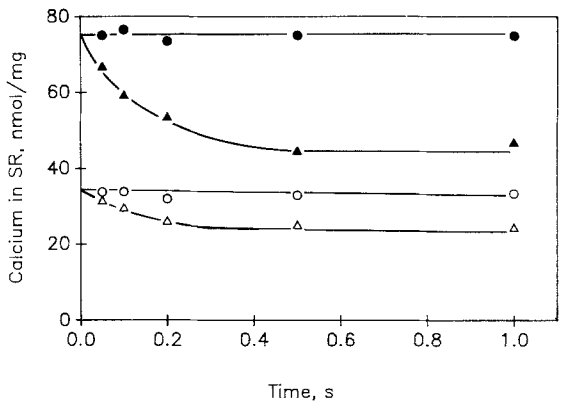

Fig. 4. Time courses of Ca^{2+} release from slow (○, △) and fast (●, ▲) twitch skeletal muscle SR determined by rapid filtration. SR vesicles (150 μg) loaded with $^{45}Ca^{2+}$ were placed onto a filter (Millipore, 0.45 μM), and Ca^{2+} release was started and stopped by controlling flow of the reaction solution. Filters were dried, and radioactivity on the filters counted by liquid scintillation counting. Reaction solutions contained 5 mM Ca^{2+} (○, ●) and 2 μM Ca^{2+} plus 2 mM caffeine (△, ▲)

Table 3. Effect of pH on ryanodine binding

pH	Ryanodine binding pmol/mg	
	Fast SR	Slow SR
6.0	0.03(1%)	0.03(3%)
6.8	2.95(100%)	0.98(100%)
7.6	4.17(141%)	2.35(240%)
8.4	6.35(215%)	4.83(492%)

SR vesicles (0.4 mg/ml) were incubated with 20 nM [^{3}H]ryanodine. The buffers used for the different pH experiments are MES (pH 6.0), MOPS (pH 6.8 and 7.6) and Tris (pH 8.4). Numbers in parentheses represent the percent of binding at pH 6.8, which was defined as 100%.

Ca^{2+} which activated release. Approximately 5 and 15 nmol Ca^{2+}/mg SR were released by 2 μM Ca^{2+} from slow and fast SR, respectively in the absence of the inhibitors. Slow SR appears to be slightly less sensitive than fast SR to each of the inhibitors, a finding similar to that obtained when the effects of ruthenium red on muscle contraction were measured in skinned fiber preparations [29]. The present values for the sensitivities of fast SR to the inhibitors are similar to those reported previously [16].

Comparison of Single-Channel Properties of Slow and Fast SR Ca^{2+} Release Channels

Channel incorporation into planar lipid bilayers differed in slow and fast SR. With SR vesicles from fast twitch muscle, fusion occurred in 5–15 min and,

Table 4. Summary of half-maximal concentrations of compounds to activate or inhibit Ca^{2+} release from passively $^{45}Ca^{2+}$-loaded slow and fast SR[a]

Effect on Ca^{2+} release	Compounds	Slow	Fast
		$C_{1/2}$	
Activation	Caffeine	0.6 mM	0.7 mM
	Doxorubicin	1.1 μM	1.1 μM
Inhibition	Mg^{2+}	0.4 mM	0.08 mM
	Ruthenium red	100 nM	40 nM
	Tetracaine	105 μM	80 μM

[a] SR vesicles were diluted with 2 μM Ca^{2+} plus various concentrations of Ca^{2+} release activators. Ca^{2+} release was terminated at 10 sec after the dilution by filtration, and washed as described in Materials and Methods. The amounts of Ca^{2+} release by the activators, calculated by subtraction of Ca^{2+} release by 2 μM Ca^{2+} alone from the total Ca^{2+} release induced by Ca^{2+} and the activators, are 3–5 and 13–15 nmol/mg for slow and fast SR, respectively. The amounts of Ca^{2+}-induced Ca^{2+} release for determination of the inhibitor effects were 5 and 15 nmol Ca^{2+}/mg for slow and fast SR, respectively. The $C_{1/2}$ values were obtained from four sets of experiments.

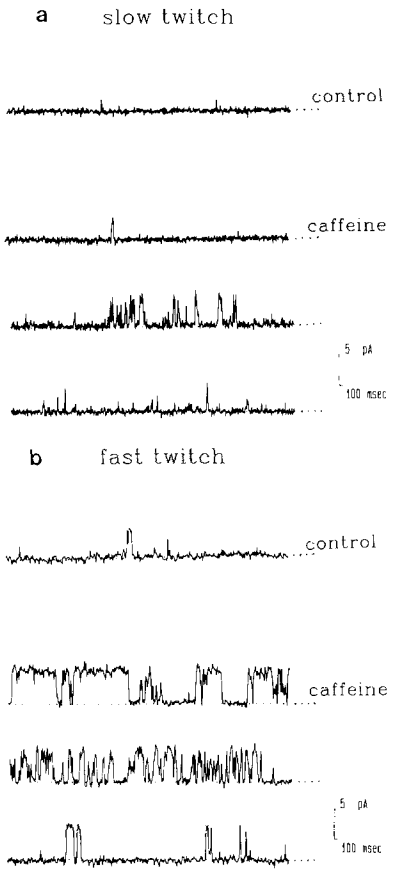

Fig. 5. Single Ca^{2+} release channel currents from SR vesicles isolated from fast and slow twitch muscle incorporated into planar lipid bilayers. In both slow (*a*) and fast (*b*), the top trace shows representative channel openings under control, unstimulated conditions. The bottom three traces show channel currents after activation by 10 μM Ca_{free} and 5 mM caffeine. Channel openings are shown as upward deflections from zero current, which is indicated by the dotted line. Holding potential was 0 mV

after perfusion of the *cis* solution, channel activity was seen in 90% of the experiments. On the other hand, fusion of SR vesicles from slow twitch muscle occurred after 20–60 min and channel activity was seen in only 20% of the experiments.

After channels were incorporated, a comparison of the channel activity showed that the single-channel conductance was similar but the kinetics were quite different. Under control (unstimulated) conditions (top trace of Fig 5*A* and *B*), channels from both muscle types were rarely open, but when open, channel conductance was ~100 pS in both muscle types (*data not shown*). In contrast, the response of the channels to addition of 10 μM free Ca^{2+} and 5 mM caffeine differed markedly (bottom three traces of Fig. 5*A* and *B*). Activity of the channel from the fast twitch muscle after stimulation by Ca^{2+} and caffeine was similar to that seen previously [6, 28, 31], where the channel opened frequently and the activity was maintained. With the slow SR channel, openings were short with long closed intervals between channel openings. The channel currents shown in the third trace of Fig. 5*A* represent the only burst with more than two or three openings seen in all the experiments done with the slow SR channel. In the absence of Ca^{2+} and caffeine, the opening of the slow channel was too infrequent to perform any analysis. Therefore, all comparisons of the SR channel from slow and fast twitch muscle were done after the channels were activated by 10 μM free Ca^{2+} and 5 mM caffeine.

A comparison of the open and closed times of the SR channels from the two muscle types showed that the mean open times differed by a factor of 3, whereas the mean closed time for the fast SR channel was 20 times shorter than the closed time for the slow SR channel (Fig. 6 and Table 5). Although

Table 5. Comparison of single-channel properties between slow and fast Ca^{2+} release channels

	Slow SR (msec)	Fast SR (msec)
Open time	5 ± 1.5	14 ± 7
Closed time	1,930 ± 1395	79 ± 57

In all cases the mean ± SD of three experiments is shown where the mean open time for 93 to 2,251 channels are averaged. Values are from caffeine-activated channels because there were insufficient number of channels open in slow muscle without activation by caffeine.

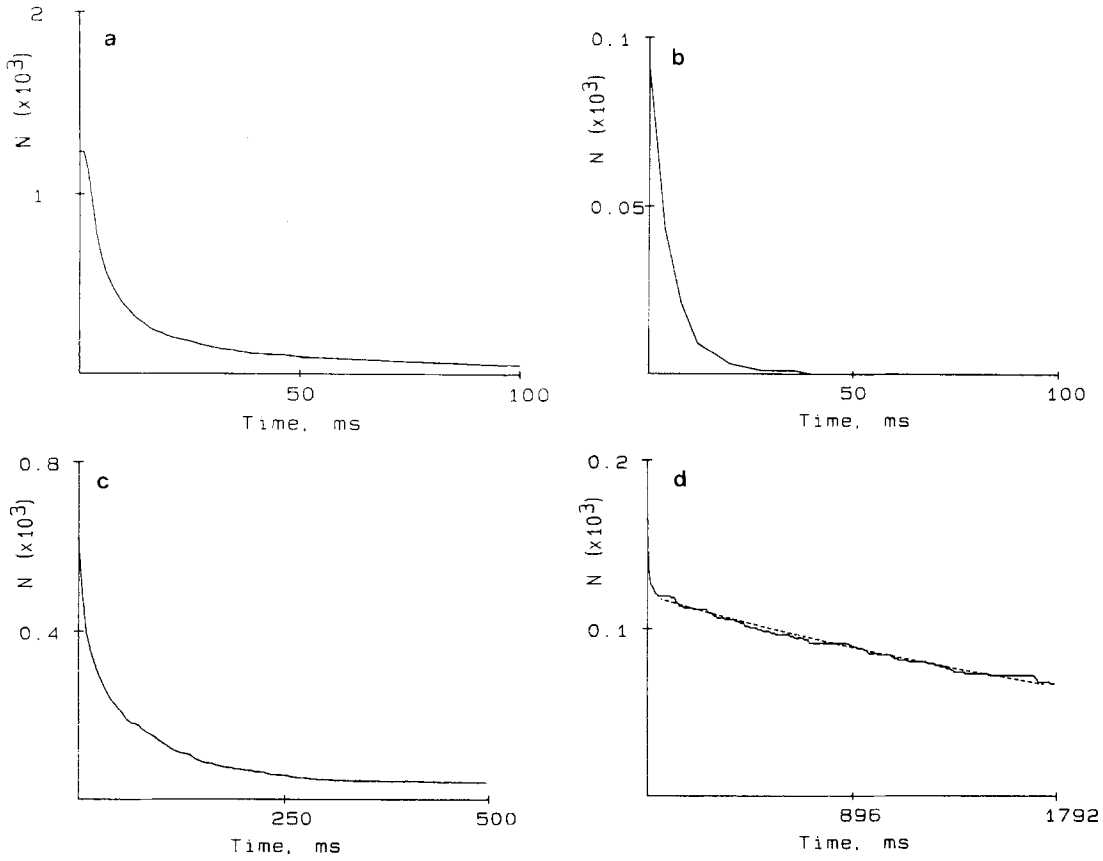

Fig. 6. Analysis of cumulative open and closed times for Ca^{2+}- and caffeine-activated Ca^{2+} release channels from slow (*b*) and (*d*) and fast (*a*) and (*c*) twitch muscle. Open and closed times for the channels from fast twitch muscle and open times for the channels from slow twitch muscle were fit by single exponential curves. Closed time for the channel from slow twitch muscle was best fit by a double exponential curve, but the dashed line represents only the longer time constant. To accumulate enough events, continuous recordings lasting 2 to 10 min were analyzed. Similar data were obtained in three experiments with each muscle type; data of one experiment from each muscle type is shown

the mean open times of the channels differed only slightly (14 msec for fast and 5 msec for slow, Table 5, $P > 0.05$), the difference between closed times were large with no overlap in the data. With the fast SR channel, both open and closed times could be fit with a single exponential. Only the open times of the slow SR channel could be fit by a single exponential. The closed times for the slow SR channel was fit with two exponentials, where the majority of the closings could be fit with the long time constant. The short time constant (mean closed time of about 7 msec) probably represents the closings seen during the infrequent "bursts" of channel openings.

Discussion

In this report, we have identified differences between Ca^{2+} release channel functions of junctional slow and fast SR preparations. The biochemical characteristics of the Ca^{2+} release channel were found to be similar in the two muscle types [25]. Functionally, however, the channels from slow twitch muscle were distinctly different from the channels from fast twitch muscle. These differences complement previous reports that SR preparations from slow and fast twitch skeletal muscles differ in morphology [30, 32], Ca^{2+} ATPase activity [8, 17], Ca^{2+} pump density [1], molecular structures of the ATPases [22], and protein profiles [34]. These differences in SR function may contribute to differences in the isometric twitch duration [2, 9, 17]. The slower half relaxation time in slow muscle may arise from differences in the amount and density of the Ca^{2+} pump. Observations of muscle contraction in skinned fiber preparations [29], suggest that the difference in time to peak contraction may result from different properties of the Ca^{2+} release channel in the two types of SR. Our results showing different channel properties support this hypothesis.

Pronounced differences were found in studies of the SR Ca^{2+} release channel in planar lipid bilayers.

Mean open and closed times for the fast SR channel were similar to the values obtained by Rousseau et al. [28]. In contrast, the Ca^{2+} channel from slow SR had a markedly longer mean closed time (Figs. 5 and 6; Table 5), despite a mean open time similar to that seen in fast SR channels. This result is qualitatively similar to the difference in the initial rates of Ca^{2+} release triggered by 2 μM Ca^{2+} and 2 mM caffeine (Table 1). However, the difference in closed time of the Ca^{2+} release channel between slow and fast SR was much larger than that in the initial rate of Ca^{2+} release between the two types of SR (20 *vs.* 3). This discrepancy could be explained as follows: (*i*) The difference in the size of slow and fast SR vesicles (Table 1) attenuates the difference in the apparent $t_{1/2}$ of Ca^{2+} release, as would be expected because a smaller vesicle will equilibrate its Ca^{2+} across the vesicle in a shorter time than a large vesicle. (*ii*) Differences in the amount of calsequestrin (Fig. 1) may also affect the initial rate of Ca^{2+} release from the SR vesicles, as evidenced by the report of Ikemoto et al. [12] that elevating the amount of Ca^{2+} bound to calsequestrin in fast SR sharply decreased the rate constant of Ca^{2+} release from fast SR.

The initial rate of Ca^{2+} release showed a threefold difference between slow and fast SR, whereas the apparent rate constant of Ca^{2+} release was found to be similar. We chose to use the initial rate of Ca^{2+} release to compare the intrinsic properties of Ca^{2+} release channels, because the equivalence of Ca^{2+} loading into the vesicles and the quantitative effect of calsequestrin binding of Ca^{2+} is uncertain. It is known, however, that the apparent rate constant of Ca^{2+} release decreases as the Ca^{2+} loading in the SR vesicles increases [12]. This factor will decrease the apparent rate constant from the fast SR, and thereby, will underestimate any difference in the efflux rate between slow and fast SR. Therefore, the initial rate of Ca^{2+} release (the amount of Ca^{2+} released times the rate constant of Ca^{2+} release, ref. [16]) appears to be a better indicator of the intrinsic channel function.

Although the present studies showed that the sensitivity of slow SR to caffeine and doxorubicin was generally similar to that of fast SR, contraction in the skinned slow muscle had a higher sensitivity to caffeine, but lower sensitivity to doxorubicin [29]. This discrepancy may be due to an effect of ATP in the skinned fiber experiments to alter the response to these drugs.

The relatively higher K_d and lower B_{max} values for fast SR, compared with results of other groups [13–15, 19, 20, 23], were due to the different experimental conditions. Our experimental conditions for the functional and binding experiments are closer to physiological conditions; e.g., our medium consisted of low ionic strength (150 mM KCl *vs.* 1 M NaCl, refs. [13, 21]), lower pH (6.8 *vs.* 7.4, [ref.15]), and a decreased $[Ca^{2+}]_{free}$ (2 *vs.* 25 μM, ref. [13]). The ryanodine binding experiments suggest that the Ca^{2+} release channel of slow and fast SR are similar. The values for the B_{max} for ryanodine binding were not significantly different between the two muscle types, because the analysis was confounded by scatter in the data. The averages, however, show that the number of binding sites in slow SR was 48% less than in fast SR, consistent with the relative amount of protein determined by densitometric scan of the SDS gel (Fig. 1). The same bell-shaped Ca^{2+} dependence of [^{3}H]ryanodine binding to both types of SR (Fig. 3) suggests that activation and inactivation of Ca^{2+} release channels occurs in the same Ca^{2+} concentration ranges. The activation and inhibition of ryanodine binding in the presence of Ca^{2+} release activators and inhibitors, respectively (Table 2), also suggest that the two types of channels are homologous. The pH dependence of ryanodine binding (Table 3) show that alkaline pH activates ryanodine binding more in slow SR, suggesting that maximal ryanodine binding at alkaline pH would be similar in the two types of SR.

In conclusion, this study indicates that functional heterogeneity of fast and slow twitch muscle can result, in part, from differences in Ca^{2+} release from the SR, where the Ca^{2+} release channel plays an important role in determining the time to peak contraction.

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