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Role of Ryanodine Receptors

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ABSTRACT: Recent findings on the ryanodine receptor of vertebrates, a Ca-release channel protein for the caffeine- and ryanodine-sensitive Ca pools, are reviewed in this article. Three distinct genes, i.e., *ryr1*, *ryr2*, and *ryr3*, express different isoforms in specific locations: Ryr1 in skeletal muscle and Purkinje cells of cerebellum; Ryr2 in cardiac muscle and brain, especially cerebellum; Ryr3 in skeletal muscle of nonmammalian vertebrates, the corpus striatum, and limbic cortex of brain, smooth muscles, and the other cells in vertebrates.

While only one isoform (Ryr1) is expressed in mammalian skeletal muscles, two isoforms (α- and β-isoforms expressed by *ryr1* and *ryr3*, respectively) are found in nonmammalian vertebrate skeletal muscles. Although the coexistence of two isoforms may merely be related to differentiation and specialization, the biological significance remains to be clarified. Ryanodine receptors in vertebrate skeletal muscles are believed to mediate two different modes of Ca release: Ca^{2+}-induced Ca release and action potential-induced Ca release. All results obtained so far with any isoform of ryanodine receptor are related to Ca^{2+}-induced Ca release and show very similar characteristics. Ca^{2+}-induced Ca release, however, cannot be the underlying mechanism of Ca release on skeletal muscle activation. Susceptibility of the ryanodine receptor's ryanodine-binding activity to modification by physical factors, such as osmolality of the medium, might be related to action potential-induced Ca release. A hypothesis of molecular interaction in view of the plunger model of action potential-induced Ca release is discussed, suggesting that the model could be compatible with Ryr1 and Ryr3, but incompatible with Ryr2. The functional relevance of ryanodine receptor isoforms, especially Ryr3, in brain also remains to be clarified.

Among *ryr1* gene-related diseases, malignant hyperthermia was the first to be identified; however, there is still the possibility of involvement of the other genes. Central core disease has been added to the list recently. A molecular approach for the diagnosis and treatment of diseases is now in progress.

KEY WORDS: Ca release channel protein, Ca^{2+}-induced Ca release, action potential-induced Ca release, ryanodine receptor isoforms, malignant hyperthermia.

I. INTRODUCTION AND OVERVIEW

Intracellular Ca^{2+} plays a critically important regulatory role in muscle contraction, metabolism, secretion, membrane excitability, and other biologic processes (Ebashi and Endo, 1968; Ebashi and Ogawa, 1988; Baker, 1988; Berridge, 1993a, b). Cytosolic intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) are regulated by the balance between the Ca removal system and the Ca supply system. The sources of cytoplasmic Ca^{2+} are Ca entry through various types of Ca channels in the cytoplasmic membrane and Ca release through Ca release channels in the Ca stores. Two main proteins for the Ca release channels have been purified: the inositol 1,4,5-trisphosphate (IP3*)

* Abbreviations: AMPOPCP, β,γ-methylene adenosine triphosphate; BAPTA, 1,2-*bis*(2-aminophenoxy) ethane-*N,N,N′,N′*-tetraacetic acid; Ca, calcium; Ca^{2+}, calcium ion; CaM, calmodulin, CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; DHP, dihydropyridine; DHP-R, dihydropyridine receptor; EC, excitation-contraction; EGTA, ethylene glycol *bis* (β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HFSR, heavy fraction of SR; IP3, inositol 1,4,5-trisphosphate; MH, malignant hyperthermia; MHS, MH susceptible; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; Ry, ryanodine; RyR, ryanodine receptor (*ryrN* and RyrN mean gene for a ryanodine receptor isoform and its product [protein], respectively. N, an integer); SR, sarcoplasmic reticulum.

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receptor for the IP3-sensitive Ca pool and the ryanodine receptor (RyR) for the caffeine- and ryanodine-sensitive Ca pool. While the IP3 receptor is mainly involved in smooth muscle and various kinds of nonmuscle cells, RyR is the principal receptor in striated muscle and is also involved in smooth muscle cells and various kinds of nonmuscle cells, including neurons. As many review articles concerning Ca channels (Catterall, 1988; Spedding and Paoletti, 1992; Bertolino and Llinas, 1992; Catterall, 1993; Putney and Bird, 1993; Putney, 1993) and IP3 receptors (Ferris and Snyder, 1992; Mikoshiba, 1993; Putney and Bird, 1993) have already been published, they should be referred to for details. Here, we only discuss directly related aspects.

In skeletal muscle, the sarcoplasmic reticulum (SR) is the main source of Ca^{2+}. The Ca release channel proteins were initially identified in SR vesicles of rabbit skeletal muscle as ryanodine-binding proteins that could be solubilized using the detergent CHAPS in the presence of a high salt concentration (Pessah et al., 1985, 1986). The protein was subsequently purified and widely designated as a RyR (Inui et al., 1987a, b; Imagawa et al., 1987; Lai et al., 1988b). The RyR incorporated in the lipid bilayer membrane shows channel activity with the properties of Ca^{2+}-induced Ca release. Electron microscopic studies of the RyR identified it morphologically as a foot which is a mass spanning the space between the transverse tubule (T-tubule) and terminal cisternae of SR and is thought to play an important role in releasing Ca from SR on skeletal muscle activation (Franzini-Armstrong, 1970, 1975). The process of Ca release on skeletal muscle activation shows characteristics distinct from those of Ca^{2+}-induced Ca release (Endo, 1977, 1985). In cardiac muscle, which has another isoform of RyR derived from a different gene (*ryr2* gene), Ca^{2+}-induced Ca release is widely accepted to be an underlying mechanism for muscle contraction (Winegrad, 1979; Fabiato, 1983). Much progress has been made, including cloning and sequence determination of cDNAs for RyRs of various origins, since RyR was first purified from rabbit skeletal muscle, which is the product of *ryr1* gene. A ryanodine receptor isoform derived from the *ryr3* gene has been recognized in cells of the limbic cortex of the brain and in other cells (Hakamata et al., 1992; Sorrentino and Volpe, 1993). Whereas only one isoform is expressed in mammalian skeletal muscle, two isoforms have been identified in nonmammalian vertebrate skeletal muscle, including that of chicken, frog, and toadfish (Airey et al., 1990; Olivares et al., 1991; O'Brien et al., 1993). Both isoforms have been purified from bullfrog skeletal muscle (Murayama and Ogawa, 1992). Their cloned cDNA sequences have been determined recently, and their primary structures have been deduced (Oyamada et al., 1994). Here, we review recent progress in the study of RyRs and discuss a possible molecular mechanism for the signal transduction from the dihydropyridine receptor (voltage sensor) to the ryanodine receptor in Ca release on skeletal muscle activation. This article concentrates on vertebrates because of the significant progress that has already been made; however, findings on invertebrates are being accumulated (Loesser et al., 1992; Seok et al., 1992; Hasan and Rosbash, 1992; Takeshima et al., 1994). In addition, several informative review articles concerning RyR have been published (Fleischer and Inui, 1989; MacLennan and Phillips, 1992; Williams, 1992; McPherson and Campbell, 1993a; Sorrentino and Volpe, 1993).

II. OVERVIEW OF THE CHEMISTRY AND PHARMACOLOGY OF RYANODINE

"The term *receptor* is often used operationally to denote any cellular macromolecule to which a drug binds to initiate its effects (Ross, 1990)." The ryanodine receptor was found to be identical to an endogenous Ca release channel protein. The ligand, ryanodine, is a tool to identify the protein that is important in physiologic functions and to investigate its properties. Therefore, it may be helpful to briefly reconsider this subject in light of the current detailed and sophisticated investigations on the molecular level, although it has been reviewed previously, concisely, and clearly by Jenden and Fairhurst (1969).

A. Chemistry of Ryanodine

Ryanodine was first characterized after isolation and crystallization from the ground stem wood

and root of *Ryania speciosa* Vahl by Rogers et al. (1948). Ryanodine is a neutral alkaloid that is soluble in water and alcohol. Advances in techniques for separation and analysis have made it possible to purify various ryanodine congeners from *Ryania* extract and to determine their chemical structures (Waterhouse et al., 1984; Ruest et al., 1985, Sutko et al., 1985b, 1990; Jefferies et al., 1992a, b). Some of these are listed in Table 1 (Pessah et al., 1985; Humerickhouse et al., 1993). Pure ryanodine, as determined by HPLC, is commercially available from only two companies: radioactive [^{3}H]ryanodine from NEN, Dupont, and nonradioactive ryanodine from Wako Co. Ltd. The other commercially available reagent, "ryanodine", is insoluble in water and contains many pharmacologically active congeners. For example, Figure 1 shows a chromatogram of "ryanodine" from Calbiochem (lot No. 801920) that was claimed to be spectrophotometrically more than 90% pure. The purity of this reagent, however, was found to be only 16% on analysis by HPLC. It was contaminated by 9,21-didehydroryanodine and other congeners. Ogawa and Harafuji (1990a) reported a downward convex Scatchard plot for [^{3}H]ryanodine binding when the analytical amount of ryanodine was supplemented with this reagent, while a linear Scatchard plot was obtained only when [^{3}H]ryanodine was used. Such a crude ryanodine, as shown in Figure 1, is not white, but brown, and insoluble in water, but soluble in alcohol. Partially purified ryanodine of white powder, which was once available from Merck, Sharp, and Dohme, and from S. B. Penick & Co. until the early 1980s, contained 9,21-didehydroryanodine in a proportion of one half to three quarters (Waterhouse et al., 1984; see Figure 1) and was soluble in water. Ryanodine and 9,21-didehydroryanodine are reported to be almost equipotent in their pharmacologic action on vertebrate skeletal and cardiac muscles (Jenden and Fairhurst, 1969; Pessah et al., 1985; Humerickhouse et al., 1993). However, the results by Pessah et al. (1985) suggested that 9,21-didehydroryanodine might have more potent effect on cardiac muscle (see Table 1).

Because some differences in ryanodine preparations could have impact on the results obtained, we must be aware of the purity of ryanodine.

B. Pharmacology of Ryanodine

Ryanodine first gained attention because of its insecticidal properties: flaccid paralysis of striated muscles accompanied by a tremendous increase in oxygen consumption. Although its effects on invertebrate muscles varied from no apparent effects to progressive contractile failure or contracture (Haslett and Jenden, 1961), the most striking actions were an irreversible

TABLE 1
Comparison of Effects of Ryanodine Congeners

Ryanoids	LD_{50} (mg/kg)[a]	IC_{50} (n*M*) for [^{3}H]ryanodine binding			
		Sk	M-SR	Card	M-SR
Ryanodine	0.1[1,b]	27[1],	12[2]	22[1],	5[2]
9,21-Didehydroryanodine	0.1[1]	23[1],	16[2]	7[1],	7[2]
9-Epiryanodine	0.47[1]	76[1]		61[1]	
9-Hydroxyryanodine (ester E)	—	185[2]		93[2]	
10-Ketoryanodine	0.55[1]	73[1]		60[1]	
8-Hydroxy, 10-epi, 9,21-didehydroryanodine (ester F)	—	749[2]		461[2]	
N,O-15-Dimethylryanodine	17[1]	384[1]		240[1]	
Anhydroryanodine	>20[1]	>10,000[1]		>10,000[1]	
Ryanodol	>20[1]	>10,000[1]		>10,000[1]	

[a] LD_{50} for mouse administered i.p.
[b] [1]Pessah et al. (1985); [2]Humerickhouse et al. (1993).

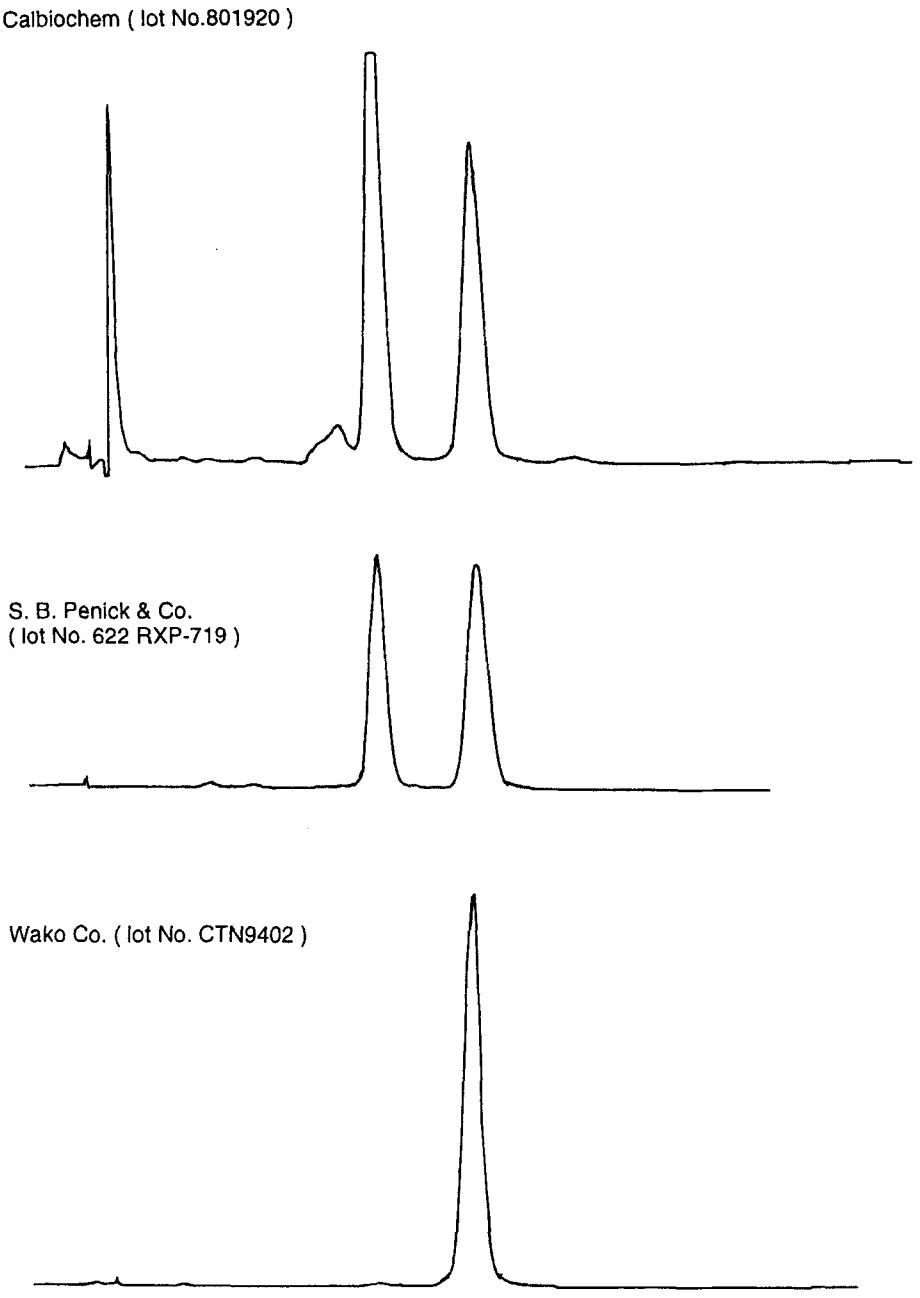

FIGURE 1. HPLC analysis of ryanodines obtained from various sources. Ryanodine was analyzed on an ODS-3 column that was eluted with ethanol:water (1:1) at a flow rate of 1 ml/min.

contracture of the skeletal muscle of vertebrates and a negative inotropic effect on mammalian cardiac muscle. The magnitude of its effect on cardiac muscle is markedly species dependent (Sutko and Willerson, 1980). Another interesting action of ryanodine is vasoconstriction, which is neurally mediated and central in origin. Jenden and Fairhurst (1969) commented that the participation of reflex pathways was not excluded and that evidence of a direct action of ryanodine on the central nervous system was lacking. When isoforms of RyR are now identified in the brain, this finding cannot be overlooked, although direct evidence is still required.

III. Ca RELEASE MECHANISMS ON SKELETAL MUSCLE ACTIVATION

The mechanism by which action potential or depolarization in the sarcolemma, including the transverse tubular membranes, causes Ca release from the sarcoplasmic reticulum is the focus of interest among investigators and the main subject of many review articles on excitation-contraction (EC) coupling (Endo, 1977; Caputo, 1983; Fleischer and Inui, 1989; MacLennan, 1990; Ashley et al., 1991; Catterall, 1991; Ebashi, 1991; Rios et al., 1991, 1992; Rios and Pizarro, 1991; Dulhunty, 1992; Lamb, 1992). Among several models proposed that concern skeletal muscle, two models still deserve consideration: the chemical transmission model and the mechanical coupling model.

Calcium ion and IP3 are plausible candidates as a chemical transmitter for Ca release from the SR. Although IP3 has been shown to be an important messenger in smooth muscle or nonmuscle cells, the role of IP3 in striated muscles is controversial. While Volpe et al. (1985) and Vergara et al. (1985) reported affirmative results (see also Nosek et al., 1986; Donaldson et al., 1987; Suárez-Isla et al., 1988), Palade (1987), Ehrlich and Watras (1988), and Ogawa and Harafuji (1989) obtained negative results (also see Movsesian et al., 1985; Scherer and Ferguson, 1985; Lea et al., 1986; Mikos and Snow, 1987; Valdivia et al., 1990). Blinks (1987) and Donaldson et al. (1988) reported that the effect of IP3 depended on conditions. Blinks and his colleagues (Hannon et al., 1992) have shown that Ca release is triggered by IP3 only under artificial conditions where the T-tubules are sealed and polarized. They argued in detail against the physiologic involvement of IP3, whereas Jaimovich (1991) reviewed this subject in favor of it. Because IP3 receptors are not found in skeletal muscles, the mechanism of Ca release by IP3, if any, would be different from that in cells containing IP3 receptors. Kijima et al. (1993) reported that IP3-binding sites were present in the intercalated discs of atrial and ventricular myocytes, while ryanodine-binding sites were on SR in myocytes. IP3 would stimulate Ca entry through intercalated discs. Although phosphatidylinositol-4,5-bisphosphate itself can release Ca from the SR, the rate of release is too slow to explain the rate of Ca release caused by action potentials (Ogawa and Harafuji, 1989; Chu and Stefani, 1991).

Since the Ca^{2+}-induced Ca release was discovered independently by Endo and colleagues (Endo et al., 1970) and by Ford and Podolsky (1970), the calcium ion has been considered a candidate for the chemical transmitter signal for Ca release channels in the SR: the increase in Ca^{2+} in a bathing medium was able to either increase or facilitate Ca release from the SR. Endo (1977, 1985), however, has raised serious doubts about the possible role of the mechanism in the physiologic regulation in vertebrate skeletal muscle for the following reasons, although Ca^{2+}-induced Ca release might be involved in the activation of cardiac muscle (Winegrad, 1979; Fabiato, 1983).

1. Ca^{2+} concentrations required to cause *net* Ca release were 100 μM or more under the physiologic conditions that simulated those in the cytoplasm (Endo, 1975). The value is certainly too high to be reached during normal action potential activation. In support of this conclusion, skeletal muscle contraction is observed even under the condition where no Ca influx can occur (Rios and Pizarro, 1991). As mentioned later, experiments by Tanabe and collaborators (1990a, b) clearly showed that a large amount of Ca current through chimeric L-type Ca channels, which was almost equivalent to that in cardiac muscle, could not be a direct trigger for skeletal muscle contraction.
2. Some local anesthetics, such as procaine and tetracaine, markedly inhibited Ca^{2+}-induced Ca release, whereas they did not inhibit depolarization-induced contraction of skeletal muscle (Thorens and Endo, 1975).
3. Although adenine or adenosine weakly stimulated Ca^{2+}-induced Ca release, they decreased Ca^{2+}-induced Ca release in the presence of ATP (Ishizuka and Endo, 1983). When these partial agonists were injected into single fibers from skeletal muscle, twitch responses to electrical stimulations were unchanged (Ishizuka et al., 1983).
4. An antagonistic action of dantrolene was markedly temperature dependent against Ca release by caffeine, but insensitive to tem-

perature against Ca release on depolarization by high K^+ (Ohta and Endo, 1986; Kobayashi and Endo, 1988).

Thus, we may conclude that Ca^{2+}-induced Ca release is not involved in physiologic Ca release on depolarization of skeletal muscle. However, it should be mentioned that the Ca^{2+}-induced Ca release mechanism is important not only in the pharmacologic actions of drugs (caffeine, quercetin, halothane, and others) on skeletal muscle, but also in some pathologic conditions, such as malignant hyperthermia, as discussed later.

In recent years, when the molecules of the Ca release channel were identified as ryanodine receptors or feet and their morphology became evident, there was renewed interest in the possibility that Ca^{2+}-induced Ca release might play some role as a modulator in normal activation in vertebrate skeletal muscle. Local Ca^{2+} concentrations in the restricted region in the neighborhood of the orifices of the channels will be increased to a level high enough to stimulate Ca^{2+}-induced Ca release. Supporting this idea, Jacquemond et al. (1991) reported decreased early peak rates of Ca release elicited with voltage-clamp depolarization when fura-2 or a mixture of fura-2 and BAPTA was injected into cut fibers from *Rana pipiens*. Fura-2 at 2 to 3 m*M* or BAPTA at 3 to 5 m*M*, which binds Ca^{2+} rapidly and maintains Ca^{2+} at a reduced level, completely eliminated the early peak, but not the following smaller steady level, of the rate of Ca release on depolarization. They attributed this removal of the transient component of SR Ca release to a prevention of Ca^{2+}-induced Ca release. Their results, however, contradict the conclusion of Baylor and associates (Baylor and Hollingworth, 1988; Hollingworth et al., 1992), who carried out similar experiments using instead intact fibers from *R. temporaria* stimulated by action potentials. Chandler and associates (Pape et al., 1993; Jong et al., 1993) performed detailed experiments to try to resolve the source of these apparent differences in the effects of fura-2. They concluded that the results were dependent on the concentration of fura-2 used: in the presence of 0.5–2 m*M* fura-2, the maximum rate of Ca release was significantly increased, which is consistent with the results of Baylor and his associates (Baylor and Hollingworth, 1988: Hollingworth et al., 1992). In the presence of 5 to 6 m*M* fura-2, however, the maximal rate of Ca release was decreased by about half, consistent with the results of Jacquemond et al. (1991). Their results were the same, regardless of the methods of activation, intact or cut fiber, and animal species (*R. pipiens* or *R. temporaria*). They concluded that the discrepancy may be caused by differences of fiber conditions or by pharmacologic actions of a high concentration of fura-2 not related to Ca buffering. They confirmed that Ca^{2+}-induced Ca release is not involved as a modulator in action potential- or depolarization-induced Ca release in vertebrate skeletal muscle (Pape et al., 1993; Jong et al., 1993).

Another suggested action of Ca^{2+} on Ca release from SR was Ca^{2+}-dependent inactivation of Ca release. The increase in the peak rate of Ca release by fura-2, which was described above, can be explained as disinhibition by fura-2 of Ca^{2+}-dependent inactivation of Ca release. The decrease in the rate of Ca release, which is observed with even small levels of Ca release in voltage-clamp experiments, was first described by Baylor et al. (1983), and characterized by Schneider and collaborators (Melzer et al., 1984; Simon et al., 1991). After a train of action potentials, the peak rate of Ca release from the SR associated with the second and subsequent action potentials was 10 to 20% of that associated with the first action potential (Jong et al., 1993). Similarly, if a voltage-clamp depolarization was given, the rate of Ca release usually increased initially, reached an early peak, and then decreased to an approximately steady level that might be as little as 10 to 20% of the peak value. This decreased rate of Ca release by prolonged depolarization may be due to inactivation of EC coupling. We do not know, however, whether this Ca^{2+}-induced inactivation of Ca release is a general underlying mechanism of the inactivation of EC coupling. These time- and Ca^{2+}-dependent activations and inactivation of Ca release was originally observed in cardiac muscle, where time-independent Ca^{2+}-induced Ca release may play a role in the Ca-release mechanism (Fabiato, 1983, 1985b). Fabiato (1984) reported similar results using microdissected fragments (3 to 6 μm in diameter) from the frog semitendinosus muscle. According to his results, the threshold Ca^{2+} concentration that triggered Ca

release increased as the rate of change in Ca^{2+} concentration was decreased. This type of Ca release was found to be insensitive to procaine, which was consistent with the Ca^{2+}-dependent inactivation of Ca release described above. This Ca^{2+}-dependent inactivation is observed at much lower Ca^{2+} concentrations than the activation of time-independent Ca^{2+}-induced Ca release. Therefore, the Ca^{2+} site for inactivation may be different from the Ca^{2+} site for activation of Ca^{2+}-induced Ca release. It is not known whether the molecule responsible for inactivation is RyR. There are no reports suggesting time-and Ca^{2+}-dependent inactivation of Ca release with isolated SR vesicles or RyR molecules (also see Ikemoto et al., 1985). It should be mentioned that the rates of Ca release from SR are calculated values using parameters assigned to various Ca buffer sites, including Ca^{2+} indicator(s), BAPTA if used, troponin C, parvalbumins, and Ca-ATPase of SR. A reexamination of the values of parameters is necessary on the following bases. First, the intracellular concentration of parvalbumin was adopted in the experiments with cut fibers where parvalbumin content may be reduced. Second, properties of Ca binding of fura-2 and other Ca indicators in the myoplasm may be different from those in cuvette (Konishi et al., 1988; Uto et al., 1992). Third, Ebashi and Ogawa (1988) reported different values of rate constants for troponin C and parvalbumins.

This time- and Ca^{2+}-dependent inactivation of Ca-release is probably distinct from "RyR adaptation" of cardiac muscle (Györke and Fill, 1993) and skeletal muscle (Györke et al., 1994). The rate for "RyR adaptation" (τ~1 to 2 s) is about one thousandth as slow as that for the time- and Ca^{2+}-dependent inactivation (τ~2 ms). "RyR adaptation" could be overcome by higher Ca^{2+}, but the inactivation was hardly reversed by stronger stimulation.

Schneider and Chandler (1973) demonstrated for the first time intramembrane charge movement that will reflect the conformation change of voltage-sensing molecules in the external membrane. Chandler and colleagues (Chandler et al., 1976) estimated the number of molecules as 500 to 600/μm^2, which is close to the density of feet structure (700/μm^2) determined by electron microscopy (Franzini-Armstrong, 1970, 1975), suggesting the idea of one voltage-sensing molecule for every foot. They proposed the mechanical coupling model, in which a plunger and plug for the pore of the Ca channel on the Ca store was moved back and forth by a voltage-sensing molecule. At first, the foot structure was thought to function as the voltage-sensing molecule as well as the plunger and plug. At present, however, it is widely accepted that the foot is the cytoplasmic part of a tetramer of the Ca release channel protein (i.e., RyR) and that the voltage-sensing molecule is the α_1-subunit of the dihydropyridine receptor* (DHP-R) or the DHP-binding protein (Schwartz et al., 1985; Beam et al., 1986; Rios and Brum, 1987; Tanabe et al., 1987, 1988; Adams et al., 1990). The general idea is as follows: some conformation change in the voltage sensor is transmitted to the RyR by an unknown mechanism to open the gate, resulting in Ca release. The DHP-R appears to form a tetramer because it has been observed as a tetrad of intramembrane particles in freeze fracture images of T-tubules by electron microscope (Block et al., 1988). In toadfish swimbladder muscle, tetrads in the T-tubules are closely apposed to feet in the SR. However, the alignment of tetrads and feet is unique in that each tetrad abuts on every other foot, which are aligned in two rows (Block et al., 1988). Every cluster of one row of tetrads faces an empty space in the other row. It is not known whether this alignment can be generalized for other skeletal muscles. Franzini-Armstrong et al. (1991) concluded that the ratio of tetrads to feet was 1:1 rather than 1:2 in the peripheral couplings of maturing skeletal muscle of mouse embryo. However, Franzini-Armstrong et al. (1991) also indicated that the center-to-center distance between tetrads along the lines of the approximately tetragonal pattern was 43 ± 4 nm, which was larger than the spacing between feet *in situ* (Block et al., 1988) and in the isolated SR (Ferguson et al., 1984) of adult skeletal muscle (28 and 33 nm, respectively). The

* DHP-R is composed of α_1-, α_2-, β-, γ-, and δ-subunits. The α_1-subunit is a main function of voltage-dependent L-type Ca channel of DHP-R. The roles of the other subunits are not known, although some of them are thought to be modulatory. In this article, "DHP-R" means α_1-subunit.

results in Figures 16 and 18 by Franzini-Armstrong et al. (1991) do not necessarily exclude the possibility of a 1:2 alignment. Franzini-Armstrong and Jorgensen (1994) acknowledged that tetrads are associated with alternate feet in the peripheral coupling of maturing skeletal muscle of mouse embryo. Bers and Stiffel (1993) reported biochemical results that the triad fraction from rabbit skeletal muscle showed a ratio of about 0.5 of B_{max} for ryanodine binding to that for DHP binding. This is consistent with a ratio of tetrads to feet of 1:2 in view of the stoichiometries of 1:4 and 1:1 of ligand binding to RyR and DHP-R, respectively. In cardiac muscle, however, the number of RyRs is reported to be much larger than that of DHP-R (Wibo et al., 1991; Bers and Stiffel, 1993).

It is not clear whether DHP-R is in direct contact with RyR, although it is strongly suggested (Franzini-Armstrong and Nunzi, 1983). Searches for an auxiliary protein or proteins that combine DHP-R and RyR have been unsuccessful (McPherson and Campbell, 1993a). In experiments in which cDNAs for DHP-Rs from skeletal and cardiac muscle were expressed in dysgenic skeletal muscle that lacks voltage sensors, Tanabe et al. (1990b) showed that the type of EC coupling was decided by the DHP-R type expressed: skeletal muscle-type EC coupling (i.e., external Ca [Ca-influx]-independent and Cd-insensitive contraction) when cDNA from skeletal muscle was expressed, and cardiac muscle-type EC coupling (i.e., external Ca [Ca-influx]-dependent and Cd-sensitive contraction) when cDNA from cardiac muscle was expressed. Using various chimeric DHP-R cDNA constructs that encode cardiac DHP-R as the basic structure in which the large, putative cytoplasmic regions are replaced by corresponding regions of skeletal muscle, they have clearly shown that the putative cytoplasmic region between repeats II and III of skeletal muscle DHP-R is an important determinant of skeletal-type EC coupling (Tanabe et al., 1990a). Properties characteristic of the cardiac L-type channel (i.e., rapid activation of large currents) were maintained even when its major cytoplasmic regions were replaced by skeletal muscle counterparts. This indicates that the putative membrane-spanning and adjacent region of the DHP-R, which form the channel and its entrances, are important in determining the properties of the L-type calcium current. However, they are not involved in signal transmission. In an accompanying paper, Adams et al. (1990) have shown that skeletal muscle, cardiac, and chimeric DHP-R constructs all restored charge movements with very similar voltage-dependent behavior in dysgenic skeletal muscle, independent of the nature of the L-type current (slowly activating small currents or rapidly activating large currents) or EC coupling type (skeletal or cardiac type) produced by them. This similarity in voltage dependence seems reasonable given that the S4 segments, which probably serve to sense voltage, are highly conserved between the skeletal muscle and cardiac DHP-Rs. The conclusion of the experiments by collaboration of Numa's and Beam's laboratories is that the putative cytoplasmic region between repeats II and III of skeletal muscle DHP-R plays a critically important role in signal transduction to RyR of dysgenic skeletal muscle (also see Section VI).

IV. Ca RELEASE CHANNELS IN SKELETAL MUSCLE

As mentioned above, the Ca release channel in skeletal muscle is thought to conduct dual modes of Ca release: action potential-induced Ca release and Ca^{2+}-induced Ca release. The results with RyR that have been reported so far are those related to Ca^{2+}-induced Ca release. Lipid bilayer experiments with RyR do not show membrane potential-dependent channel activation. This may be reasonable in view of the equal distribution of various kinds of ions, except calcium ions, between cytoplasmic and intraluminal regions (Somlyo et al., 1977a, b, 1981). The SR membrane is shown to be permeable to monovalent ions, indicating the absence of membrane potential difference across the SR membrane (Kasai and Kometani, 1981; Ogawa et al., 1981; Kasai et al., 1985). This, however, does not necessarily exclude the possibility of action potential-induced Ca release. What is important is how the conformation change in the voltage sensor of DHP-R in the T-tubule membrane is transmitted to RyR. In that sense, the experimental systems used so far may not be appropriate to observe the action po-

tential-induced Ca release. Reevaluation of experimental conditions is required. A skinned fiber with the closed orifice of a T-tubule (i.e., Natori's skinned fiber [Natori, 1954]) may be useful for the investigation of coupling between DHP-R and RyR.

Here, the general properties of Ca^{2+}-induced Ca release are reviewed briefly in order to deepen our understanding of RyR. Because Ca^{2+}-induced Ca release has been studied in detail using skeletal muscle SR, and because the basic idea can be extended to other kinds of cells, the discussion concentrates on skeletal muscles. Endo (1977, 1985) has reviewed Ca^{2+}-induced Ca release in detail.

A. Ca^{2+}-Induced Ca Release

Weber (Weber and Herz, 1968; Weber, 1968) first showed that caffeine caused Ca release from SR vesicles. Her results are summarized as follows:

1. A higher Ca release can be observed with a fraction sedimented with lower centrifugal force, that is, a heavy fraction of SR vesicles (HFSR) in present terms.
2. The action of caffeine was stimulated by Ca^{2+} and high concentrations of ATP and inhibited by Mg^{2+} and procaine.
3. At a lower temperature, more Ca^{2+} was released.
4. The release experiments were more successful with SR from frog skeletal muscle and less successful with SR from rabbit skeletal muscle.

Confirming Weber's results, Ogawa (1970) also observed that the released Ca was reaccumulated by SR, indicating that the Ca release was not due to inhibition of Ca uptake. In those days, the idea that inhibition of Ca uptake would simply lead to Ca release prevailed. Ebashi and colleagues, in contrast, believed that Ca release was a process distinct from Ca uptake (Ogawa et al., 1971). Ogawa and Ebashi (1973, 1976) reported that β,γ-methylene adenosine triphosphate (AMPOPCP), a nonhydrolyzable analog of ATP, can cause Ca release. This indicates that ATP hydrolysis is not required for stimulation of Ca release, unlike Ca uptake by SR. AMPOPCP and caffeine markedly potentiate each other in their Ca-releasing action, indicating that their sites of action are different. Although adenylylimidodiphosphate (AMP-PNP) can be substituted for AMPOPCP, this analog was found to be hydrolyzed very slowly by SR Ca-ATPase. The Ca release by these reagents is significantly affected by temperature (Weber and Herz, 1968; Ogawa, 1970; Ogawa and Ebashi, 1976). The critical temperature for Ca release was found to be around 10°C, which is consistent with the critical temperature for rapid-cooling contracture reported by Sakai (Sakai, 1963, 1965; Lüttgau and Oetliker, 1968).

Endo and colleagues (Endo et al., 1970) examined the Ca-releasing action of caffeine using skinned fibers (fibers split in a relaxing solution containing EGTA and ATP). In the presence of appropriate concentrations of Ca^{2+} and EGTA as Ca^{2+} buffer, 0.2 to 1 m*M* caffeine caused repeated contractions of low frequency. The peak tension of each contraction was usually more than 80% of the maximum tension that could be developed by the same fiber with application of sufficient Ca^{2+}. This suggests that Ca release is a regenerative process in which Ca^{2+} itself causes the Ca release from SR. They demonstrated that Ca^{2+} did in fact release calcium from the SR. The Ca^{2+}-induced Ca release was also independently and simultaneously reported by Ford and Podolsky (1970). To deepen understanding of the properties of Ca^{2+}-induced Ca release, Endo devised procedures to measure Ca release in the absence of Ca uptake. His conclusions were as follows (Endo, 1981, 1985; Endo et al., 1981):

1. The threshold concentration of Ca^{2+} for Ca release was more than 1 μ*M* in the absence of Mg^{2+} or adenine nucleotides. The rate of Ca release increased with an increase in Ca^{2+} concentrations, with a maximum rate at approximately 0.1 m*M* Ca^{2+}. At concentrations higher than 0.1 m*M*, however, the rate decreased with an increase in Ca^{2+} concentration.
2. Adenine nucleotides, including ATP, AMPOPCP, ADP, and AMP, increased the

rate of Ca release without changing the Ca^{2+} dependence described above. The stimulating effect of nucleotides is highly specific for adenine derivatives.

3. Caffeine exerted two kinds of effects: the first was to increase the Ca^{2+} sensitivity of Ca^{2+}-induced Ca release, and the second was to increase the maximum rate of release at the optimum Ca^{2+} concentration. The increase in the maximum rate of release by caffeine is less conspicuous than that by ATP; while the maximal effect of caffeine (50 m*M*) was less than a 100-fold increase, that of ATP was more than 1000-fold.
4. Procaine reduced the rate of Ca release more or less evenly over the whole range of Ca^{2+} concentrations in the presence or absence of caffeine. The increased Ca^{2+} sensitivity by caffeine was not affected by procaine.
5. Mg^{2+}, on the other hand, appears to have two kinds of inhibitory effect: to decrease the Ca^{2+} sensitivity of Ca^{2+}-induced Ca release and to reduce the maximal rate of Ca release at the optimum concentration of Ca^{2+}. Inhibition by Mg^{2+} at higher Ca^{2+} concentrations was weaker than that at lower Ca^{2+} concentrations. The results, however, cannot be completely explained by competition with Mg^{2+} for the Ca^{2+} site of Ca^{2+}-induced Ca release.

These results were confirmed by many investigators. Figure 2 shows the Ca^{2+} dependencies of Ca^{2+}-induced Ca release under various conditions that were obtained by Kurebayashi using skinned fibers from frog iliofibularis muscles. It should be noted that significant Ca release in the absence of Ca^{2+} could be observed in the presence of adenine nucleotides or caffeine and was rather remarkable in the presence of both agents (Figure 2; also see Ogawa et al., 1985; Kurebayashi and Ogawa, 1986).

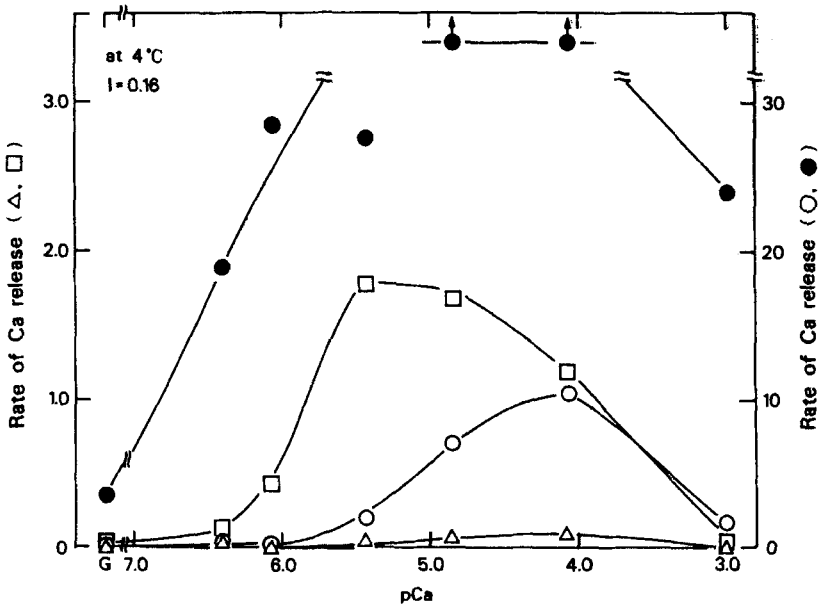

FIGURE 2. Ca^{2+}-induced Ca release in skinned frog iliofibularis muscle fibers. For details of the method, refer to Kurebayashi and Ogawa (1986). △, Ca^{2+} alone (10 m*M* Ca-EGTA buffer); □, 5 m*M* caffeine added; ○, 4 m*M* AMP added; ●, 4 m*M* AMP and 5 m*M* caffeine added. The medium was at an ionic strength of 0.16, the temperature was 4°C, and sarcomere length was 2.8 μm. Closed circles with arrows mean that the rate of Ca release was too fast to be determined, G means that 10 m*M* EGTA was added in the absence of Ca, and the ordinates reflect the relative rate of Ca release. Note that the scale in the ordinate was increased by a factor of ten in the presence of AMP (○, ●).

The SR membrane of frog skeletal muscle has very low permeability to Ca^{2+}. Therefore, SR can hold Ca inside its lumen in a Ca^{2+}-free medium that is made by the addition of EGTA in the absence of ATP. The HFSR vesicles isolated from bullfrog skeletal muscle hold a significant amount of calcium even 3 d after preparation. Therefore, passive loading is frequently unsuccessful, unlike the case with SR vesicles from rabbit skeletal muscle (unpublished observation). Generally speaking, SR membranes from mammalian skeletal muscle are more permeable. The permeability of SR membrane to Ca^{2+} increases from mammalian skeletal muscle to cardiac and smooth muscle to nonmuscle cell. For example, rat liver microsomes discharged Ca^{2+} very quickly when ATP was depleted or when Ca-ATPase activity was inhibited by thapsigargin (Thastrup et al., 1990). Because the permeability at rest increases, the analysis of specific Ca release becomes more difficult.

Activated channels of RyR appear to have large pores because measured flux rates for monovalent cations are reported to be high (Meissner and McKinley, 1976; Kasai and Kometani, 1981; Kasai et al., 1985). This is also reflected in unit conductance for K^+ or Na^+ as large as 750 pS in lipid bilayer experiments (also see Table 2). Magnesium ion can pass through activated channels of RyR (Nagasaki and Kasai, 1980; Ogawa et al., 1981; Somlyo et al., 1981).

Many drugs, for example, thymol, quercetin, halothane, doxorubicin, and others have been reported to cause caffeine-like Ca release (i.e., stimulated by Ca^{2+} and ATP and inhibited by high Mg^{2+}, procaine, and ruthenium red) (see Endo, 1985; Fleischer and Inui, 1989). However, the site of action of these drugs may not always be the same as that of caffeine. For example, the site of action of halothane is not identical with that of caffeine, although they may be close or overlapping in part (Ogawa and Kurebayashi, 1982) (also see Section X.A). All these drugs are exogenous. Recently, an endogenous reagent, cADP-ribose (cADPR), was reported to cause Ca release (Galione, 1992, 1993; Berridge, 1993b). The first report was that the reagent caused Ca release in sea urchin eggs that had been desensitized to IP3 (Clapper et al., 1987; Lee, 1993). It was then reported to stimulate insulin release from pancreatic β-cells through increased $[Ca^{2+}]_i$ (Takasawa et al., 1993). Although it has no effect on skeletal muscle RyR (Mészáros et al., 1993; Morrissette et al., 1993), it is reported to cause Ca release from RyR of cardiac muscle SR (Mészáros et al., 1993). Berridge (1993b), however, had reservations, stating that "a note of caution is required because unpublished observations circulating within the calcium fraternity indicate that cADPR has no effect in certain cells." Even in nonmuscle cells, the effect of cADPR on pancreatic β-cells is still controversial (see, e.g., Islam et al., vs. Takasawa et al., *Science,* **262:** 584–586, 1993).

Channel activity of HFSR or RyR has been examined after incorporation into lipid bilayers. Channel activity is dependent on Ca^{2+} concentrations in the *cis* chamber. (The compartment where biological specimens, such as vesicles to be fused or RyR to be incorporated, are added is referred to as *cis,* which is considered to be equivalent to the cytoplasmic side. The other compartment is referred to as *trans,* which corresponds to the luminal side.) With the increase in Ca^{2+} concentrations in the *cis* chamber, the probability of an open channel is increased. With a fixed Ca^{2+} concentration, ATP or adenine nucleotide derivatives and caffeine increase the probability of an open channel. Procaine, ruthenium red, and Mg^{2+}, in contrast, decrease this probability (Smith et al., 1986, 1988; Meissner and Henderson, 1987; Lai et al., 1988b; Bull et al., 1989; Fill et al., 1990; Murayama and Ogawa, 1992). Ryanodine, up to a concentration of approximately 10 to 20 μ*M*, keeps the channel open but with a decreased conductance (open lock in the subconductance state). At a higher concentration, perhaps 30 to 50 μ*M* or more (in some cases, 10 μ*M*), ryanodine closes the channel (Meissner, 1986; Lattanzio et al., 1987; Meissner et al., 1989). The effect of ryanodine, open lock in the subconductance state, could be observed at a concentration as low as the nanomolar range. With a nanomolar concentration of the reagent, however, it takes so long for the effect to become detectable that such low concentrations of ryanodine are impractical for lipid bilayer experiments (Smith et al., 1988). To shorten the latency period, most investigators use a micromolar or greater concentrations of ryanodine. While the closed state caused by high concentrations of ryanodine was observed in Ca

flux experiments with SR vesicles or in lipid bilayer experiments, no definite evidence for channel closing by ryanodine up to 1 m*M* was obtained in frog skinned fibers (Oyamada et al., 1993) in contrast to the results with rabbit skinned fibers (Su, 1987). We must be alert for the possible effects of contaminating ryanodine congeners when using high concentrations of ryanodine.

The remarkable effect of caffeine is to increase the Ca^{2+} sensitivity of the Ca-release channel, with moderate enhancement in the maximum rate of Ca release. Adenine nucleotides, on the other hand, increase the rate of Ca release with the smallest change in the Ca^{2+} sensitivity (see Figure 2). The results from lipid bilayer experiments that have been reported so far, however, did not seem to differentiate the effects, although a prolonged dwell time in the open state was reported in the presence of ATP in some cases. We sometimes observed results with an isoform of bullfrog skeletal muscle that would indicate that ATP may increase the magnitude of unit conductance as well as open-channel probability. We must be aware that the lipid compositions (PE:PS = 1:1 or PE:PS:PC = 25:15:10) used in lipid bilayer membrane experiments are actually artificial. Whereas much is known about the significant effects of lipids on the activity of Ca-ATPase protein in SR (Martonosi and Beeler, 1983), their effect on channel activities still needs to be examined. Hymel et al. (1988a, b), who used soybean phospholipid for membrane formation, reported a unique response of channels to ryanodine (stepwise change by integral multiples of 3.8 pS in 50 m*M* Ca^{2+}), whereas modulation by Ca^{2+}, ATP, Mg^{2+}, and ruthenium red was qualitatively similar to findings already reported. The presence of multiple conductance states (or subconductance states) was also reported by investigators who adopted the popular Müller-Rudin system (Smith et al., 1988; Lai et al., 1988b), although the minimum value of unit conductance was much higher. Recently, palmitoyl carnitine was shown to activate Ca-release channel activity (El-Hayek et al., 1993). Murayama observed that PS decreased [^{3}H]ryanodine binding activity (personal communication).

B. Ryanodine Binding

It has been shown that ryanodine binds to the open Ca channel and keeps it open. The binding is modulated by many reagents in the same way that they modulate Ca release (Pessah et al., 1985, 1986; Fleischer et al., 1985; Meissner, 1986). However, the relationship between channel activity and ryanodine binding was not clear. Ogawa and Harafuji (1990a) showed that the channel opening is not identical to ryanodine binding, but that the open state is only a requirement for ryanodine binding. The affinity of the channel for ryanodine is decreased with a decrease in temperature with no change in B_{max}, while Q_{10} for the rate of binding was estimated to be about 3 (also see Carroll et al., 1991). This observation leads to the conclusion that ryanodine binding is an endothermic process, whereas many binding reactions are exothermic. This suggests the following possibilities: (1) ryanodine binding may involve at least two independent processes, i.e., channel opening and binding to the open channel, and (2) hydrophobic interaction may be involved in ryanodine binding. Because they are not mutually exclusive, both may participate. As mentioned later, the channel is composed of a tetramer of RyR. Therefore, hydrophobic interaction may be involved in the polymerization of monomers as well as in the interaction between channel protein and ryanodine.

With HFSR from bullfrog skeletal muscle, Ca^{2+} alone did not cause any significant ryanodine binding in a 0.17 *M* KCl (or NaCl) medium (Figure 3A). This may correspond to the very low Ca release activity of SR in skinned fibers (Figure 2). The effects of ATP and caffeine on ryanodine binding are very similar to their effects on Ca release (Figures 2 and 3A). When AMPOPCP was added to the 0.17 *M* KCl medium, Ca^{2+}-dependent ryanodine binding increased without any change in the bell-shaped Ca^{2+}-dependence curve, where Ca^{2+} is stimulatory in low concentrations and inhibitory in high concentrations. The optimum Ca^{2+} concentration in the presence of various concentrations of AMPOPCP was almost the same, about 70 μM Ca^{2+}. The stimulatory Ca^{2+} concentrations that would give half the maximum value was 20 μM at 25°C and approximately

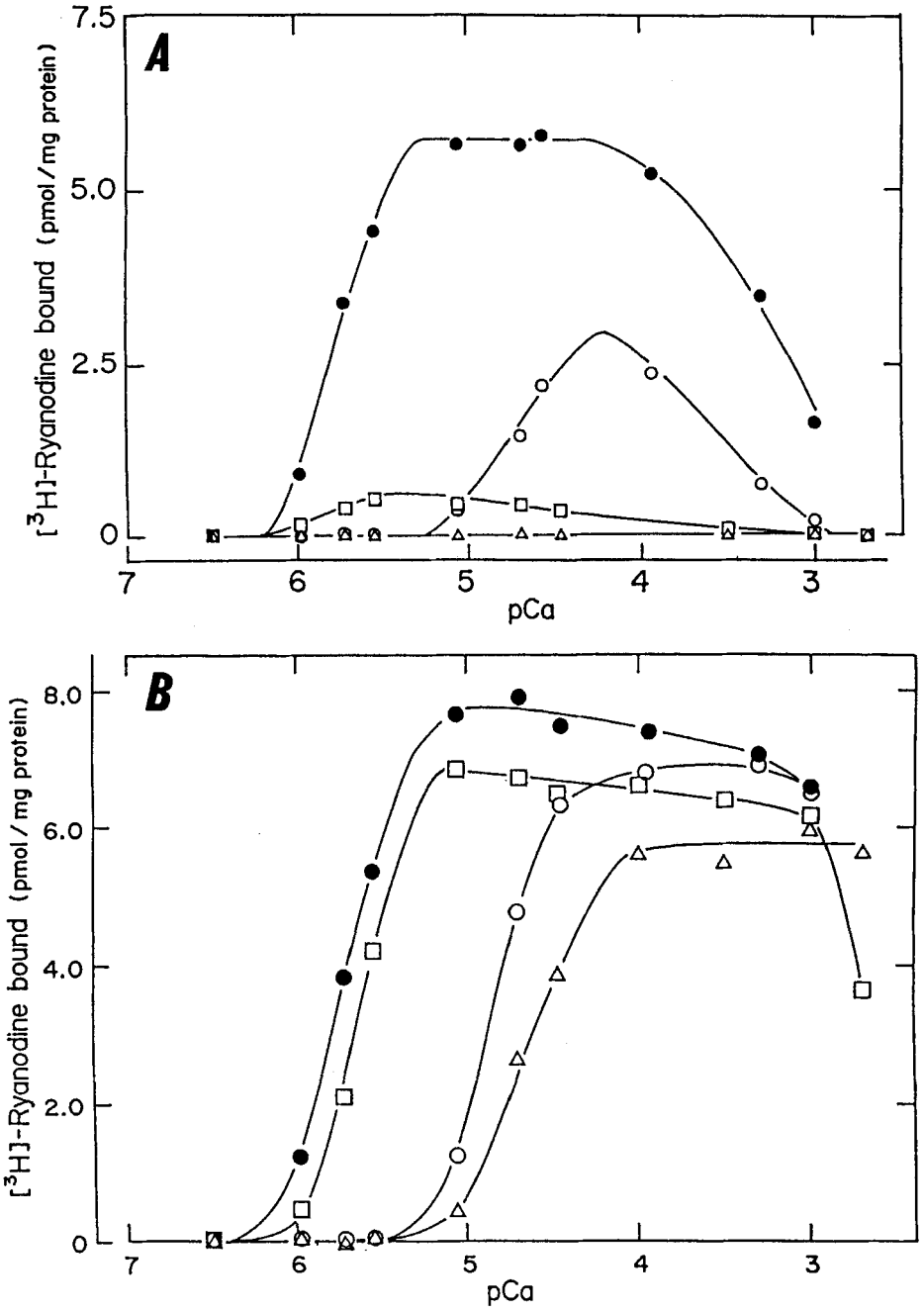

FIGURE 3. [^{3}H]Ryanodine binding to HFSR from bullfrog skeletal muscle at different salt concentrations. The reaction media contained 0.17 *M* KCl (A) and 1 *M* NaCl (B) at 25°C. The results were not different between NaCl or KCl. △, Ca^{2+} alone; □, 10 m*M* caffeine added; ○, 1 m*M* AMPOPCP added; ●, 10 m*M* caffeine and 1 m*M* AMPOPCP added. For details, refer to Ogawa and Harafuji (1990a, b). Compare the results with those in Figure 2, which show Ca^{2+} dependence of Ca^{2+}-induced Ca release at 4°C.

7 μ*M* at 0°C. The effect of AMPOPCP approaches saturation at around 3 m*M*, with half the maximum effect at 0.46 m*M* AMPOPCP, regardless of temperature. The AMPOPCP dependence of [^{3}H]ryanodine binding at 25°C showed a Hill coefficient of about 2.2, whereas the coefficient at

0°C was about 1.0. This indicates that the number of sites for the nucleotide may be two or more per channel. Caffeine, in contrast to AMPOPCP, shifted the Ca^{2+} dependence to a lower Ca^{2+} concentration range in a dose-dependent manner. In addition to this Ca^{2+}-sensitizing effect, caffeine also showed multiple effects: (1) to make Ca^{2+} dependence steeper, (2) to increase the peak amount of ryanodine binding, which is less than that by ATP, (3) to lower the inhibitory Ca^{2+} concentration range, and (4) to increase the inhibition due to a high Ca^{2+} concentration. While both Ca^{2+} sensitization and the increase in the Hill coefficient for Ca^{2+} dependence by caffeine were saturated at 10 m*M*, the increase in the peak amount of ryanodine binding continued to increase up to at least 15 m*M*. Therefore, it is quite likely that the sites for caffeine comprise at least two classes: a site for Ca^{2+} sensitization and steeper Ca^{2+} dependence of Ca^{2+} activation, and a site for increasing the peak amount. Because caffeine and AMPOPCP showed different actions, and because they markedly potentiated each other in ryanodine binding as well as in Ca release, the sites of action are distinct. Ryanodine binding was markedly inhibited by Mg^{2+} and ruthenium red, as already reported (Fleischer and Inui, 1989). Procaine at 10 m*M* moderately reduced the amount of ryanodine bound. Although dantrolene also decreased binding, the degree of inhibition appears to be weaker than that expected from the antagonistic action to muscle contracture in malignant hyperthermia. In contrast, heparin, an inhibitor of the IP3 receptor, slightly increased the amount of ryanodine binding in a dose-dependent manner. In a 1.0 *M* NaCl (or KCl) medium, on the other hand, Ca^{2+}-dependent ryanodine binding was markedly enhanced and reached a point of near saturation, even in the absence of caffeine or AMPOPCP (Figure 3B). The Ca^{2+} dependencies were very similar to those in the 0.17 *M* KCl medium, except for the absence of inhibition up to 1 m*M* Ca^{2+}. Very little inhibition by high Ca^{2+} concentrations is one of marked changes in ryanodine binding in the 1.0 *M* NaCl medium. The effect of AMPOPCP was an increase in the amount of ryanodine binding, but to a much lesser extent than that in 0.17 *M* KCl medium. It is now clear that AMPOPCP causes only weak Ca^{2+} sensitization. The effect of caffeine was Ca^{2+} sensitization. The other effects of caffeine in the 0.17 *M* KCl medium were obscure in the 1 *M* NaCl medium. The effects of caffeine and AMPOPCP were additive but not potentiating, unlike the case in the 0.17 *M* KCl medium. The affinity for ryanodine ($1/K_d$) was increased by AMPOPCP and caffeine, with a slight change in the maximum binding sites (B_{max}) in the 1.0 *M* NaCl medium. This is in contrast to the results in the 0.17 *M* KCl medium, where B_{max} as well as $1/K_d$ was increased in the order of Ca^{2+} + caffeine, Ca^{2+} + AMPOPCP, and Ca^{2+} + AMPOPCP + caffeine.

With HFSR from rabbit skeletal muscle, most [^{3}H]ryanodine-binding experiments were carried out in a high-salt medium. Some investigators (for example, Pessah et al., 1987), however, showed significant Ca^{2+}-stimulated ryanodine binding in a low-salt medium without ATP or caffeine, where negligible amounts of ryanodine binding were obtained with bullfrog HFSR. While stimulation by a high concentration of salt was assumed to be due to ionic strength (Imagawa et al., 1987, 1989; Michalak et al., 1988; Meissner and El-Hashem, 1992), Ogawa and Harafuji (1990b) concluded that the stimulating effect of a high salt concentration was due to osmolality, but not ionic strength, because a similar amount of ryanodine binding was obtained when the osmolality of the solution was adjusted with sucrose instead of salt. This is also consistent with the inference that hydrophobic interaction may be important in ryanodine binding. While Kasai et al. (1992) reported that KCl, but not glucose or sucrose, stimulated the flux of neutral molecules as well as Ca flux through SR vesicles from rabbit skeletal muscle, Kurebayashi (personal communication) observed a stimulatory effect of 1 *M* glucose on Ca release from SR in frog skinned skeletal muscle fibers. In lipid bilayer experiments for channel activity measurements, Herrmann-Frank et al. (1991) reported that the unit conductance of K^+-conducting channel of RyR from aorta was dependent on the concentration of KCl: 367, 700 (half the maximum), and 1400 pS (the maximum), in the presence of 250, 400, and 1000 m*M* KCl, respectively. Similar findings were obtained with RyR from rabbit skeletal muscle (Smith et al., 1988; Meissner and El-Hashem, 1992). In some cases, 10% glycerol, in

addition to 0.5 *M* NaCl or KCl, was used in spite of the difficulty in balancing the hydrostatic pressure on the lipid membrane between *cis* and *trans* compartments (Lai et al., 1988b). These may be the same reason that high osmolality is favorable for the opening of channels (see page 246).

The kinetics of ryanodine binding apparently follow pseudo-first order kinetics (Pessah et al., 1987; Lai et al., 1988b; Ogawa and Harafuji, 1990a, b; Hasselbach and Migala, 1992). The rate constants at 25°C were 0.23 to 0.55 h^{-1} in the 0.17 *M* KCl medium and 1.2 to 1.9 h^{-1} in the 1 *M* NaCl medium, depending on the presence of AMPOPCP and caffeine. Ryanodine binding is very slow in the physiologic ionic conditions, which is consistent with the slow onset of action of ryanodine. The dissociation of bound ryanodine, however, cannot be explained if the binding is a simple second-order reaction (Ogawa and Harafuji, 1990b; Hasselbach and Migala, 1992). Especially in the case where bound [^{3}H]ryanodine was to be chased by 10 μM or more nonradioactive ryanodine, dissociation slowed remarkably (Lai et al., 1988b; Ogawa and Harafuji, 1990b). These results remain to be clarified.

V. GENERAL CHARACTERISTICS OF PURIFIED RYANODINE RECEPTORS

The results mentioned above have also been observed with RyRs purified from rabbit skeletal muscle and from other sources, including partially purified ones, as summarized in Table 2.

The molecular weight of RyR purified from rabbit skeletal muscle was estimated to be 360 or 400 to 450 kDa from the mobility on SDS-PAGE using the Laemmli system. It is calculated to be approximately 565 kDa from the amino acid sequence deduced from its cDNA (Takeshima et al., 1989; Zorzato et al., 1990). This discrepancy is partly due to the lack of appropriate markers of molecular weights and partly due to the system of electrophoresis. Murayama and Ogawa (1992) estimated the molecular weight to be 590 kDa, which is in reasonable agreement with the calculated value, using the Weber-Osborn system and molecular markers of polymerized phosphorylase. With the Laemmli system, the mobility of RyR was far from the linear relationship of the logarithm of the molecular weight. The monomer was found to have a sedimentation coefficient of 9S and was inactive in [^{3}H]ryanodine binding (Lai et al., 1989). The active fraction had a sedimentation coefficient of about 30 S and showed a single band of the same mobility as that of the monomer on SDS-PAGE (Lai et al., 1989). These results led to the conclusion that the active form of RyR is a tetramer in ryanodine binding as well as in channel activity. This is in marked contrast to the IP3 receptor, of which a monomer shows IP3 binding activity, and a tetramer forms the Ca channel (Mignery and Sudhof, 1990; Mignery et al., 1990; Miyawaki et al., 1991). The mobilities of RyRs from cardiac muscle and brain on SDS-PAGE were similar to each other and higher than that of rabbit skeletal muscle, indicating smaller estimated molecular weights. [^{3}H]ryanodine-binding activities of these preparations were detected in the zone of equivalent density after centrifugation on a linear sucrose-density gradient. The negative staining image of RyR from rabbit skeletal muscle was in the shape of a quatrefoil on electron microscopic examination with dimensions of $26 \times 26 \times 8$ to $21 \times 21 \times 12$ nm (Inui et al., 1987a, b; Lai et al., 1988b). This is consistent with the morphology and dimensions of the foot (Ferguson et al., 1984; Block et al., 1988; Saito et al., 1988; Radermacher et al., 1992). Assuming the density of densely packed protein to be 1.37 g/cm^3, the molecular weight of the quatrefoil (i.e., foot) is calculated to be approximately 4.4×10^6, which is much higher than the molecular weight of the tetramer, 2.3×10^6. This suggests that the foot molecule may have a cavity, or that the foot protein may be loosely packed. Wagenknecht et al. (1989) showed that the foot has a pinwheel appearance with a complex path or cavity running through it (also see Radermacher et al., 1992). On the other hand, Anderson et al. (1989) reported that a lower proportion of cardiac RyR showed a complete characteristic quatrefoil structure. The dimensions of the cardiac quatrefoil were slightly larger than that from skeletal muscle, in marked contrast to its greater mobility on SDS-PAGE (Anderson et al., 1989). The RyR from brain seems to be similar to cardiac RyR in size and shape. Although the B_{max} of ryanodine binding for any RyR is 1 mol of ryanodine bound per 1 mol of tetramer (i.e., 4 mol of RyR monomer) the actual

TABLE 2
Characteristics of Purified Ryanodine Receptors

	Skeletal muscle (Sk M)			Cardiac (ventricle)	Brain	Aorta
		Bullfrog[12]		Dog,[2,7,8]	Rabbit,[9]	Dog or pig[10]
	Rabbit [1–6]	α-RyR	β-RyR	sheep[11]	bovine[13]	
Molecular weight						
cDNA[a]	565 k	571 k	553 k		565 k (Ryr2), 552 k (Ryr3)	
SDS-PAGE	360 k, 400–450 k, 590 k[12]	690 k	570 k	340 k, <400 k	<400 k	
Active form	Tetramer	Tetramer		Tetramer	Tetramer	Tetramer
Sedimentation coefficient	~30 S	~30 S		~30 S	~30 S	~30 S
Negative-staining image	Quatrefoil, 26 × 26 × 8 –21 × 21 × 12 nm	Quatrefoil, similar to rabbit Sk M		Quatrefoil, 22 × 22 nm	Quatrefoil, 28 × 28– 35 × 37 nm	Quatrefoil
Ryanodine binding						
K_d (n*M*)	4–7	2–5	2–5	3–6	3	
B_{max} (mol/mol tetramer)	1	1	1	1	1	
Channel activity main conductance (pS) in						
50–100 m*M* Ca^{2+}	3.8 × N[b], ~110			70, 80[11]	140[13]	110
250 m*M* KCl	360–750			730[11]	107,[9] 800[13]	360
500 m*M* NaCl	600	200	200	550		
Regulation						
Activation by μ*M* Ca^{2+}	Yes	Yes	Yes	Yes	Yes	Yes
Activation by ATP	Yes	Yes	Yes	Yes	Yes	Yes
Activation by caffeine	Yes	Yes	Yes	Yes	Yes	Yes
Inhibition by Mg^{2+}	Yes	Yes	Yes	Yes	Yes	Yes
Inhibition by ruthenium red	Yes	Yes	Yes	Yes	Yes	Yes
Modification by Ry						
Long-term open, subconductance state	Yes	Yes	Yes	Yes	Yes[13] or no[c]	No[d]
Fully closed state	Yes (≥10 μ*M*)			Yes	Yes (≥4 μ*M*)	Yes (≥500 μ*M*)

Note: [1]Inui et al. (1987a), [2]Inui et al. (1987b), [3]Imagawa et al. (1987), [4]Lai et al. (1988), [5]Smith et al. (1988), [6]Hymel et al. (1988), [7]Anderson et al. (1989), [8]Imagawa et al. (1989), [9]McPherson et al. (1991), [10]Herrmann-Frank et al. (1991), partial purification, [11]Lindsay and Williams (1991), [12]Murayama and Ogawa (1992), [13]Lai et al. (1992a).

[a] See Table 4.

[b] N stands for an integer. The system, including the composition of lipids, differs from that of Müller-Rudin, which was adopted in the other reports.

[c] According to McPherson et al. (1991), caffeine is absolute necessary to open the channel of which the unit conductance was one-fourth of that of skeletal muscle and 4-fold that of IP3 receptor. No subconductance states, as Smith et al. (1988) described on skeletal muscle RyR, were observed. Ryanodine did not modify the channel activity at 10–100 n*M*, and it completely closed the channel at 4 μ*M*.

[d] Hermann-Frank et al. (1991) reported that they could not observe a long-term open, subconducting state in spite of extensive efforts in lipid bilayer experiments. On the other hand, Wagner-Mann et al. (1992) reported that 10 μ*M* or higher ryanodine caused a significant increase in $[Ca^{2+}]_i$ with bovine and porcine coronary arteries (for details, see the text).

results were reported to be 220 to 490 pmol/mg protein. Because the molecular weights for monomer calculated from amino acid sequences deduced from cDNAs were 550 to 570 k, the expected values are 440 to 450 pmol/mg protein. The reasons for the varied results can be considered as follows. First is the difficulty in determining protein concentration. On the basis of its high sensitivity and freedom from interference from such coexisting reagents as dithiothreitol and detergents, a dye-binding method is now commonly used for the protein concentration determi-

nation of RyR. The amount of protein is calibrated with bovine serum albumin as a standard. Whether the absorption coefficient for a unit weight is identical to the standard, however, is not known. Second is the difficulty of completely trapping bound [^{3}H]ryanodine, which must be separated from free [^{3}H]ryanodine. When Whatman GF/B treated with polyethyleneimine was used, the results varied depending on the amount of protein charged on the filter, suggesting that some protein may flow through the filter. GF/C often gave rise to lower results than GF/B. Last, but not least, is inactivation of the preparation. When an RyR preparation was subjected to freezing and thawing, B_{max} decreased with no change in K_d (Murayama and Ogawa, 1992). From our experience, the concentrating procedure with a Centriprep-30 may deteriorate RyR, and a decrease in B_{max} can be obtained without a change in K_d. Campbell also reported that the use of digitonin, instead of CHAPS, gave rise to a reduced B_{max} without an effect on K_d (Imagawa et al., 1987; Smith et al., 1988). This may also be the case with cardiac and brain RyR, which sometimes gave lower B_{max} values and appear to be more labile, as reported (Anderson et al., 1989; McPherson et al., 1991; McPherson and Campbell, 1993b).

It was reported that the sites for ryanodine binding were heterogeneous and composed of two or more classes. Some workers reported that SR vesicles from cardiac muscle characteristically had two classes of binding sites, in contrast to a single class for SR vesicles from skeletal muscle (Pessah et al., 1985; Inui et al., 1987b; Michalak et al., 1988). Some claimed that SR vesicles from skeletal muscle also had two classes of binding sites, implying separate sites for open lock and for channel closing (Lai et al., 1989; McGrew et al., 1989). Imagawa et al. (1989) reported that microsomes and purified RyR from canine cardiac ventricle muscles showed only a single class of binding sites (also see Anderson et al., 1989; Lindsay and Williams, 1991). The heterogeneous binding sites may arise from impurities of the biologic specimen. Microsomes from cardiac muscle may contain more membranes of organelles other than SR (e.g., cytoplasmic membrane and mitochondria) than microsomes from skeletal muscle. Moreover, the SDS-PAGE pattern of microsomes shows that many specimens are contaminated to a surprising extent by actomyosin. Impurities in the reagent ryanodine can also be a cause (see Lai et al., 1989; Carroll et al., 1991). As mentioned above, Ogawa and Harafuji (1990a) notice that the Scatchard plot for [^{3}H]ryanodine binding was curvilinear when the analytical amount of ryanodine was supplemented with impure ryanodine, while the plot was linear when pure [^{3}H]ryanodine alone was used. Some papers reported that the K_d for the high-affinity site was in the nanomolar range, while that for the low-affinity site was in the micromolar range or higher (McGrew et al., 1989; Lai et al., 1989). The authors of those papers, on the other hand, reported that the background was obtained from radioactivity in the presence of cold ryanodine of the order of 1 to 10 μM. Under these conditions, significant counts of radioactivity above the background are very difficult or almost impossible to obtain. Therefore, K_ds in the micromolar range will be subject to a great deal of variation. We do not necessarily deny the possibility of the existence of a low-affinity site with a K_d in the micromolar range or higher, as suggested by the findings of channel closing or a marked retardation of the dissociation of bound ryanodine by a high concentration of ryanodine. However, the low-affinity site cannot be determined by conventional binding experiments.

Channel activity in lipid bilayer experiments, especially the values of unit conductance, vary among investigators, as shown in Table 2. This may be due to differences in experimental conditions. Even though the conditions seem to be similar, the results may differ by a factor of two or more. The variation is more marked with labile preparations, such as RyRs from cardiac muscle, brain, and smooth muscles. This is also the case with ryanodine. While Lai et al. (1992a) observed the long-term open state at a decreased conductance with RyR from bovine brain, as was the case with skeletal muscle RyR (Rousseau et al., 1987; Smith et al., 1988), McPherson et al. (1991) showed only the fully closed state in the presence of 4 μM ryanodine and no modification of channel activity by 0.1 μM ryanodine. Herrmann-Frank et al. (1991) also reported no open lock, subconductance state with microsomes from aorta. Only the closed state was observed at 500 μM

ryanodine or higher. These results, however, are difficult to reconcile with what is known of ryanodine binding and changes of $[Ca^{2+}]_i$ caused by ryanodine.

The two isoforms α-RyR and β-RyR* were purified from bullfrog skeletal muscle (Murayama and Ogawa, 1992). From mobilities on disc SDS-PAGE with the Weber-Osborn system, molecular weights were estimated to be 690 and 570 kDa for α- and β-RyR, respectively. Recently, their deduced amino acid sequences were determined (Oyamada et al., 1994). The total numbers of amino acid residues and calculated molecular weights were 5037 (or 5031 with a deletion) (about 571 kDa) and 4868 (about 553 kDa) for α-and β-RyR, respectively. α-RyR shows atypical mobility on SDS-PAGE. This may be consistent with findings that the α-isoforms from frog, toad, fish, and chicken skeletal muscle varied in their mobilities, in contrast to the similar mobilities for β-RyRs. The atypical mobility of the α-isoform from bullfrog skeletal muscle may be due to characteristics intrinsic to the amino acid composition or possible posttranslational modifications, such as glycosylation. Because the estimate for rabbit skeletal muscle RyR was reasonable, the latter possibility is more likely. The other characteristics were similar to those mentioned above, except for their distinct Ca^{2+} dependencies in ryanodine binding. Interestingly, β-RyR was approximately 20 times more sensitive to Ca^{2+} than α-RyR in the presence of 1.0 m*M* AMPOPCP and 10 m*M* caffeine in a 1.0 *M* NaCl medium. While ryanodine binding to α-RyR was negligible in a Ca^{2+}-free medium that was made by the addition of 9 m*M* EGTA, ryanodine binding to β-RyR was about one fifth of the maximum under the same conditions. Half the maximum binding was obtained at pCa 5.8 ($(pCa)_{1/2} = 5.8$) with α-RyR, in contrast to pCa 7.1 ($(pCa)_{1/2} = 7.1$) with β-RyR (Murayama and Ogawa, 1992). SR vesicles, in contrast, showed $(pCa)_{1/2}$ of 5.60, which was similar to that of α-RyR under similar conditions. When SR vesicles were solubilized by CHAPS and phospholipids, the value of $(pCa)_{1/2}$ increased to 6.39, which can be expected from $(pCa)_{1/2}$ values for α- and β-RyRs with the assumption that the two isoforms coexist in equal amounts in SR (Murayama and Ogawa, 1993). In a 0.17 *M* NaCl medium, however, solubilized SR did not show increased Ca^{2+} sensitivity ($(pCa)_{1/2}$). The SR vesicles showed similar Ca^{2+} sensitivities in 0.17 and 1 *M* NaCl media (Ogawa and Harafuji, 1990a, b; also see Figure 3). In a 0.17 *M* NaCl medium, in contrast, both α- and β-RyRs showed Ca^{2+} dependencies similar to that for SR vesicles. It was in the 1.0 *M* NaCl medium that β-RyR had the very much increased Ca^{2+} sensitivity (Murayama and Ogawa, in preparation). The increase in Ca^{2+} sensitivity was caused by a combination of 1 *M* NaCl and CHAPS in the presence of phospholipids. This effect was additive to the Ca^{2+}-sensitizing effect of caffeine, indicating that the underlying mechanism was distinct from that of caffeine. This Ca^{2+} sensitization was also observed in a 1 *M* KCl medium, but not in a 1 *M* Na methanesulfonate or Na propionate medium (Murayama and Ogawa, in preparation). Therefore, the Ca^{2+}-sensitizing effect of NaCl and KCl cannot be ascribed simply to ionic strength. It may be related to the redistribution of ions and accompanying water molecules between RyR molecules and their surrounding medium. Furthermore, two other effects were noted in a medium with a high salt concentration (also see Figures 3A and B). The first effect is the enhancement of ryanodine binding at the optimal Ca^{2+} concentration, and the second is disinhibition due to high Ca^{2+} concentrations. Glucose of an equivalent osmolality could be substituted for NaCl or KCl in terms of enhancement of ryanodine binding at the optimal Ca^{2+} concentration, but only partly in terms of the disinhibition in the presence of very high Ca^{2+} concentrations (Murayama and Ogawa, in preparation). These findings suggest that many factors, including osmolality of the medium, can induce conformation change in ryanodine receptor molecules, which may in turn result in enhanced ryanodine-binding activity, channel opening, and Ca release.

Inhibition by Mg^{2+}, procaine, ruthenium red, and high Ca^{2+} became very much weaker or neg-

* Of two isoforms in nonmammalian vertebrate skeletal muscles, the isoform of lower mobility on SDS-PAGE is referred to as α-RyR, and that of higher mobility as β-RyR. This nomenclature is consistent with that used in references already published.

ligible in a 1.0 *M* NaCl medium (also see Murayama and Ogawa, 1992). β-RyR in a 1.0 *M* NaCl medium was more resistant to inhibition by Mg^{2+} and ruthenium red than was α-RyR. For example, 10 μ*M* ruthenium red decreased the ryanodine-binding activity of β-RyR to only 65% of control in the 1 *M* NaCl medium, whereas α-RyR binding activity was reduced to 19%. In the 0.17 *M* NaCl medium, negligible binding to α- or β-RyR was observed in the presence of 10 μ*M* ruthenium red (Murayama and Ogawa, in preparation). This insensitivity to inhibitory reagents in a high-salt medium was also observed with mammalian skeletal and cardiac muscle RyRs (Pessah et al., 1986; Imagawa et al., 1987), which was erroneously ascribed to solubilization. In contrast, the inhibitory effect of dantrolene, although weak, was unchanged or increased in the 1.0 *M* NaCl medium. Dantrolene might exert an inhibitory effect on intensely opened channels; however, this remains to be verified.

RyR or SR vesicles of cardiac muscle are reported to differ from those of skeletal muscles in the following respects: higher sensitivity to Ca^{2+}, lower proportion of complete quatrefoil morphology, less stimulation by high salt concentration or ATP, and less sensitivity to inhibition by high Ca^{2+}, Mg^{2+}, and ruthenium red (Lai et al., 1988a, 1992b; Michalak et al., 1988; Anderson et al., 1989; Imagawa et al., 1989). Most of these findings could be reproduced with skeletal muscle RyR solubilized in a 1 *M* NaCl medium, as mentioned above. These findings might be characteristic for RyRs in a unique conformation, taking into consideration that cardiac quatrefoils looked swollen (Anderson et al., 1989). Therefore, the alleged characteristics for cardiac RyR need to be reexamined as to whether they are intrinsic. While some investigators reported a higher Ca^{2+} sensitivity of cardiac SR than of skeletal muscle SR (Rousseau et al., 1986; Meissner and Henderson, 1987; Lai et al., 1992b), others reported near equality or a reverse relationship (Pessah et al., 1985; Michalak et al., 1988; Imagawa et al., 1989). These comparisons are made between dog or rat ventricle muscle and rabbit skeletal muscle. Comparisons of the Ca^{2+} sensitivity of RyR between cardiac muscle and skeletal muscle must be performed with material from the same species of animals. Otherwise, we cannot determine whether the difference, if any, is due to organ specificity or merely to species differences. Murayama observed no difference in the Ca^{2+} dependence of [^{3}H]ryanodine binding between skeletal and cardiac muscles of rabbit (personal communication).

VI. MOLECULAR STRUCTURE OF RYANODINE RECEPTORS

Since the first report by Takeshima et al. (1989) of the amino acid sequence of rabbit skeletal muscle RyR that was deduced from its cDNA sequence, amino acid sequences of RyRs of various origin have been deduced from their cloned cDNA sequences: human skeletal muscle (Zorzato et al., 1990), which was recently partially revised (Zhang et al., 1993), rabbit cardiac muscle (Otsu et al., 1990; Nakai et al., 1990), pig skeletal muscle (normal and MHS) (Fujii et al., 1991), and rabbit brain (Hakamata et al., 1992). The gene for cardiac muscle RyR (*ryr2*) was found to be distinct from that for skeletal muscle RyR (*ryr1*) (Otsu et al., 1990) (see Table 3). The same mRNA for skeletal muscle RyR is expressed not only in white fast-twitch skeletal muscle, but also in red slow-twitch skeletal muscle (Otsu et al., 1990). On the other hand, the mRNA for cardiac muscle RyR is detected in brain as well as in cardiac muscle (Otsu et al., 1990; Hakamata et al., 1992). The RNA that is probed by cDNA for brain-specific RyR (Ryr3) was detected not only in brain, but also in smooth muscle (Hakamata et al., 1992). Hakamata et al. (1992), however, concluded that the mRNA in smooth muscle may be different from that in brain because of potential splicing. Mink lung epithelial cells treated with transforming growth factor-β (TGF-β) expressed an RyR encoded by a gene, β4; the receptor was similar to muscle RyRs, but differed by showing no response to caffeine. A portion of the 3′-terminal region of the cDNA for the RyR isoform was sequenced (Giannini et al., 1992). The deduced amino acid sequence has very high homology to the C-terminal fifth of the brain-specific RyR described by Hakamata et al. (1992). Recently, the human ryanodine receptor gene (*ryr3*) has been localized to 15q14-q15 by *in situ* hybridization (Sorrentino et al., 1993). Although *ryr3*-origin isoforms occur in various cells, their charac-

TABLE 3
Classification of Ryanodine Receptor Genes and the Main Sites of Their Expression

Gene	Locus on human chromosome	Expression sites
ryr1	19q13.1[1,4,5 b]	Skeletal muscle (white fast twitch and red slow twitch),[a] Purkinje cell in cerebellum
ryr2	1q42.1–q43[1,2,6]	Ventricle muscle (not detectable in bullfrog), brain (ubiquitous, but rich in cerebellum), endothelial cells[7]
ryr3	15q14–q15[2,3]	Brain (specific regions, such as corpus striatum, thalamus, and hippocampus), skeletal muscle (as β-RyR of nonmammalian vertebrate),[a] smooth muscles, nonmuscle cells, including epithelial cells

[a] In nonmammalian vertebrate skeletal muscle, two isoforms of ryanodine receptor (α-RyR and β-RyR) are detected, while only one isoform is present in mammalian skeletal muscle. Amino acid sequences of bullfrog RyRs deduced from cDNAs revealed that α-RyR is most homologous to the rabbit skeletal muscle-type isoform, and β-RyR to rabbit brain-specific isoform (see Table 5). This is likely to be the case with avian and piscine skeletal muscles, although it has not been proved rigorously.

[b] [1]Otsu et al. (1990), [2]Hakamata et al. (1992), [3]Sorrentino et al. (1993), [4]McCarthy et al. (1990), [5]MacKenzie et al. (1990), [6]Otsu et al. (1993), [7]Lesh et al. (1993).

teristics may vary, probably because of potential multiple splicing.

The three isoforms of RyRs from skeletal muscle, cardiac muscle, and brain should be compared in the same species of animals. Otherwise, it is unclear whether the difference is due to organ or species specificity. So far, only cDNAs from rabbit are suitable for this purpose, although closer examination revealed individual specificity. Because the characterizations of purified RyR proteins were carried out with samples from different animal species, as shown in Table 2, comparison here is limited to the deduced amino acid sequences. The results are summarized in Table 4. Generally speaking, the homology between any two of the three isoforms from different genes is 66 to 70%. The C-terminal tenth of the three isoforms is well conserved. Although differences are scattered throughout the sequences, they are concentrated in about 100 residues after residue 1300 and in about 400 residues just before the C-terminal tenth. On the other hand, RyRs of mammalian skeletal muscles are well conserved: identity among human, rabbit, and pigs is more than 95%. However, we must keep in mind that the difference between normal and malignant hyperthermia-susceptible (MHS) pig is only one of a total of 5035 residues (mutation of R615C) (Fujii et al., 1991).

Recently, cDNA sequences for α- and β-RyR from bullfrog skeletal muscle were determined and their amino acid sequences deduced. The identity in the deduced amino acid sequences between α- and β-RyRs is 69%. α-RyR shows the highest identity (80%) to rabbit skeletal muscle RyR, while β-RyR has 86% identity to rabbit brain RyR. These identity values were significantly higher than those among isoforms of different gene origins (Table 5). The general characteristics of the primary sequences of α- and β-RyRs are comparable to those between rabbit skeletal muscle and brain RyRs, as shown in Table 4.

Although information about cDNA sequences and the deduced amino acid sequences of RyRs is accumulating, we know little about the relationship of structure and function. While a sequence of 400 amino acids (Mignery and Sudhof, 1990) or 650 amino acids (Miyawaki et al., 1991) at the N terminus of the IP3 receptor is known to be necessary for IP3 binding, the ryanodine binding site was just determined to be the 76-kDa C-terminal tryptic fragment of rabbit skeletal

TABLE 4
Comparisons among Amino Acid Sequences of RyR Isoforms in Rabbit

	Skeletal muscle (S)	Heart (H)	Brain (B)
Gene	*ryr1*	*ryr2*	*ryr3*
Number of residues	5037[1 a]	4968 or 4976[3 b]	4872[5]
	5032[2 c]	4969[4]	
Calculated MW	564–565 kDa	565 kDa	552 kDa
Identity		66% (S/H)	67% (S/B)
			70% (H/B)
Regions of marked difference[d]	1302–1408	1315–1408	Absence of about 100 residues after the residue 1301
	4249–4626	4210–4562	4100–4400
One tenth C-terminal		Extremely homologous among the three	

[a] [1]Takeshima et al. (1989), [2]Zorzato et al. (1990), [3]Nakai et al. (1990), [4]Otsu et al. (1990), [5]Hakamata et al. (1992).

[b] Nakai et al. reported that some rabbits have the version deleted at 3716–3723 (VTGSQRSK). The following replacements of amino acids in their sequence were observed when compared with the sequence determined by Otsu et al. (1990): 74L → S, 1806G → A, 1885V → M, 2267V → deleted, 3501A → Q (3500), 4496L → F (4495). Hakamata et al. (1992) adopted the shorter sequence for comparison among the three isoforms.

[c] Results of Zorzato et al. (1990) differ from those of Takeshima et al. in the following respects: deletion of 3481–3485 (AGDAQ) and 2015E → D.

[d] Differences among the three RyR isoforms are observed over the entire sequence.

TABLE 5
Identities (%) between RyRs of Various Origins

	Frog Sk M		Rabbit		
	α-RyR	β-RyR	Sk M (Ryr1)	Heart (Ryr2)	Brain (Ryr3)
Frog Sk M					
α-RyR	—	69	80	68	68
β-RyR		—	67	70	86
Rabbit					
Sk M (Ryr1)			—	65	67
Heart (Ryr2)				—	70
Brain (Ryr3)					—

Note: RyrN, a product of the *ryrN* gene; Sk M, skeletal muscle.

muscle RyR (Witcher et al., 1994). The C-terminal tenth of any RyR is well conserved and contains four highly hydrophobic segments. Takeshima et al. (1989) believed them to be potential transmembrane domains (M1–M4) in the deduced sequence of rabbit skeletal muscle RyR. These domains, which show very high homology to counterparts of the IP3 receptor (Furuichi et al., 1989; Ferris and Snyder, 1992), are thought to be involved in tetramer formation by hydrophobic interaction as well as in channel formation. Zorzato et al. (1990), on the other hand, proposed eight additional putative transmembrane domains in the C-terminal fifth in addition to the M1–M4 of Takeshima et al. (1989) on the basis of criteria that MacLennan and collaborators adopted for transmembrane sequences in the elucidation of the structure of the Ca-ATPase of SR (MacLennan,

1990). Hydropathy indices for the alleged transmembrane segments of other channel proteins are not always as high as those for M1–M4. Some segments lining channels, (e.g., S4 of the α_1-subunit of DHP-R (Tanabe et al., 1987; Mikami et al., 1989)) show lower hydrophobicity. Because both the N and C termini are reasonably assumed to be on the cytoplasmic side, the number of transmembrane domains would be even. We can certainly find more than four candidates for them. The binding sites for modulators of Ca^{2+}-induced Ca release (e.g., Ca^{2+}, ATP, and Mg^{2+}) have also not yet been fixed. Takeshima et al. (1989) proposed residues 3614-4457 as plausible modulator binding sites, whereas Otsu et al. (1990) proposed residues 2619-3016 for them. Numa (Nakai et al., 1990; Hakamata et al., 1992) then appeared to move closer to the opinion of MacLennan (Otsu et al., 1990), and MacLennan (Chen et al., 1992, 1993b) vice versa. An EF-hand structure for Ca binding (Fuchs, 1991; Bairoch, 1992) cannot be detected in any of the deduced amino acid sequences of RyRs. However, Takeshima et al. (1989) reported that amino acid sequences 4253-4264, 4407-4416, and 4489-4499 in rabbit skeletal muscle RyR resemble EF-hand structures. Nakai et al. (1990) assigned EF-handlike sequences to residues 1336-1347 and 2010-2021 in cardiac muscle RyR, although they were not conserved in the skeletal muscle counterpart. Hakamata et al. (1992) found an EF-handlike sequence in residues 3934-3945 of rabbit brain RyR that is well conserved not only in skeletal muscle, but also in cardiac muscle. Otsu et al. (1990) assigned potential Ca-binding sites of the EF type to residues 1873-1924 in rabbit cardiac muscle RyR that is not conserved in the skeletal muscle counterpart. Chen et al. (1993b) proposed that Ca^{2+}-activation sites would be residues 4485-4494 because an antibody against 13C2P1 (4485-4494) of rabbit skeletal muscle RyR inhibited Ca-channel activity, whereas an antibody against 13C2 (4478-4512) increased Ca-channel activity (Chen et al., 1992, 1993b). In contrast, the mutations for MHS in pig and human, where Ca^{2+} sensitivity for Ca release is increased, indicate that the site would be close to the N terminus (see Section X.A). The site for Ca^{2+} regulation remains to be fixed. It should be pointed out that Ca-ATPase of SR, which shows Ca^{2+} sensitivity similar to or higher than that of RyR, does not have an EF-hand structure either (MacLennan, 1990). In the search for potential ATP-binding sites, the sequences [AG]X(4)GK[ST], GXGXXG, or GX(4)G were adopted as consensus sequences. Although both Numa et al. and MacLennan et al. proposed some candidates, they were not satisfactory. These sequences are only part of the consensus sequence for ribose and phosphate group-binding sites of nucleotides (Wierenga and Hol, 1983; Bourne et al., 1991). They are not specific to adenine nucleotides, whereas the potentiating effect on Ca release is specific to adenosine derivatives (Endo et al., 1981). Recently, binding sites for the purine ring of ATP were reported in chaperonin proteins GroEL and GroES, which have an affinity of submillimolar concentration (Martin et al., 1993). The tyrosine residue was found to be close to the purine ring of the nucleotide bound to GroES. Consensus sequences inferred from the results are Y[GAST][VG][KTQSN] for GroES and GY[ND][AT][AMLTS] for GroEL, and they have been searched for in RyR sequences. Whereas GY[ND][AT][AMLTS] for GroEL were not found in any RyRs, Y[GAST][VG][KTQSN] were detected. The finding that ATP bound to the site for GroES was not hydrolyzed is consistent with the role of adenine nucleotide moieties in stimulating Ca release. The results are shown in Figure 4A. In rabbit skeletal muscle RyR, Y1081 and Y3937 are the putative sites. The consensus sequence is well conserved in the other RyRs that have had sequences determined: segment A7 has a higher degree of coincidence than segment A3. The putative segment A3 would be consistent with the result reported by Iino et al. (1992), which showed that the removal of about 1000 residues from the N terminus of rabbit skeletal muscle RyR enhanced the rate of Ca release without changing the Ca^{2+} sensitivity of Ca release, which is very similar to the effect of adenine nucleotide. We would like to propose these sites as putative adenine nucleotide-binding sites.

Furthermore, two interesting motifs were revealed: leucine zipper and RGD motifs, that are not detected in all IP3 receptors. The leucine zipper motif consists of a periodic repetition of leucine residues at every seventh position over a distance of eight helical turns. The segments containing these periodic leucine residues are pro-

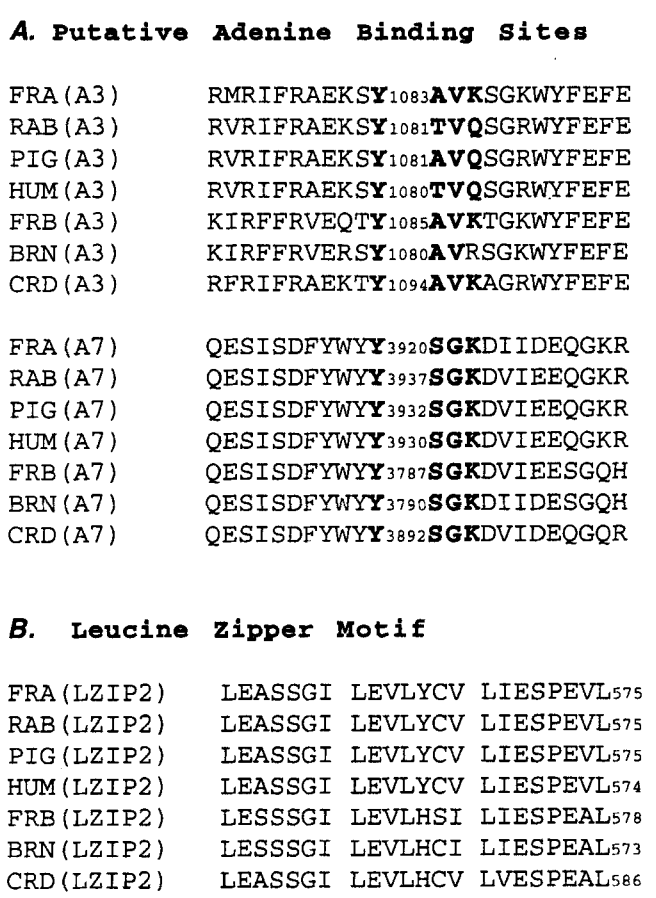

FIGURE 4. Some interesting motifs in RyRs. (A) Putative adenine binding sites; (B) leucine zipper motif. FRA, FRB, α-RyR, and β-RyR of bullfrog skeletal muscle, respectively; RAB, rabbit skeletal muscle RyR; HUM, PIG, human and pig skeletal muscle RyRs, respectively; BRN, rabbit brain RyR; CRD, rabbit cardiac muscle RyR.

posed to exist in an α-helical conformation, and the leucine side chains extending from one α-helix interact with those from a similar α-helix of a second polypeptide. Although this pattern was originally proposed to explain a nuclear DNA-binding protein, it was also found to be important in tetramer formation (Harbury et al., 1993). Rabbit skeletal muscle and bullfrog α-RyR have one leucine zipper motif (554-575), whereas other RyRs (bullfrog β-RyR, rabbit cardiac and brain, and human and pig skeletal muscle RyR) contain two motifs in overlap. For example, bullfrog β-RyR has them in residues 550-571 and 557-578 (Figure 4B). While some or all of M1–M4 are assumed to be potential candidates, the leucine zipper pattern may be an additional site for polymerization. In IP3 receptor, where the motif cannot be found, the part responsible for tetramer formation is only the putative transmembrane domains in the C-terminal region that show high homology to sequences of RyR. Multiple sites for the polymerization of RyR, however, may be quite reasonable in view of the morphology of the foot, a huge mass in the cytoplasm that is larger than expected from the density of closely packed molecules and has a cavity with a complex configu-

ration. Takeshima et al. (1993) reported that brain contains an RNA species derived from the skeletal muscle ryanodine receptor gene (*ryr1*) using a different transcriptional start site and that the ATG triplet encoding Met4382 of skeletal muscle RyR can function as a translation initiation codon, resulting in production of the C-terminal 656 amino acid residues of Ryr1. The expressed protein was detected on the endoplasmic reticulum membrane of CHO cells. According to their model of RyR, this expressed protein contains the channel region, including M1–M4 and putative sites for Ca^{2+} binding and Ca^{2+}-induced Ca release in skeletal muscle RyR. However, neither [^{3}H]ryanodine-binding activity nor Ca^{2+} release in response to caffeine could be detected. It might simply be due to a lack of ryanodine- and caffeine-binding sites. It may also be possible that the protein could not form an active tetramer because of the lack of an N-terminal region. Marks et al. (1990) determined the amino acid sequences of limited proteolytic fragments of RyR from rabbit skeletal muscle and clarified the surface topography of RyR molecules. Along a similar line of investigation, Chen et al. (1993a) identified the position of tryptic fragments in RyR, resulting from partial digestion of HFSR from rabbit skeletal muscle using various site-specific antibodies, and inferred the parts involved in polymerization by determining the density of the site-containing fragments on sucrose-density gradient centrifugation after solubilization of the partially trypsinized HFSR by CHAPS. They concluded that one of the putative portions involved in tetramer formation consists of amino acid residues 460-1260 of rabbit skeletal muscle RyR (Chen et al., 1993a). The site directly responsible for tetramer formation is probably only a part of the suggested portion. These results are consistent with the inference that the sites of the leucine zipper motif are involved in tetramer formation.

Another interesting motif is the RGD pattern. The tripeptide was originally found in fibronectin, which is crucial for interaction with its cell surface receptor, an integrin. Later, it was also found in the sequences of a number of other proteins, where it has been shown to play a role in cell adhesion, or it may serve the same purpose as recognition system, although it is not always (Ruoslahti and Pierschbacher, 1986; Bairoch, 1992; Fuchs, 1991). For example, it was reported that an RGD sequence in the cell-extracellular matrix junction was probably involved in the gravity-induced polarity of cytoplasmic streaming in the internodal cells of *Chara corallia* (Wayne et al., 1992). Recently, the specificity of RGD was examined in the immune response against foot-and-mouth disease virus, which contained RGD in antigenic site A. The contribution of RGD to the interaction of site A with site A-specific monoclonal antibodies was analyzed (Novella et al., 1993) and found to be so specific that KGD was no longer functional. Among 32,000 entries in SWISS-PROT 28 database, 1845 polypeptides have the RGD tripeptide. The RGD tripeptide is found in every RyR sequence determined: residues 3484-3486 in α-RyR, 3359-3361 in β-RyR, 3499-3501 in rabbit skeletal muscle, 3345-3347 in rabbit cardiac muscle, 3359-3361 in rabbit brain, 3493-3495 (3494-3496 in revised sequence) in human skeletal muscle, and 3495-3497 in pig skeletal muscle (Figure 5A). The segment containing RGD in rabbit skeletal muscle RyR is in the trypsin-sensitive region F as described by Chen et al. (1993a), indicating the exposed surface. The surface exposure of RGD-containing segments will also be the case with other RyR isoforms. In rabbit heart and brain RyRs, additional RGDs are found at residues 4343-4345 and 4274-4276, respectively. The discussion is limited to the patterns of the earlier sequences because they are found ubiquitously. More interestingly, in all RyRs examined except cardiac RyR, the sequences of 12 residues preceding RGD and 28 residues following it are well conserved (Figure 5A). The preceding sequence contains many cationic residues (R and K), and the following sequence is hydrophobic. In cardiac RyR, in contrast, RGD is flanked by anionic groups, although the part following RGD showed very weak hydrophobicity. The segment containing RGD of cardiac RyR is not equivalent to the counterparts of other RyRs in the multiple sequence alignments. In the equivalent counterpart of rabbit cardiac muscle, R of the tripeptide RGD is replaced by K 3455. The targeting motif RGD is now lost, although the neighboring sequences are rather well conserved (Figure 5A).

A. RGD-Containing Segments in Ryanodine Receptors

```
FRA(RGD) GGSDQGRTKK KRR3484GDRYSVQ TSLIVATLKK MLPIGLNMCS PTD
RAB(RGD) GGSDQERTKK KRR3499GDRYSVQ TSLIVATLKK MLPIGLNMCA PTD
PIG(RGD) GGSDQERTKK KRR3495GDRYSVQ TSLIVATLKK MLPIGLNMCA PTD
HUM(RGD) GGSDQERTKK KRR3494GDRYSVQ TSLIVATLKK MLPIGLNMCA PTD

FRB(RGD) GGQDQERKRT KRR3359GDLYSIQ TSLIVAALKK MLPIGLNMCT PGD
BRN(RGD) GGQDQERKKT KRR3359GDLYSIQ TSLIVAALKK MLPIGLNMCT PGD

CRD(KGD) AVSDQERKKM KRK3455GDRYSMQ TSLIVAALKR LLPIGLNICA PGD
CRD(RGD) VVSEEDHLKA EAR3345GDMSEAE LLILDEFTTL ARDLYAFYPL LIR
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B. Characterization of Putative Cytoplasmic Loops between Repeats II and III of DHP-Rs

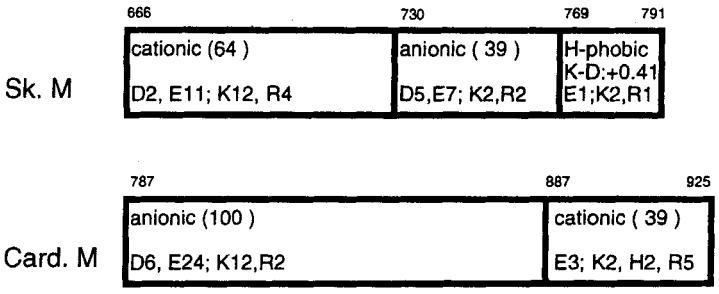

FIGURE 5. Putative coupling sites between RyR and DHP-R in EC coupling. (A) RGD motifs in RyRs; (B) characterization of putative cytoplasmic loops between repeats II and III of rabbit DHP-Rs. Characteristics of subdivisions are shown by the words cationic, anionic, and H-phobic (hydrophobic), with numbers of amino acid residues in parentheses. Polar amino acid residues with their numbers are shown in single alphabetical code. The sequence of residues 769-782 in skeletal muscle DHP-R is highly hydrophobic, with an average Kyte-Doolittle (K-D) score of +0.87, while the sequence of residues 905–913, flanked with cationic segments in cardiac muscle DHP-R, is hydrophobic.

This finding may be related to the results of a series of experiments carried out by Tanabe and collaborators on the expression of DHP-R cDNA in dysgenic mice (Adams et al., 1990; Tanabe et al., 1990a, b). They concluded that the putative cytoplasmic region between repeats II and III of skeletal muscle DHP-R (i.e., CSk3) is an important determinant for skeletal muscle-type EC coupling. It is quite natural to consider that the segment containing RGD in an RyR may interact with the putative cytoplasmic region between repeats II and III of DHP-R. The RGD-containing segments in RyRs have a unique feature, as mentioned above: a cationic portion followed by a hydrophobic portion. Therefore, a suitable counterpart of DHP-R would contain anionic and hydrophobic segments in tandem. It was shown that 211 C-terminal residues of skeletal muscle DHP-R were not involved in signal transmission, using intact and truncated cDNA expression (Beam et al., 1992). The pI values for putative cytoplasmic loops of DHP-Rs from skeletal muscle, cardiac muscle, and chimeric constructs were calculated. The putative cytoplasmic loops between repeats

II and III were found to be highly likely candidates. Therefore, the question is why the loop of skeletal muscle, or CSk3, can be discriminated from that of cardiac muscle. Thus, the putative cytoplasmic loop from skeletal muscle (residues 666-791) is compared with that of cardiac muscle DHP-R (residues 787-925) (Figure 5B). Although both loops are anionic as a whole, closer examination revealed that the loop of skeletal muscle can be divided into three subdivisions: a cationic region of 64 amino acid residues, an anionic region of 39 residues, and a hydrophobic group of 23 residues. In contrast, the loop of cardiac DHP-R is composed of two subdivisions: a highly hydrophilic anionic region of 100 residues (average Kyte-Doolittle score of –1.46) and a cationic region of 39 residues (average Kyte-Doolittle score of –0.41). It is quite probable that the putative cytoplasmic loop of skeletal muscle DHP-R can appose to the RGD-containing domain of skeletal muscle RyR. Both ionic and hydrophobic interactions may be important for coupling between DHP-R and the RyR. Tanabe et al. (1990a) reported that CSk2 also gave rise to positive results, although with lesser probability (3/12) and weaker response. CSk2 (pI: 7.29) was found to be composed of an anionic region of 60 amino acid residues, a cationic region of 30 residues, and a hydrophobic region (average Kyte-Doolittle score of 1.40) of 21 C-terminal residues. These results suggest that the hydrophobic interaction may be more important for coupling. This proposal has yet to be proved by examining the type of EC coupling between various types of DHP-Rs or their chimeras and RyRs mutated in the RGD-containing segment. Lu et al. (1994) showed that the putative cytoplasmic loops between repeats II and III of both skeletal and cardiac muscle DHP-Rs reversibly stimulated the channel activity of rabbit skeletal muscle SR vesicles, but not of dog cardiac SR vesicles. However, this finding may not be directly relevant to action potential-induced Ca release because the specificity for the effective polypeptides was lacking and bovine serum albumin was also effective. These polypeptides also stimulated ryanodine-binding activity, but more than 200 times higher protein concentrations were required. Further experiments are required to evaluate these findings. On the other hand, Oyamada et al. (1994) proposed the potential involvement of a certain portion near residue 1360 in α-RyR of bullfrog skeletal muscle in EC coupling, assuming that β-RyR is not involved in EC coupling.

Homologous RGD-containing segments in RyRs are found in β-RyR and brain-specific isoforms (i.e., Ryr3 isoform). It is easily assumed that the same coupling mechanism as that in skeletal muscle may exist in neuronal cells containing brain-specific isoforms. A structure similar to the foot structure was observed in mouse cerebellum (Henkart et al., 1976). On the other hand, cardiac type RyR (i.e., Ryr2 isoform) has an RGD motif in the segment with distinct features. Therefore, the putative loop of DHP-R for coupling may be different, even though coupling exists, as indicated by morphologic observations (Jewett et al., 1971; Sommer and Johnson, 1979; Jorgensen et al., 1993). It would be interesting if the brain-specific isoform released Ca^{2+} on electrical activation in neuronal cells, whereas the cardiac type isoform released Ca^{2+} through a Ca^{2+}-induced Ca release mechanism that may be activated by IP3-induced Ca release from IP3 receptors in brain. These possibilities must be examined.

This discussion does not necessarily exclude the possibility of involvement of auxiliary proteins to bind DHP-R and RyR together (Kawamoto et al., 1986; Chadwick et al., 1988; Knudson et al., 1983a, b; McPherson and Campbell, 1993a). The coupling between them as determined by Ca release on electrical stimulation survives even in hypertonic solutions where the morphology of triads is modified (Sperelakis and Rubio, 1971; Page and Upshaw-Earley, 1977; Somlyo et al., 1977b; Franzini-Armstrong et al., 1978). In a hypertonic solution, enhanced Ca release is observed on electrical stimulation (Taylor et al., 1975; Parker and Zhu, 1987), while muscle contraction is diminished or lost, mainly because of an increased ionic strength that interferes with the interaction of actin and myosin (Fujino et al., 1961; Koch-Weser, 1963; Blinks, 1965; Lännergren and Noth, 1973; Homsher et al. 1974; Lamb et al., 1993). The coupling between DHP-R and RyR must be tight in view of these observations. Although not a coupling protein, FK 506-binding proteins, which can modulate the activity of RyR,

promises to attract significant attention (Collins, 1991; Jayaraman et al., 1992; Timerman et al., 1993).

VII. BIOLOGICAL MEANINGS OF TWO ISOFORMS COEXISTING IN NONMAMMALIAN VERTEBRATE SKELETAL MUSCLES

Whereas only one isoform has been detected in mammalian skeletal muscle, Sutko and colleagues first identified the existence of two isoforms (α- and β-RyR) in chicken pectoral muscles (Airey et al., 1990). They found that there are also two isoforms in bullfrog skeletal muscle, toadfish swimbladder muscle and body wall skeletal muscle, and chicken leg muscle (Olivares et al., 1991), although O'Brien et al. (1993) showed only α-RyR expressed in toadfish swimbladder muscle (see later in this section). Differential immunologic reactions to monoclonal and polyclonal antibodies and distinct peptide maps on limited proteolysis led them to conclude that β-RyR is not a proteolytic fragment of α-RyR, but is an isoform. Both the α- and β-RyR function as a homotetramer. Immunofluorescent localization of the two isoforms on 1-μm-thick serial cryosections of chicken breast muscles indicated that they were colocalized to the triad junction in the same cells. In view of findings by Block et al. (1988) that each tetrad was apposed to an alternate foot, it will be interesting to see whether one of the two isoforms is consistently contiguous to a tetrad. It was shown that they were present in equal amounts in microsomes from chicken breast muscles, but that the α-isoform was more abundant in microsomes from chicken thigh muscle. Therefore, the two isoforms do not necessarily exist in equal amounts. Sutko et al. (1991) have shown that the two isoforms are expressed at different times during embryonic chick skeletal muscle development. α-RyR is immunologically detected in a majority of fibers in day E10 muscle when myofibril assembly, Z-line formation, and measurable [^{3}H]nitrendipine binding begin to be observed. On the other hand, β-RyR is first observed immunohistochemically at low levels in a few fibers in day E15 muscle. Using the immunoprecipitation method, they demonstrated that α-RyR showed [^{3}H]epiryanodine-binding activity at day E10, while β-RyR did so significantly between day E15 and day E20. They concluded that the two isoforms make unique contributions to muscle cell structure and functions. α-RyR may participate in events involving muscle differentiation. Recently, Airey et al. (1993a) showed an autosomal recessive lethal mutant in the chick, crooked neck dwarf (*cn*) mutant, in which the mRNA for α-RyR is specifically at a reduced level, while the levels of mRNAs for other proteins, including Ca-ATPase, calsequestrin, and soluble enzymes, are normal. Mutant skeletal muscles form and exhibit a normal morphology during the first week of embryonic development. However, beginning at day E7.5, progressive changes in the organization and integrity of appendicular and axial skeletal muscles can be detected in *cn/cn* embryos (Kieny et al., 1983, 1988a, b). Morphologic changes include disarray of myofibrils, dilated sarcoplasmic vesicles, blebbing of the outer nuclear membrane, mitochondrial swelling, and nuclear inclusions (Kieny et al., 1988a, b). Connective tissues and extracellular matrix components are also affected, owing to alterations in muscle properties. *cn/cn* skeletal muscles do not appear to develop beyond the myotube stage, and by hatching, a general degeneration of all mutant skeletal muscles has occurred. The defect associated with the *cn* mutation is specific for skeletal muscle, as cardiac and smooth muscles appear normal (Kieny et al., 1988b). Airey et al. concluded that a failure to make normal α-RyR appears to be closely associated with the *cn* mutation. However, mutant cells in low-density primary cultures that failed to express normal α-RyR exhibited only a part of the mutant phenotype (Airey et al., 1993b). Because they reported that the *cn/cn* phenotype observed in embryonic *cn/cn* skeletal muscle was exhibited more completely by mutant muscle cells plated at high cell densities (Airey et al., 1993b), many of the changes in phenotype described above may be secondary to the failure of expression of α-RyR or to other unidentified conditions.

Murayama and Ogawa (1992) reported that Ca^{2+} sensitivities in [^{3}H]ryanodine binding were markedly different in α-RyR and β-RyR from bullfrog skeletal muscle. This might indicate that

β-RyR is an amplifier for Ca release, which α-RyR first initiates. However, they showed that the enhanced Ca^2 sensitivity was due to the combined effects of high salt concentration and solubilization by CHAPS in the presence of phospholipids (Murayama and Ogawa, 1993) and observed no difference in Ca^{2+} sensitivity between α- and β-RyRs in a 0.17 *M* NaCl medium (Murayama and Ogawa, in preparation). Bull and Marengo (1993) reported two types of Ca^{2+} dependence in SR Ca-release channels from frog skeletal muscle. Because their channel activity in lipid bilayer experiments was determined in a low-salt medium, however, their results may not correspond to results reported by Murayama and Ogawa (1992).

Using their affinity-purified specific polyclonal antibodies (anti-α and anti-β, respectively) against α-RyR and β-RyR from bullfrog skeletal muscle, Murayama and Ogawa (in preparation) examined the relationships among vertebrate striated muscles. Occurrence of a third isoform of RyR in frog ventricle muscles was doubted, unlike in chicken ventricle muscle (Airey et al., 1993c). It cannot be concluded that two isoforms in skeletal muscle of nonmammalian vertebrates are composed of mammalian skeletal muscle-type and cardiac-type isoforms, a situation that is contrary to the conclusion of Lai et al. (1992b). When skeletal muscles from various species of animals were investigated with anti-β, the antibody cross-reacted broadly with β-RyRs from all nonmammalian vertebrate skeletal muscles examined, including chicken, various species of frogs and toads, and carp (although weaker), while it gave negative results with mammalian skeletal muscles and mammalian and nonmammalian cardiac ventricular muscles. In contrast, the cross-reactivity of anti-α was limited to the genus *Rana* (i.e., frogs), and a very weak reaction (less than one tenth) was observed with *Xenopus* (clawed toad). Negative reactions were obtained with carp, chicken, and mammals. These results were similar regardless of muscle fiber types (fast-twitch, slow-twitch, and slow-tonic fibers) except for chicken anterior latissimus dorsi, a slow-tonic fiber that did not show cross-reactivity to anti-α or anti-β. These results indicate that the β-RyR isoform of one animal species is related to the β-RyRs of the other nonmammalian vertebrates, and that the relationship is probably also true for α-RyR. This is at variance with the suggestion by Airey et al. (1993c) that chicken β-RyR is the counterpart of bullfrog α-RyR on the basis of the extent of phosphorylation by calmodulin (CaM) kinase. Furthermore, our results indicate that the β-RyR isoform is widely homologous among nonmammalian vertebrate skeletal muscles and that it might not be related to the skeletal muscle type of mammals or cardiac muscle of any other vertebrates. The α-RyRs, on the other hand, may be subjected to diversification, which is also reflected in the variety of mobilities on SDS-PAGE, in contrast to the uniform mobilities of β-RyRs. The deduced amino acid sequence for β-RyR of bullfrog skeletal muscle consistently showed the highest identity to the deduced amino acid sequence from rabbit brain (*ryr3*-gene origin), while α-RyR showed identity to the rabbit skeletal muscle type isoform (see Table 5; Oyamada et al., 1994). They also examined the distribution of total RNA by reverse transcription-polymerase chain reaction (RT-PCR) analysis with cDNA-specific probes. RNA for α-RyR was detected strongly in skeletal muscles and very weakly in brain, and RNA for β-RyR was detected strongly in skeletal muscles, moderately in brain, and weakly in lung and stomach. In cardiac ventricle and kidney, RNA for neither α- nor β-RyR was detected.

Recently, an extensive examination of the occurrence and distribution of these two isoforms was carried out using skeletal muscles from a variety of fish, reptiles, and birds with immunologic techniques (O'Brien et al., 1993). These studies confirmed in part previously published results, but found two unique key results. (1) Snakes and lizards share with mammals the pattern of expressing only the α-RyR isoform, while turtles and alligators express both isoforms. (2) Rapidly contracting muscles of fish and birds (extraocular muscles and toadfish swimbladder muscle) as well as the rattlesnake tail-shaker muscle express only the α-RyR isoform. The finding that only α-RyR is expressed in toadfish swimbladder contradicts the results of Olivares et al. (1991). O'Brien et al. (1993) dismissed the possibility that expression of the two isoforms is related to muscle type, consistent with our results described above. They

suggested that the existence of the α-isoform alone would be related to specialization for high contraction speed. However, it does not appear to correlate with the absolute value of contraction speed. According to Franzini-Armstrong and associates (Ferguson and Franzini-Armstrong, 1988; Franzini-Armstrong et al., 1988; Appelt et al., 1989, 1991), the number of feet per fiber volume did not vary so much among fiber types from various animals of different contraction speeds, but the number of Ca-ATPase per fiber volume varied to a great extent, indicating that fast relaxation is more critical for rapid contraction. Murayama and Ogawa (in preparation) observed that β-RyRs (Ryr3) seem to be widely homogeneous among nonmammalian vertebrate skeletal muscles, while α-RyRs (Ryr1) are diversified with respect to mobilities on SDS-PAGE and immunoreactivities. The expression of one or two isoforms may simply be related to the extent of cell differentiation and specialization. The coexistence of multiple isoforms can also be seen in brain, especially in cerebellar Purkinje cells of mammals (Kuwajima et al., 1992) as well as those of nonmammalian vertebrates (Ellisman et al., 1990; Walton et al., 1991; Airey et al., 1993a; Ouyang et al., 1993). Further characterization of the functional and structural properties of these RyR isoforms is required to further our understanding of their biologic significance.

VIII. RYANODINE RECEPTORS IN CARDIAC AND SMOOTH MUSCLES

The general characteristics of RyRs are qualitatively very similar, as shown in Table 2. This raises the question of why ryanodine exerts a negative inotropic effect on cardiac muscle, but causes contracture in skeletal muscle.

The magnitude of negative inotropism by ryanodine depends on the species of mammal (Sutko and Willerson, 1980). Rat ventricle is the most sensitive to ryanodine and significantly affected by subnanomolar ryanodine. The magnitude and rate of the developed force were reduced dose-dependently by half by a concentration of about 1 n*M* and became negligible at 10 μ*M*. Dog, cat, and rabbit preparations exhibited similar threshold concentrations of 1 to 5 n*M*, and the concentrations for half the maximum effect were approximately 10 n*M*. The magnitudes and rates of force development in these preparations were affected in a biphasic manner by increasing concentrations of ryanodine: the maximum effects were obtained with 1 μ*M* ryanodine, and the magnitude of depression was reversed at higher concentrations. The greatest decreases in developed forces were about 60, 40, and 10% for dog, cat, and rabbit preparations, respectively. In contrast, no effect was observed in frog ventricle. These differences among animal species are reported to parallel the estimated relative dependence on the contribution of SR to intracellular Ca^{2+} for contraction (Fabiato and Fabiato, 1978; Sutko and Willerson, 1980; Bers, 1985). We were consistently unable to detect any high molecular weight mass corresponding to RyR on SDS-PAGE of microsomes from bullfrog ventricles when the gel was stained by Coomassie brilliant blue or when Western blot analysis was performed on anti-α or anti-β antibodies (Murayama and Ogawa, in preparation). Because mRNA for Ca-ATPase of SR was undetectable (Vilsen and Andersen, 1992), bullfrog ventricle muscle is likely to contain no or only a minor amount of SR. In mammalian cardiac muscles, which are inhibited by ryanodine, the resting membrane potential and action potential were not significantly affected (Sutko and Kenyon, 1983; Marban and Wier, 1985). Slight prolongation of the plateau duration of the action potential was, instead, observed (Marban and Wier, 1985; Wier et al., 1985). Ryanodine abolished the oscillatory aftercontractions and transient depolarizations or transient inward currents in guinea pig papillary muscles and calf cardiac Purkinje fibers in K^+-free solution, while not preventing the Ca-overload state caused by K^+ removal (Sutko and Kenyon, 1983). Ca influx through voltage-activated Ca channels was unchanged or slightly increased (Mitchell et al., 1984), although Valdivia and Coronado (1989) reported that ryanodine from Calbiochem at micromolar or high concentrations inhibited DHP-sensitive Ca channels from rabbit skeletal muscles. The Na/Ca exchange rate was not significantly affected either (Marban and Wier, 1985; Wier et al., 1985). The Ca^{2+} sensitivity of

the contractile system of a skinned fiber was unchanged by ryanodine (Fabiato, 1985a; Wier et al., 1985; Su, 1988). These results are consistent with ryanodine effecting a decreased availability of intracellular Ca^{2+}, perhaps through a diminishment of its release from the SR. A plausible explanation is that Ca^{2+} released from the SR by ryanodine is removed from the cytoplasm by a Ca pump in the sarcolemma or by the Na/Ca exchange reaction with a minimal increase in $[Ca^{2+}]_i$, if any, resulting in an SR depleted of Ca (Hilgemann et al., 1983; Inui et al., 1987b; Rousseau et al., 1987). Hansford and Lakatta (1987) confirmed, by monitoring $[Ca^{2+}]_i$ in single adult rat cardiac myocytes by a fluorescent dye, quin 2, that ryanodine consistently depleted SR of Ca^{2+}. In contrast, Marban and Wier (1985) examined the effect of ryanodine on aequorin-injected canine cardiac Purkinje fibers by measuring membrane potential, calcium transients, and contraction. During exposure to a K^+-free, low-Na^+ solution, 10 m*M* caffeine evoked a large increase in Ca^{2+} and contractile force in the presence of 1 μ*M* ryanodine, above and beyond the increases induced by Na^+ withdrawal alone. This may indicate that SR contains a large amount of Ca^{2+}, even in the presence of ryanodine, leading to the conclusion that ryanodine may inhibit Ca release itself (also see Wier, 1990). This agrees with the conclusion of Sutko, who claimed that ryanodine inhibited the Ca release process (Sutko and Kenyon, 1983; Sutko et al., 1985a). Fabiato (1985a) showed, in single skinned cardiac cells from rat ventricles, that ryanodine inhibited three types of Ca release from the SR. Su (1988) showed, in skinned fiber bundles from papillary muscle of rabbit, that ryanodine at concentrations up to 100 n*M* inhibited Ca release by 25 m*M* caffeine, although there was no change in Ca contents in the Ca store, which was releasable by Triton X-100. She indicated that the negative inotropism by ryanodine at 100 n*M* or more was due to a decrease in calcium accumulation in the SR. However, ryanodine did not inhibit the Ca^{2+}-ATPase activity of cardiac SR, and net Ca uptake may be increased under some conditions (Jones et al., 1979; DuBell et al., 1993). In conclusion, the underlying mechanism by which ryanodine prevents an increase in the cytoplasmic Ca^{2+} concentration of cardiac muscles, whether by depletion of calcium in the SR or by interference with the normal Ca release from the SR, needs to be clarified.

A similar situation exists in smooth muscles, neurons, and other cells. The most widely accepted hypothesis favors depletion of Ca in the Ca store. Taking advantage of the nature of ryanodine to remove the functional contribution of the caffeine-sensitive Ca store, Iino et al. (1988) estimated the relative contributions of IP3 receptor and RyR in smooth muscle contraction by various stimuli. They interpreted their results with the assumption that the effect of ryanodine was to keep RyR open, but in the long-term subconductance state, and concluded that certain compartments of the Ca store (Sα, according to their nomenclature) have both IP3 receptor and RyR, while the others (Sβ) have only the IP3 receptor. Sα and Sβ coexist in a single smooth muscle cell (Yamazawa et al., 1992). These findings indicate that some parts of the IP3-sensitive Ca pool might be eliminated by ryanodine. Kanmura et al. (1988), however, reported that 10 μ*M* ryanodine depleted not only caffeine-sensitive stores, but also IP3-sensitive stores in rabbit ear artery. Some lipid bilayer experiments failed to demonstrate the long-term open state of channels caused by ryanodine with RyRs from brain (McPherson et al., 1991) and aorta (Herrmann-Frank et al., 1991). However, they did show complete closure of the channel. It is noteworthy that brain RyR was not affected by 10 to 100 n*M* ryanodine, but was closed by concentrations of 4 μ*M* or more. This would be consistent with the conclusion by Ito et al. (1986) that ryanodine closes the channel. If this is the case, the basis of determinations by Iino and associates (1988) will be challenged. On the other hand, there are many experiments showing that intracellular Ca^{2+} concentrations were increased transiently or persistently by micromolar or 10 μ*M* concentrations of ryanodine (Hwang and van Breemen, 1987; Ashida et al., 1988; Joulou-Schaeffer and Freslon, 1988; Kanmura et al., 1988; Friel and Tsien, 1992; Wagner-Mann et al., 1992; Ivanenko et al., 1993). Since Oyamada et al. (1993) reported the absence of channel closing by 1 m*M* ryanodine in frog skinned skeletal muscle fibers, it is important to gain accurate and defini-

tive information concerning the behavior of channels affected by ryanodine. We cannot exclude at present the possibility that the capability of SR for Ca release may be regulated by the extent of filling of the Ca store.

IX. RYANODINE RECEPTORS IN BRAIN AND NEURONS

The ryanodine receptors in brain were initially identified in the microsomal fraction using Ca^{2+}-stimulated [^{3}H]ryanodine binding (Ashley, 1989; McPherson and Campbell, 1990; Ellisman et al., 1990; Damiani et al., 1991; Mészarós and Volpe, 1991; Kawai et al., 1991; Padua et al., 1991; Zimanyi and Pessah, 1991). The specific activity for [^{3}H]ryanodine binding of microsomes from brain or cerebellum was about 2 to 4% (up to 10%) of those from skeletal muscle and about 10% of those from cardiac muscle (McPherson and Campbell, 1990; Damiani et al., 1991; Kuwajima et al., 1992; Witcher et al., 1992). Because any purified RyR shows an activity of 1 mol of ryanodine bound per tetramer, those figures may reflect the difference in the contents of the receptor. Immunohistochemical study revealed that the distribution of RyR was heterogeneous in brain (McPherson and Campbell, 1990; Kuwajima et al., 1992; Nakanishi et al., 1992; Padua et al., 1992; Stein et al., 1992; Lai et al., 1992a). It may be concluded that the cerebral cortex, hippocampus, and olfactory bulb have more RyR than the cerebellum in the mammalian brain, with the reverse being the case in brains of nonmammalian vertebrates (Zupanc et al., 1992; Ouyang et al., 1993). The details of intracellular distributions are not always consistent among investigators. For example, Sutko and associates (Walton et al., 1991; Ouyang et al., 1993) and Lai et al. (1992a) reported that RyR was detectable not only in soma, but also in dendrites, whereas Mikoshiba et al. reported very few RyR in dendrites (Nakanishi et al., 1992; Kuwajima et al., 1992).

Recently, it has been demonstrated that several isoforms of RyR are expressed in brain. Otsu et al. (1990) for the first time showed that mRNA of cardiac type (*ryr2* origin) occurs in brain. Furthermore, a brain-specific cDNA that shows approximately 70% identity in its deduced amino acid sequence with either cardiac or skeletal muscle RyRs has recently been cloned (Hakamata et al., 1992) and shown to be of *ryr3* origin. The mRNA was found to be expressed in specific regions of the brain: corpus striatum, thalamus, and hippocampus (Hakamata et al., 1992). This type of mRNA is also detected in tissues containing smooth muscle, such as aorta, esophagus, taenia coli, urinary bladder, ureter, and uterus. In contrast, mRNA from *ryr2* is ubiquitously expressed in brain, but is most abundant in cerebellum (Hakamata et al., 1992) (see Table 3). Antibodies against peptide sequences specific to cardiac RyR consistently detect a protein throughout the central nervous system (Lai et al., 1992a; also see McPherson and Campbell, 1993b). In mouse brain, a protein that was immunoreactive with an antibody raised against a peptide sequence specific to skeletal muscle RyR was found to be present in cerebellar Purkinje cells (Kuwajima et al., 1992). This is consistent with results with chicken Purkinje cells by Sutko and collaborators (Ellisman et al., 1990; Walton et al., 1991; Airey et al., 1993a; Ouyang et al., 1993). They detected two bands on Western blot analysis of microsomes from cerebellum, whereas only one band was detected with microsomes from cerebrum using monoclonal antibody 34C, which reacted positively with α- and β-isoforms of skeletal muscle RyR and cardiac muscle RyR isoform (Ellisman et al., 1990). For interpretation of these results, the following information may be helpful. Although the relative mobilities of three chicken RyR isoforms were β-RyR > cardiac RyR > α-RyR, cardiac RyR moved much closer to β-RyR than to α-RyR (Airey et al., 1993c). When different animal species were compared, the mobilities of β-RyRs were similar to each other, while those of α-RyRs varied (Olivares et al., 1991; Murayama and Ogawa, in preparation). On the basis of cDNA sequences, β-RyR from bullfrog skeletal muscle has the same number of amino acid residues as brain-specific RyR of rabbit and shows the highest homology to it (see Table 5). These results indicate the difficulty of determining whether the band that moves faster is the β-isoform of skeletal muscle, cardiac isoform, or brain-specific isoform, although most investigators assume it to be the

cardiac isoform. Monoclonal antibody specific to the α-isoform of skeletal muscle (110F) detected a protein in chicken cerebellar Purkinje cells (Ouyang et al., 1993). The cerebellar Purkinje cells of crooked neck dwarf mutant of chicks do not express α-RyR (Airey et al., 1993a). In fish brain, this is probably also the case because microsomes from fish brain showed two bands on Coomassie staining and Western blot analysis by monoclonal antibody 34C (Zupanc et al., 1992). Finally, Ryr1 originated from *ryr1* is certainly expressed in Purkinje cells in cerebellum. However, it should be determined what other isoforms are expressed in the specific regions of brain.

McPherson and Campbell (1993b) investigated which of the three isoforms is the major form of RyR purified from rabbit brain. On the basis of immunologic reactions and structural studies of limited proteolytic fragments and reactivity to a hydrophobic reagent, 3-(trifluoromethyl)-3-(m-[^{125}I]iodophenyl)dizaerine, they concluded that purified brain RyR is very similar to the cardiac type, but is distinct from the skeletal type. This conclusion is reasonable because cardiac-type mRNA is the major type of RyR in brain. They did, however, note subtle differences in the structure of proteins expressed in heart and brain. Because the brain-specific isoform, Ryr3, had not yet been purified, they did not compare RyR purified from brain with Ryr3. The subtle differences might be attributed to the coexistence of Ryr3 and Ryr1. It should be made clear whether the difference is intrinsic between cardiac-specific and brain-specific RyRs, or whether it can be ascribed to the splicing of cardiac-type mRNA. Another interesting finding in their paper is the marked inhibition of [^{3}H]ryanodine binding by calmodulin. In their discussion on this matter, they underestimated the CaM content of brain. They reported that CaM at 5 μ*M* almost completely inhibited ryanodine binding and that an IC_{50} was about 0.4 μ*M*. Because their system is unlikely to be contaminated with Ca-CaM-dependent protein kinase, the effect of Ca-CaM is probably due to direct interaction between CaM and RyR. The brain is one of the organs richest in CaM and has a CaM concentration of about 50 μ*M* or more. The affinity of CaM for Ca^{2+} may be about 10^5 M^{-1} in the cytoplasm (Ebashi and Ogawa, 1988). Intracellular Ca^{2+} may increase from about 0.1 to 1 μ*M* on cell activation. About half of the RyR would be occupied by Ca-CaM at rest in the presence of 0.1 μ*M* Ca^{2+} and be incapable of binding ryanodine, that is, in the closed state if it is assumed that CaM will be active when two of four Ca sites in CaM are occupied by calcium. At 1 μ*M* Ca^{2+}, almost all RyR would be in the closed state. This may be unfavorable to the regulatory role of Ca-CaM. However, the effect of ligand-free CaM on RyR cannot be determined, as they discussed. Therefore, a definite conclusion must be deferred.

The inhibitory action of CaM on Ca release from SR vesicles was first reported by Meissner and Henderson (1987). They attributed this inhibition to a direct action, but not via phosphorylation. Skeletal and cardiac muscle are tissues that contain small amounts of CaM — no more than 6 μ*M* (Ebashi and Ogawa, 1988). Because the affinity of CaM for Ca^{2+} in the myoplasm is probably as low as 10^5 M^{-1}, a physiologic regulatory role for CaM will be less likely in view of an IC_{50} of 0.1 to 0.2 μ*M*. This would also be true even though the modification is exerted through phosphorylation. Witcher et al. (1991) showed that Ca-CaM-dependent protein kinase phosphorylated a unique site of cardiac RyR and that the phosphorylation stimulated channel activity. However, this is unlikely to be a physiologic regulatory mechanism *in vivo* because of the low concentration of CaM, as discussed above. Because neuronal Ca-CaM-dependent protein kinase has a lower affinity for CaM than does smooth muscle myosin light-chain kinase (Hanson and Schulman, 1992), phosphorylation through the kinase is less likely to be a physiologic modulator, even in neurons. For a final answer, however, more information is needed.

Although IP3 receptor and RyR are Ca-release channel proteins, their distributions in the brain of mammals are different. While IP3 receptor is highest in cerebellum, RyR is most abundant in either hippocampus or olfactory bulb or both in mammals (McPherson and Campbell, 1990; Lai et al., 1992a; Ferris and Snyder, 1992). IP3 receptor and RyR coexist in avian cerebellar Purkinje neuron cells, but their intracellular localizations are somewhat differential. Both receptors

were detected in cell body, dendritic processes (main branch), and dendritic shafts. In dendritic spines that receive input from parallel fibers coming from cerebellar granule cells, however, IP3 receptor was detected, but not RyR (Walton et al., 1991). These specific localizations may have functional significance. IP3 receptors are more abundant than RyR by a factor of four- to tenfold in rabbit cerebellum (McPherson and Campbell, 1990). According to Walton et al. (1991), the difference is as large as 50-fold in chicken cerebellum. Unitary conductance of the RyR channel is estimated to be about four times as high as that of the IP3 receptor (McPherson and Campbell, 1990; Ehrlich and Watras, 1988; Maeda et al., 1991; also see Table 2). At least 50% of the endoplasmic reticulum Ca^{2+} stores in brain can be consistently released by IP3 (Ferris and Snyder, 1992). While the isoform of RyR in brain is largely Ryr2, Ryr1 and Ryr3 are detected in Purkinje cells and in hippocampus, respectively. As discussed above (see pages 252 to 254), Ryr1 and Ryr3 isoforms can conduct action potential-induced signal transduction, as seen in skeletal muscle-type EC coupling. On the other hand, Ryr2 isoform might work through Ca^{2+}-induced Ca release. These possibilities are closely related to the hypothesis that intracellular Ca^{2+} is involved in long-term potentiation in the hippocampus (Bliss and Collingridge, 1993). The β-RyR-like (Ryr3) isoform in the corpus striatum might be responsible for malignant syndrome, one of the neurologic side-effects of neuroleptic drugs, because dantrolene is effective despite the lack of evidence of abnormality of Ca^{2+} handling in skeletal muscle.

X. DISEASES RELATED TO THE RYANODINE RECEPTOR GENE (*ryr1*)

A. Malignant Hyperthermia

Although anesthesia provides little cause for concern for most humans, inhalation anesthetics in those genetically predisposed to malignant hyperthermia (MH) can induce metabolic abnormalities, including marked metabolic acidosis, cardiac tachycardia, an abrupt rise in body temperature, and extremely high fever, which can lead to tissue damage or death. Skeletal muscle contracture occurs in most cases, but not all. Use of depolarizing muscle relaxants (e.g., succinylcholine) in combination with general inhalation anesthetics precipitates an episode. The following discussion is limited to cases accompanied by skeletal muscle contracture. Dantrolene is a potent antidote for MH (Denborough and Lovell, 1960; Britt and Kalow, 1970; Aldrete and Britt, 1978).

Similar conditions also occur in domestic animals, especially swine. Swine homozygous for the abnormality respond to stresses in the same way that humans respond to anesthetics — with muscle contracture and high fever (porcine stress syndrome, PSS). These animals produce pale, soft, exudative pork in large segments of their carcasses, which causes serious economic loss to stock-raising and meat-producing industries. Skeletal muscles from PSS animals are more sensitive to caffeine and halothane than that from normal ones, resulting in muscle contracture in lower concentrations of those drugs (Britt and Kalow, 1970; Aldrete and Britt, 1978).

Kalow et al. (1970) first demonstrated that the patients' muscles have a higher sensitivity than muscles from normal individuals to the contracture-inducing action of caffeine applied with or without halothane. Dysfunction of SR was believed to be the main underlying cause of MH. The effect of halothane on the Ca^{2+}-ATPase activity of SR were contradictory: some reported that halothane decreased activity, but others reported the opposite (Aldrete and Britt, 1978). Kurebayashi and Ogawa (1982) reported that halothane dose-dependently shifted the pCa-activity curves to higher Ca^{2+} concentration ranges. The relationship of pCa-EP (phosphorylated intermediate) level was also dose dependently shifted in a similar way, with little effect on the rate of EP hydrolysis. The main action is on the steps leading to EP formation, with a reduced apparent affinity of Ca-ATPase for Ca^{2+}. The results are similar with HFSRs from bullfrog and rabbit skeletal muscles. The results explain why the previous results appeared contradictory: the determinations were carried out at a fixed concentration of Ca^{2+}. The effect of halothane on Ca^{2+}-ATPase activity is related to the inhibition of Ca

uptake by SR, which caused only a slow Ca leakage and could not be the cause of rapid Ca release, as mentioned later.

Takagi et al. (1976) demonstrated the Ca-releasing action of halothane, which was similar to that of caffeine on the SR in skinned fibers, and also showed that SR in skinned fibers from susceptible persons was more sensitive to halothane than that from normal individuals. Endo et al. (1983) showed that the Ca^{2+}-induced Ca release mechanism in muscles from a MH patient was observable at lower concentrations of Ca^{2+} than that in normal muscles, that is, the mechanism is about twice as sensitive to Ca^{2+} as that in non-MH muscles. The maximum rate of Ca release at a sufficiently high concentration of Ca^{2+} was also higher (by a factor of about two). Halothane accelerated Ca^{2+}-induced Ca release to a similar extent in muscles from both MH and non-MH patients. However, no difference was observed in the properties of Ca uptake by SR or of the contractile protein system between MH and non-MH muscles. Ogawa and Kurebayashi (1982) examined the Ca-releasing action of halothane on HFSRs from bullfrog and rabbit skeletal muscles (also see Endo, 1985; Fleischer and Inui, 1989; MacLennan and Phillips, 1992). The Ca release was more marked at pH 6.5 than at pH 6.8. Halothane caused more Ca release from rabbit HFSR at 25 than at 15°C, whereas in frog HFSR the effect of temperature was the reverse, similar to caffeine. These findings may be consistent with the observation that metabolic acidosis precedes muscle contracture and that succinylcholine is a precipitating agent that may cause an increase in local temperature. An underlying mechanism for the metabolic acidosis may be that phosphorylase kinase, a regulatory enzyme of glycogenolysis, can be activated at lower Ca^{2+} concentrations than the contractile system (Ozawa et al., 1967). Ca release by halothane was accelerated by ATP and inhibited by high Mg^{2+} and procaine. In these respects, halothane is similar to caffeine. Finding the potentiating effect of caffeine at a fixed concentration with various doses of halothane, Ogawa and Kurebayashi (1982) concluded that the site of action of halothane could not be the same as that of caffeine, although the sites were very close. Dantrolene is very effective as a treatment for MH. Endo and collaborators (Ohta and Endo, 1986) showed that the effect of dantrolene was critically dependent on temperature: at 37°C, it inhibited Ca^{2+}-induced and caffeine-induced Ca release, but had no effect at 20°C. The inhibitory action of dantrolene on K-contracture, however, was independent of temperature. Dantrolene shows no effect on Ca release from SR of cardiac muscle or smooth muscle, in contrast to local anesthetics, such as procaine. In addition, [^{3}H]ryanodine binding to α-RyR and β-RyR from frog skeletal muscle were reduced to a similar extent by dantrolene.

An abnormality in the Ca release channel of skeletal muscle (i.e., ryanodine receptor) may account for the dysfunction in MH. The locus of MHS was found on human chromosome 19q13.1 (MacLennan et al., 1990; McCarthy et al., 1990), where the *ryr1* gene was also located (MacKenzie et al., 1990). cDNAs of *ryr1* genes from normal and homozygous MHS swine were cloned and sequenced. The deduced amino acid sequences from an MHS and a normal pig differed by a single amino acid: in the MHS pig, Cys was substituted for Arg 615 (R615C) (Fujii et al., 1991; Otsu et al., 1991). This mutation was confirmed by analysis of trypsin fragments of RyRs from normal and MHS pigs (Mickelson et al., 1992). This result suggests that the region including this site was near the cytoplasmic surface (also see Chen et al., 1993a). Corresponding to the porcine MH mutation, R614C was found in 1 of 35 human families studied, and this mutation also cosegregated with MH (Gillard et al., 1991). The same mutation has been found in 2 to 5% of unrelated human MH families investigated throughout the world. More recently, a second MH mutation, G248R, was reported (Gillard et al., 1992). This mutation has been detected in only a single MHS pedigree. As discussed later, R2434H and R163C are other candidates for producing the abnormality. On the other hand, there was a recent report of linkage to markers on chromosome 17q11.2-24, which contains genes for two subunits of DHP-R and a subunit of the adult muscle sodium channel. However, the locus of the chromosome that is responsible for MHS in more than 50% of European MHS families has not yet been identified, and is claimed to be clearly not on chromosome 17 or 19 (Johnson, 1993).

B. Central Core Disease (CCD)

Central core disease of muscle is a rare myopathy usually characterized by hypotonia and proximal muscle weakness in infancy. Muscle weakness of the lower extremities is frequently the main symptom, and the clinical course of the disorder can be slow or nonprogressive. However, the severity of symptoms may vary from nonexistent to severe, with a wide variation in muscle involvement. Diagnosis of CCD is by histologic examination of skeletal muscle biopsy specimens. Typical CCD samples show amorphous central cores in type 1 fibers. Identification of cores is facilitated by staining for oxidative enzyme activity. As the cores are depleted of mitochondria, they appear as negative areas within the normal enzyme activity areas of the surrounding muscle fibers. Genetic analysis of several CCD pedigrees indicates that the disorder is inherited as an autosomal dominant trait and is closely associated with a predisposition to MH. Attempts to link the CCD gene to chromosome 19q13.1, the location of *ryr1*, have been successful (Haan et al., 1990; Kausch et al., 1991). Recently, two groups have independently and simultaneously succeeded in identifying the mutation in CCD. Zhang et al. (1993) reported R2434H substitution in a large Canadian family. Those who have the mutation were also found to have MH. Quane et al. (1993) reported R163C mutation in a CCD pedigree of Italian descent and in an unrelated MHS pedigree of Danish origin and I403M substitution in an unrelated CCD pedigree of Italian descent.

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REFERENCES

Adams, B. A., Tanabe, T., Mikami, A., Numa, S., and Beam, K. G. (1990), Intramembrane charge movement restored in dysgenic skeletal muscle by injection of dihydropyridine receptor cDNAs, *Nature (London)*, **346:**569–572.

Airey, J. A., Beck, C. F., Murakami, K., Tanksley, S. J., Deerinck, T. J., Ellisman, M. H., and Sutko, J. L. (1990), Identification and localization of two triad junctional foot protein isoforms in mature avian fast twitch skeletal muscle, *J. Biol. Chem.*, **265:** 14187–14194.

Airey, J. A., Baring, M. D., Beck, C. F., Chelliah, Y., Deerinck, T. J., Ellisman, M. H., Houenou, L. J., McKemy, D. D., Sutko, J. L., and Talvenheimo, J. (1993a), Failure to make normal α ryanodine receptor is an early event associated with the crooked neck dwarf (cn) mutation in chicken, *Dev. Dyn.*, **197:** 169–188.

Airey, J. A., Deerinck, T. J., Ellisman, M. H., Houenou, L. J., Ivanenko, A., Kenyon, J. L., McKemy, D. D., and Sutko, J. L. (1993b), Crooked neck dwarf (cn) mutant chicken skeletal muscle cells in low density primary cultures fail to express normal α ryanodine receptor and exhibit a partial mutant phenotype, *Dev. Dyn.*, **197:** 189–202.

Airey, J. A., Grinsell, M. M., Jones, L. R., Sutko, J. L., and Witcher, D. (1993c), Three ryanodine receptor isoforms exist in avian striated muscle, *Biochemistry*, **32:** 5739–5745.

Aldrete, J. A. and Britt, B. A., Eds. (1978), *Second International Symposium on Malignant Hyperthermia*, Grune & Stratton, New York.

Anderson, K., Lai, F. A., Liu, Q.-Y., Rousseau, E., Erickson, H. P., and Meissner, G. (1989), Structural and functional characterization of the purified cardiac ryanodine receptor-Ca^{2+} release channel complex, *J. Biol. Chem.*, **264:** 1329–1335.

Appelt, D., Buenviaje, B., Champ, C., and Franzini-Armstrong, C. (1989), Quantitation of 'junctional feet' content in two types of muscle fiber from hind limb muscles of the rat, *Tissue Cell*, **21:** 783–794.

Appelt, D., Shen, V., and Franzini-Armstrong, C. (1991). Quantitation of Ca ATPase, feet and mitochondria in superfast muscle fibres from the toadfish, *Opsanus tau*, *J. Muscle Res. Cell Motil.*, **12:** 543–552.

Ashida, T., Schaeffer, J., Goldman, W. F., Wade, J. B., and Blaustein, M. P. (1988), Role of sarcoplasmic reticulum in arterial contraction: comparison of ryanodine's effect in a conduit and a muscular artery, *Circ. Res.*, **62:** 854–863.

Ashley, C. C., Mulligan, I. P., and Lea, T. J. (1991), Ca^{2+} and activation mechanisms in skeletal muscle, *Q. Rev. Biophys.*, **24:** 1–73.

Ashley, R. H. (1989), Activation and conductance properties of ryanodine-sensitive calcium channels from brain microsomal membranes incorporated into planar lipid bilayers, *J. Membr. Biol.*, **111:** 179–189.

Bairoch, A. (1992), PROSITE: a dictionary of sites and patterns in proteins, *Nucleic Acids Res.*, **20:** 2013–2018.

Baker, P. F., Ed. (1988), *Handbook of Experimental Pharmacology, Vol. 83, Calcium in Drug Actions*, Springer-Verlag, Berlin.

Baylor, S. M., Chandler, W. K., and Marshall, M. W. (1983), Sarcoplasmic reticulum calcium release in frog skeletal muscle fibres estimated from arsenazo III calcium transients, *J. Physiol.*, **344:** 625–666.

Baylor, S. M. and Hollingworth, S. (1988), Fura-2 calcium transient in frog skeletal muscle fibres, *J. Physiol.*, **403:** 151–192.

Beam, K. G., Adams, B. A., Niidome, T., Numa, S., and Tanabe, T. (1992), Function of a truncated dihydropyridine receptor as both voltage sensor and calcium channel, *Nature (London)*, **360:** 169–171.

Beam, K. G., Knudson, C. M., and Powell, J. A. (1986), A lethal mutation in mice eliminates the slow calcium current in skeletal muscle cells, *Nature (London)*, **320:** 168–170.

Berridge, M. J. (1993a), Inositol triphosphate and calcium signalling, *Nature (London)*, **361:** 315–325.

Berridge, M. J. (1993b), A tale of two messengers, *Nature (London)*, **365:** 388–389.

Bers, D. M. (1985), Ca influx and sarcoplasmic reticulum Ca release in cardiac muscle activation during postrest recovery, *Am. J. Physiol.*, **248:** H366–H381.

Bers, D. M. and Stiffel, V. M. (1993), Ratio of ryanodine to dihydropyridine receptors in cardiac and skeletal muscle and implications for E-C coupling, *Am. J. Physiol.*, **264:** C1587–C1593.

Bertolino, M. and Llinás, R. R. (1992), The central role of voltage-activated and receptor-operated calcium channels in neuronal cells, *Annu. Rev. Pharmacol. Toxicol.*, **32:** 399–421.

Blinks, J. R. (1965), Influence of osmotic strength on cross-section and volume of isolated single muscle fibres, *J. Physiol.*, **177:** 42–57.

Blinks, J. R. (1987), The use of photoproteins as calcium indicators in muscle; inositol 1,4,5-triphosphate causes calcium release in frog skeletal muscle only when transverse tubules have been interrupted, *J. Muscle Res. Cell Motil.*, **8:** 462–463.

Bliss, T. V. P. and Collingridge, G. L. (1993), A synaptic model of memory: long-term potentiation in the hippocampus, *Nature (London)*, **361:** 31–39.

Block, B. A., Imagawa, T., Campbell, K. P., and Franzini-Armstrong, C. (1988), Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle, *J. Cell Biol.*, **107:** 2587–2600.

Bourne, H. R., Sanders, D. A., and McCormick, F. (1991), The GTPase superfamily: conserved structure and molecular mechanism, *Nature (London)*, **349:** 117–127.

Britt, B. A. and Kalow, W. (1970), Malignant hyperthermia; aetiology unknown!, *Can. Anaesth. Soc. J.*, **17:** 316–330.

Bull, R. and Marengo, J. J. (1993), Sarcoplasmic reticulum release channels from frog skeletal muscle display two types of calcium dependence, *FEBS Lett.*, **331:** 223–227.

Bull, R., Marengo, J. J., Suárez-Isla, B. A., Donoso, P., Sutko, J. L., and Hidalgo, C. (1989), Activation of calcium channels in sarcoplasmic reticulum from frog muscle by nanomolar concentrations of ryanodine, *Biophys. J.*, **56:** 749–756.

Caputo, C. (1983), Pharmacological investigations of excitation-contraction coupling, in *Handbook of Physiology, Skeletal Muscle*, Peachey, L. D., Adrian, R. H., and Geiger, S. R., Eds., American Physiological Society, Bethesda, MD, 381–415.

Carroll, S., Skarmeta, J. G., Yu, X., Collins, K. D., and Inesi, G. (1991), Interdependence of ryanodine binding, oligomeric receptor interactions, and Ca^{2+} release regulation in junctional sarcoplasmic reticulum, *Arch. Biochem. Biophys.*, **290:** 239–247.

Catterall, W. A. (1988), Structure and function of voltage-sensitive ion channels, *Science*, **242:** 50–61.

Catterall, W. A. (1991), Excitation-contraction coupling in vertebrate skeletal muscle: a tale of two calcium channels, *Cell*, **64:** 871–874.

Catterall, W. A. (1993), Structure and function of voltage-gated ion channels, *Trends Neurosci.*, **16:** 500–506.

Chadwick, C. C., Inui, M., and Fleischer, S. (1988), Identification and purification of a transverse tubule coupling protein which binds to the ryanodine receptor of terminal cisternae at the triad junction in skeletal muscle, *J. Biol. Chem.*, **263:** 10,872–10,877.

Chandler, W. K., Rakowski, R. F., and Schneider, M. F. (1976), A non-linear voltage dependent charge movement in frog skeletal muscle, *J. Physiol.*, **254:** 245–283.

Chen, S. R. W., Zhang, L., and MacLennan, D. H. (1992), Characterization of a Ca^{2+} binding and regulatory site in the Ca^{2+} release channel (ryanodine receptor) of rabbit skeletal muscle sarcoplasmic reticulum, *J. Biol. Chem.*, **267:** 23,318–23,326.

Chen, S. R. W., Airey, J. A., and MacLennan, D. H. (1993a), Positioning of major tryptic fragments in the Ca^{2+} release channel (ryanodine receptor) resulting from partial digestion of rabbit skeletal muscle sarcoplasmic reticulum, *J. Biol. Chem.*, **268:** 22,642–22,649.

Chen, S. R. W., Zhang, L., and MacLennan, D. H. (1993b), Antibodies as probes for Ca^{2+} activation sites in the Ca^{2+} release channel (ryanodine receptor) of rabbit skeletal muscle sarcoplasmic reticulum, *J. Biol. Chem.*, **268:** 13,414–13,421.

Chu, A. and Stefani, E. (1991), Phosphatidylinositol 4,5-bisphosphate-induced Ca^{2+} release from skeletal muscle

sarcoplasmic reticulum terminal cisternal membranes: Ca^{2+} flux and single channel studies, *J. Biol. Chem.*, **266:** 7699–7705.

Clapper, D. L., Walseth, T. F., Dargie, P. J., and Lee, H. C. (1987), Pyridine nucleotide metabolites stimulate calcium release from sea urchin egg microsomes desensitized to inositol trisphosphate, *J. Biol. Chem.*, **262:** 9561–9568.

Collins, J. H. (1991), Sequence analysis of the ryanodine receptor: possible association with a 12K, FK506-binding immunophilin/protein kinase C inhibitor, *Biochem. Biophys. Res. Commun.*, **178:** 1288–1290.

Damiani, E., Tobaldin, G., Volpe, P., and Margreth, A. (1991), Quantitation of ryanodine receptor of rabbit skeletal muscle, heart and brain, *Biochem. Biophys. Res. Commun.*, **175:** 858–865.

Denborough, M. A. and Lovell, R. R. H. (1960), Anaesthetic deaths in a family, *Lancet*, **2:** 45.

Donaldson, S. K., Goldberg, N. D., Walseth, T. F., and Huetteman, D. A. (1987), Inositol trisphosphate stimulates calcium release from peeled skeletal muscle fibers, *Biochim. Biophys. Acta*, **927:** 92–99.

Donaldson, S. K., Goldberg, N. D., Walseth, T. F., and Huetteman, D. A. (1988), Voltage dependence of inositol 1,4,5-trisphosphate-induced Ca^{2+} release in peeled skeletal muscle fibers, *Proc. Natl. Acad. Sci. U.S.A.*, **85:** 5749–5753.

DuBell, W. H., Lewartowski, B., Spurgeon, H. A., Silverman, H. S., and Lakatta, E. G. (1993), Repletion of sarcoplasmic reticulum Ca after ryanodine in rat ventricular myocytes, *Am. J. Physiol.*, **265:** H604–H615.

Dulhunty, A. F. (1992), The voltage-activation of contraction in skeletal muscle, *Prog. Biophys. Mol. Biol.*, **57:** 181–223.

Ebashi, S. (1991), Excitation-contraction coupling and the mechanism of muscle contraction, *Annu. Rev. Physiol.*, **53:** 1–16.

Ebashi, S. and Endo, M. (1968), Calcium ion and muscle contraction, *Prog. Biophys. Mol. Biol.*, **18:** 123–183.

Ebashi, S. and Ogawa, Y. (1988), Troponin C and calmodulin as calcium receptors: mode of action and sensitivity to drugs, in *Handbook of Experimental Pharmacology*, Vol. 83, *Calcium in Drug Actions*, Baker, P. F., Ed., Springer-Verlag, Berlin, 31–56.

Ehrlich, B. E. and Watras, J. (1988), Inositol 1,4,5-trisphosphate activates a channel from smooth muscle sarcoplasmic reticulum, *Nature (London)*, **336:** 583–586.

El-Hayek, R., Valdivia, C., Valdivia, H. H., Hogan, K., and Coronado, R. (1993), Activation of the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum by palmitoyl carnitine, *Biophys. J.*, **65:** 779–789.

Ellisman, M. H., Deerinck, T. J., Ouyang, Y., Beck, C. F., Tanksley, S. J., Walton, P. D., Airey, J. A., and Sutko, J. L. (1990), Identification and localization of ryanodine binding proteins in the avian central nervous system, *Neuron*, **5:** 135–146.

Endo, M. (1975), Mechanism of action of caffeine on the sarcoplasmic reticulum of skeletal muscle, *Proc. Jpn. Acad.*, **51:** 479–484.

Endo, M. (1977), Calcium release from the sarcoplasmic reticulum, *Physiol. Rev.*, **57:** 71–108.

Endo, M. (1981), Mechanism of calcium-induced calcium release in the SR membrane, in *The Mechanism of Gated Calcium Transport across Biological Membranes*, Ohnishi, S. T. and Endo, M., Eds., Academic Press, New York, 257–264.

Endo, M. (1985), Calcium release from sarcoplasmic reticulum, *Curr. Top. Membr. Trans.*, **25:** 181–230.

Endo, M., Kakuta, Y., and Kitazawa, T. (1981), A further study of the Ca-induced Ca release mechanism, in *The Regulation of Muscle Contraction: Excitation-Contraction Coupling*, Grinnell, A. D. and Brazier, M. A., Eds., Academic Press, New York, 181–195.

Endo, M., Tanaka, M., and Ogawa, Y. (1970), Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres, *Nature (London)*, **228:** 34–36.

Endo, M., Yagi, S., Ishizuka, T., Horiuti, K., Koga, K., and Amaha, K. (1983), Changes in the Ca-induced Ca release mechanism in the sarcoplasmic reticulum of the muscle from a patient with malignant hyperthermia, *Biomed. Res.*, **4:** 83–92.

Fabiato, A. (1983), Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum, *Am. J. Physiol.*, **245:** C1–C14.

Fabiato, A. (1984), Dependence of the Ca^{2+}-induced release from the sarcoplasmic reticulum of skinned skeletal muscle fibres from the frog semitendinosus on the rate of change of free Ca^{2+} concentration at the outer surface of the sarcoplasmic reticulum, *J. Physiol.*, **353:** 56P.

Fabiato, A. (1985a), Effects of ryanodine in skinned cardiac cells, *Fed. Proc.*, **44:** 2970–2976.

Fabiato, A. (1985b). Calcium-induced release of calcium from the sarcoplasmic reticulum, *J. Gen. Physiol.*, **85:** 189–320.

Fabiato, A. and Fabiato, F. (1978), Calcium-induced release of calcium from the sarcoplasmic reticulum of skinned cells from adult human, dog, cat, rabbit, rat, and frog hearts and from fetal and new-born rat ventricles, *Ann. N.Y. Acad. Sci.*, **307:** 491–522.

Ferguson, D. G. and Franzini-Armstrong, C. (1988), The Ca^{2+} ATPase content of slow and fast twitch fibers of guinea pig, *Muscle Nerve*, **11:** 561–570.

Ferguson, D. G., Schwartz, H. W., and Franzini-Armstrong, C. (1984), Subunit structure of junctional feet in triads of skeletal muscle: a freeze-drying, rotary-shadowing study, *J. Cell Biol.*, **99:** 1735–1742.

Ferris, C. D. and Snyder, S. H. (1992), Inositol 1,4,5-trisphosphate-activated calcium channels, *Annu. Rev. Physiol.*, **54:** 469–488.

Fill, M., Coronado, R., Mickelson, J. R., Vilven, J., Ma, J., Jacobson, B. A., and Louis, C. F. (1990), Abnormal ryanodine receptor channels in malignant hyperthermia, *Biophys. J.*, **57:** 471–475.

Fleischer, S. and Inui, M. (1989), Biochemistry and biophysics of excitation-contraction coupling, *Annu. Rev. Biophys. Biophys. Chem.*, **18:** 333–364.

Fleischer, S., Ogunbunmi, E. M., Dixon, M. C., and Fleer, E. A. M. (1985), Localization of Ca^{2+} release channels with ryanodine in junctional terminal cisternal of sarcoplasmic reticulum of fast skeletal muscle, *Proc. Natl. Acad. Sci. U.S.A.*, **82:** 7256–7259.

Ford, L. E. and Podolsky, R. J. (1970), Regenerative calcium release within muscle cells, *Science*, **167:** 58–59.

Franzini-Armstrong, C. (1970), Studies of the triad. I. Structure of the junction in frog twitch fibers, *J. Cell Biol.*, **47:** 488–499.

Franzini-Armstrong, C. (1975), Membrane particles and transmission at the triad, *Fed. Proc.*, **34:** 1382–1389.

Franzini-Armstrong, C., Ferguson, D. G., and Champ, C. (1988), Discrimination between fast- and slow-twitch fibres of guinea pig skeletal muscle using the relative surface density of junctional transverse tubule membrane, *J. Muscle Res. Cell Motil.*, **9:** 403–414.

Franzini-Armstrong, C., Heuser, J. E., Reese, T. S., Somlyo, A. P., and Somlyo, A. V. (1978), T-tubule swelling in hypertonic solutions: a freeze substitution study, *J. Physiol.*, **283:** 133–140.

Franzini-Armstrong, C. and Jorgensen, A. O. (1994), Structure and development of E-C coupling units in skeletal muscle, *Annu. Rev. Physiol.*, **56:** 509–534.

Franzini-Armstrong, C. and Nunzi, G. (1983), Junctional feet and particles in the triads of a fast-twitch muscle fibre, *J. Muscle Res. Cell Motil.*, **4:** 233–252.

Franzini-Armstrong, C., Pincon-Raymond, M., and Rieger, F. (1991), Muscle fibers from dysgenic mouse *in vivo* lack a surface component of peripheral couplings, *Dev. Biol.*, **146:** 364–376.

Friel, D. D. and Tsien, R. W. (1992), A caffeine- and ryanodine-sensitive Ca^{2+} store in bullfrog sympathetic neurones modulates effects of Ca^{2+} entry on $[Ca^{2+}]i$, *J. Physiol.*, **450:** 217–246.

Fuchs, R. (1991), MacPattern: protein pattern searching on the Apple Macintosh, *Comput. Applic. Biosci.*, **7:** 105–106.

Fujii, J., Otsu, K., Zorzato, F., DeLeon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J., and MacLennan, D. H. (1991), Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia, *Science*, **253:** 448–451.

Fujino, M., Yamaguchi, T., and Suzuki, K. (1961), "Glycerol effect" and the mechanism linking excitation of the plasma membrane with contraction, *Nature (London)*, **192:** 1159–1161.

Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N., and Mikoshiba, K. (1989), Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400, *Nature (London)*, **342:** 32–38.

Galione, A. (1992), Ca^{2+}-induced Ca^{2+} release and its modulation by cyclic ADP-ribose, *Trends Pharmacol. Sci.*, **13:** 304–306.

Galione, A. (1993), Cyclic ADP-ribose: a new way to control calcium, *Science*, **259:** 325–326.

Giannini, G., Clementi, E., Ceci, R., Marziali, G., and Sorrentino, V. (1992), Expression of a ryanodine receptor-Ca^{2+} channel that is regulated by TGF-β, *Science*, **257:** 91–94.

Gillard, E. F., Otsu, K., Fujii, J., Duff, C., DeLeon, S., Khanna, V. K., Britt, B. A., Worton, R. G., and MacLennan, D. H. (1992), Polymorphisms and deduced amino acid substitutions in the coding sequence of the ryanodine receptor (*RYR1*) gene in individuals with malignant hyperthermia, *Genomics*, **13:** 1247–1254.

Gillard, E. F., Otsu, K., Fujii, J., Khanna, V. K., DeLeon, S., Derdemezi, J., Britt, B. A., Duff, C. L., Worton, R. G., and MacLennan, D. H. (1991), A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia, *Genomics*, **11:** 751–755.

Györke, S. and Fill, M. (1993) Ryanodine receptor adaptation: control mechanisms of Ca^{2+}-induced Ca^{2+} release in heart, *Science*, **260:** 807–809.

Györke, S., Véléz, P., Suárez-Isla, B., and Fill, M. (1994), Activation of single cardiac and skeletal ryanodine receptor channels by flash photolysis of caged Ca^{2+}, *Biophys. J.*, **66:** 1879–1886.

Haan, E. A., Freemantle, C. J., McCure, J. A., Friend, K. L., and Mulley, J. C. (1990), Assignment of the gene for central core disease to chromosome 19, *Hum. Genet.*, **86:** 187–190.

Hakamata, Y., Nakai, J., Takeshima, H., and Imoto, K. (1992), Primary structure and distribution of a novel ryanodine receptor/calcium release channel from rabbit brain, *FEBS Lett.*, **312:** 229–235.

Hannon, J. D., Lee, N. K.-M., Yandong, C., and Blinks, J. R. (1992), Inositol trisphosphate (InsP3) causes contraction in skeletal muscle only under artificial conditions: evidence that Ca^{2+} release can result from depolarization of T-tubules, *J. Muscle Res. Cell Motil.*, **13:** 447–456.

Hansford, R. G. and Lakatta, E. G. (1987), Ryanodine releases calcium from sarcoplasmic reticulum in calcium-tolerant rat cardiac myocytes, *J. Physiol.*, **390:** 453–467.

Hanson, P. I. and Schulman, H. (1992), Neuronal Ca^{2+}/calmodulin-dependent protein kinases, *Annu. Rev. Biochem.*, **61:** 559–601.

Harbury, P. B., Zhang, T., Kim, P. S., and Alber, T. (1993), A switch between two-, three-, and four-stranded coiled coils in GCN4 leucine zipper mutants, *Science*, **262:** 1401–1407.

Hasan, G. and Rosbash, M. (1992), *Drosophila* homologs of two mammalian intracellular Ca^{2+}-release channels: identification and expression patterns of the inositol 1,4,5-triphosphate and the ryanodine receptor genes, *Development*, **116:** 967–975.

Haslett, W. L. and Jenden, D. J. (1961), A comparative study of the effect of ryanodine on muscle, *J. Cell. Comp. Physiol.*, **57:** 123–133.

Hasselbach, W. and Migala, A. (1992), How many ryanodine binding sites are involved in caffeine induced calcium release from sarcoplasmic reticulum terminal cysternae vesicles?, *Z. Naturforsch. Teil C*, **47:** 136–147.

Henkart, M., Landis, D. M. D., and Reese, T. S. (1976), Similarity of junctions between plasma membranes and endoplasmic reticulum in muscle and neurons, *J. Cell Biol.*, **70:** 338–347.

Herrmann-Frank, A., Darling, E., and Meissner, G. (1991), Functional characterization of the Ca^{2+}-gated Ca^{2+} release channel of vascular smooth muscle sarcoplasmic reticulum, *Pflugers Arch.*, **418:** 353–359.

Hilgemann, D. W., Delay, M. J., and Langer, G. A. (1983), Activation-dependent cumulative depletions of extracellular free calcium in guinea pig atrium measured with antipyrylazo III and tetramethylmurexide, *Circ. Res.*, **53:** 779–793.

Hollingworth, S., Harkins, A. B., Kurebayashi, N., Konishi, M., and Baylor, S. M. (1992), Excitation-contraction coupling in intact frog skeletal muscle fibers injected with mmolar concentration of fura-2, *Biophys. J.*, **63:** 224–234.

Homsher, E., Briggs, F. N., and Wise, R. M. (1974), Effects of hypertonicity on resting and contracting frog skeletal muscles, *Am. J. Physiol.*, **226:** 855–863.

Humerickhouse, R. A., Besch, H. R., Jr., Gerzon, K., Ruest, L., Sutko, J. L., and Emmick, J. T. (1993), Differential activating and deactivating effects of natural ryanodine congeners on the calcium release channel of sarcoplasmic reticulum: evidence for separation of effects at functionally distinct sites, *Mol. Pharmacol.*, **44:** 412–421.

Hwang, K. S. and van Breemen, C. (1987), Ryanodine modulation of ^{45}Ca efflux and tension in rabbit aortic smooth muscle, *Pflugers Arch.*, **408:** 343–350.

Hymel, L., Inui, M., Fleischer, S., and Schindler, H. (1988a), Purified ryanodine receptor of skeletal muscle sarcoplasmic reticulum forms Ca^{2+}-activated oligomeric Ca^{2+} channels in planar bilayers, *Proc. Natl. Acad. Sci. U.S.A.*, **85:** 441–445.

Hymel, L., Schindler, H., Inui, M., and Fleischer, S. (1988b), Reconstitution of purified cardiac muscle calcium release channel (ryanodine receptor) in planar bilayers, *Biochem. Biophys. Res. Commun.*, **152:** 308–314.

Iino, M., Kobayashi, T., and Endo, M. (1988), Use of ryanodine for functional removal of the calcium store in smooth muscle cells of the guinea-pig, *Biochem. Biophys. Res. Commun.*, **152:** 417–422.

Iino, M., Takano-Ohmuro, H., Kawana, Y., and Endo, M. (1992), Enhancement of Ca^{2+}-induced Ca^{2+} release in calpain treated rabbit skinned muscle fibers, *Biochem. Biophys. Res. Commun.*, **185:** 713–718.

Ikemoto, N., Antoniu, B., and Mészáros, L. G. (1985), Rapid flow chemical quench studies of calcium release from isolated sarcoplasmic reticulum, *J. Biol. Chem.*, **260:** 14,096–14,100.

Imagawa, T., Smith, J. S., Coronado, R., and Campbell, K. P. (1987), Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca^{2+}-permeable pore of the calcium release channel, *J. Biol. Chem.*, **262:** 16,636–16,643.

Imagawa, T., Takasago, T., and Shigekawa, M. (1989), Cardiac ryanodine receptor is absent in type I slow skeletal muscle fibers: immunochemical and ryanodine binding studies, *J. Biochem.*, **106:** 342–348.

Inui, M., Saito, A., and Fleischer, S. (1987a), Purification of the ryanodine receptor and identity with feet structures of junctional terminal cisternae of sarcoplasmic reticulum from fast skeletal muscle, *J. Biol. Chem.*, **262:** 1740–1747.

Inui, M., Saito, A., and Fleischer, S. (1987b), Isolation of the ryanodine receptor from cardiac sarcoplasmic reticulum and identity with the feet structures, *J. Biol. Chem.*, **262:** 15,637–15,642.

Ishizuka, T. and Endo, M. (1983), Effects of adenine on skinned fibers of amphibian fast skeletal muscle, *Proc. Jpn. Acad.*, **59:** 93–96.

Ishizuka, T., Iijima, T., and Endo, M. (1983), Effects of adenine on twitch and other contractile responses of single fibers of amphibian fast skeletal muscle, *Proc. Jpn. Acad.*, **59:** 97–100.

Ito, K., Takakura, S., Sato, K., and Sutko, J. L. (1986), Ryanodine inhibits the release of calcium from intracellular stores in guinea-pig aortic smooth muscle, *Circ. Res.*, **58:** 730–734.

Ivanenko, A., Baring, M. D., Airey, J. A., Sutko, J. L., and Kenyon, J. L. (1993), A caffeine- and ryanodine-sensitive Ca^{2+} store in avian sensory neurons, *J. Neurophysiol.*, **70:** 710–722.

Jacquemond, V., Csernoch, L., Klein, M. G., and Schneider, M. F. (1991), Voltage-gated and calcium-gated calcium release during depolarization of skeletal muscle fibers, *Biophys. J.*, **60:** 867–873.

Jaimovich, E. (1991), Chemical transmission at the triad: InsP3?, *J. Muscle Res. Cell Motil.*, **12:** 316–320.

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

Jefferies, P. R., Lam, W.-W., Toia, R. F., and Casida, J. E. (1992a), Ryania insecticide: structural assignments of four natural 8_{ax}-hydroxy-10-epiryanoids, *J. Agric. Food Chem.*, **40:** 509–512.

Jefferies, P. R., Toia, R. F., Brannigan, B., Pessah, I., and Casida, J. E. (1992b), Ryania insecticide: analysis and biological activity of 10 natural ryanoids, *J. Agric. Food Chem.*, **40:** 142–146.

Jenden, D. J. and Fairhurst, A. S. (1969), The pharmacology of ryanodine, *Pharmacol. Rev.*, **21:** 1–25.

Jewett, P. H., Sommer, J. R., and Johnson, E. A. (1971), Cardiac muscle: its ultrastructure in the finch and hummingbird with special reference to the sarcoplasmic reticulum, *J. Cell Biol.*, **49:** 50–65.

Johnson, K. (1993), Malignant hyperthermia hots up!, *Hum. Mol. Genet.*, **2:** 849.

Jones, L. R., Besch, H. R., Jr., Sutko, J. L., and Willerson, J. T. (1979), Ryanodine-induced stimulation of net Ca^{++} uptake by cardiac sarcoplasmic reticulum vesicles, *J. Pharmacol. Exp. Ther.*, **209:** 48–55.

Jong, D.-S., Pape, P. C., Chandler, W. K., and Baylor, S. M. (1993), Reduction of calcium inactivation of sarcoplas-

mic reticulum calcium release by fura-2 in voltage-clamped cut twitch fibers from frog muscle, *J. Gen. Physiol.*, **102:** 333–370.

Jorgensen, A. O., Shen, A. C. Y., Arnold, W., McPherson, P. S., and Campbell, K. P. (1993), The Ca^{2+}-release channel/ryanodine receptor is localized in junctional and corbular sarcoplasmic reticulum in cardiac muscle, *J. Cell Biol.*, **120:** 969–980.

Joulou-Schaeffer, G. and Freslon, J. L. (1988), Effects of ryanodine on tension development in rat aorta and mesenteric resistance vessels, *Br. J. Pharmacol.*, **95:** 605–613.

Kalow, W., Britt, B. A., Terreau, M. E., and Haist, C. (1970), Metabolic error of muscle metabolism after recovery from malignant hyperthermia, *Lancet*, **2:** 895–898.

Kanmura, Y., Missiaen, L., Raeymaekers, L., and Casteels, R. (1988), Ryanodine reduces the amount of calcium in intracellular stores of smooth-muscle cells of the rabbit ear artery, *Pflugers Arch.*, **413:** 153–159.

Kasai, M., Kawasaki, T., and Yamamoto, K. (1992), Permeation of neutral molecules through calcium channel in sarcoplasmic reticulum vesicles, *J. Biochem.*, **112:** 197–203.

Kasai, M. and Kometani, T. (1981), Ion permeable channels in sarcoplasmic reticulum membrane, in *Nerve Membrane: Biochemistry and Function of Channel Proteins*, Matsumoto, G. and Kotani, M., Eds., University of Tokyo Press, Tokyo, 151–164.

Kasai, M., Nunogaki, K., Nagasaki, K., Tanifuji, M., and Sokabe, M. (1985), Ion channels of sarcoplasmic reticulum vesicles and calcium release, in *Structure and Function of Sarcoplasmic Reticulum*, Fleischer, S. and Tonomura, Y., Eds., Academic Press, Orlando, FL, 537–559.

Kausch, K., Lehmann-Horn, F., Janka, M., Wieringa, B., Grimm, T., and Müller, C. R., (1991), Evidence for linkage of the central core disease locus to the proximal long arm of human chromosome 19, *Genomics*, **10:** 765–769.

Kawai, T., Ishii, Y., Imaizumi, Y., and Watanabe, M. (1991), Characteristics of [^{3}H]ryanodine binding to the rabbit cerebral microsomes, *Brain Res.*, **540:** 331–334.

Kawamoto, R. M., Brunschwig, J.-P., Kim, K. C., and Caswell, A. H. (1986), Isolation, characterization, and localization of the spanning protein from skeletal muscle triads, *J. Cell Biol.*, **103:** 1405–1414.

Kieny, M., Boutineau, A.-M., Pautou, M.-P., and Goetinck, P. F. (1988a), Muscular dysgenesis in fowl: ultrastructural study of skeletal muscles in the crooked neck dwarf (cn/cn) mutant, *Biol. Struct. Morphol.*, **1:** 15–27.

Kieny, M., Boutineau, A.-M., Pautou, M.-P., and Jouk, P. S. (1988b), The crooked neck dwarf muscular dysgenesis in fowl is due to a selective alteration of the somitic myogenic cell line, *Roux's Arch. Dev. Biol.*, **197:** 49–55.

Kieny, M., Mauger, A., Hedayat, I., and Goetinck, P. F. (1983), Ontogeny of the leg muscle tissue in the crooked neck dwarf mutant (cn/cn) chick embryo, *Arch. Anat. Micro. Morphol. Exp.*, **72:** 1–17.

Kijima, Y., Saito, A., Jetton, T. L., Magnuson, M. A., and Fleischer, S. (1993), Different intracellular localization of inositol 1,4,5-trisphosphate and ryanodine receptors in cardiomyocytes, *J. Biol. Chem.*, **268:** 3499–3506.

Knudson, C. M., Stang, K. K., Jorgensen, A. O., and Campbell, K. P. (1993a), Biochemical characterization and ultrastructural localization of a major junctional sarcoplasmic reticulum glycoprotein (Triadin), *J. Biol. Chem.*, **268:** 12,637–12,645.

Knudson, C. M., Stang, K. K., Moomaw, C. R., Slaughter, C. A., and Campbell, K. P. (1993b), Primary structure and topological analysis of a skeletal muscle-specific junctional sarcoplasmic reticulum glycoprotein (Triadin), *J. Biol. Chem.*, **268:** 12,646–12,654.

Kobayashi, T. and Endo, M. (1988), Temperature-dependent inhibition of caffeine contracture of mammalian skeletal muscle by dantrolene, *Proc. Jpn. Acad.*, **64:** 76–79.

Koch-Weser, J. (1963), Influence of osmolarity of perfusate on contractility of mammalian myocardium, *Am. J. Physiol.*, **204:** 957–962.

Konishi, M., Olson, A., Hollingworth, S., and Baylor, S. M. (1988), Myoplasmic binding of fura-2 investigated by steady-state fluorescence and absorbance measurements. *Biophys. J.*, **54:** 1089–1104.

Kurebayashi, N. and Ogawa, Y. (1982), Effect of halothane on the calcium activated ATPase reaction of fragmented sarcoplasmic reticulum in reference to the Ca-releasing action, *J. Biochem.*, **92:** 907–913.

Kurebayashi, N. and Ogawa, Y. (1986), Characterization of increased Ca^{2+} efflux by quercetin from the sarcoplasmic reticulum in frog skinned skeletal muscle fibres, *J. Muscle Res. Cell Motil.*, **7:** 142–150.

Kuwajima, G., Futatsugi, A., Niinobe, M., Nakanishi, S., and Mikoshiba, K. (1992), Two types of ryanodine receptors in mouse brain: skeletal muscle type exclusively in Purkinje cells and cardiac muscle type in various neurons, *Neuron*, **9:** 1133–1142.

Lai, F. A., Anderson, K., Rousseau, E., Liu, Q.-Y., and Meissner, G. (1988a), Evidence for a Ca^{2+} channel within the ryanodine receptor complex from cardiac sarcoplasmic reticulum, *Biochem. Biophys. Res. Commun.*, **151:** 441–449.

Lai, F. A., Erickson, H. P., Rousseau, E., Liu, Q.-Y., and Meissner, G. (1988b), Purification and reconstitution of the calcium release channel from skeletal muscle, *Nature (London)*, **331:** 315–319.

Lai, F. A., Dent, M., Wickenden, C., Xu, L., Kumari, G., Misra, M., Lee, H. B., Sar, M., and Meissner, G. (1992a), Expression of a cardiac Ca^{2+}-release channel isoform in mammalian brain, *Biochem. J.*, **288:** 553–564.

Lai, F. A., Liu, Q. Y., Xu, L., El-Hashem, A., Kramarcy, N. R., Sealock, R., and Meissner, G. (1992b), Amphibian ryanodine receptor isoforms are related to those of mammalian skeletal or cardiac muscle, *Am. J. Physiol.*, **263:** C365–C372.

Lai, F. A., Misra, M., Xu, L., Smith, H. A., and Meissner, G. (1989), The ryanodine receptor-Ca^{2+} release channel complex of skeletal muscle sarcoplasmic reticulum:

evidence for a cooperatively coupled, negatively charged homotetramer, *J. Biol. Chem.*, **264:** 16,776–16,785.

Lamb, G. D. (1992), DHP receptors and excitation-contraction coupling, *J. Muscle Res. Cell Motil.*, **13:** 394–405.

Lamb, G. D., Stephenson, D. G., and Stienen, G. J. M. (1993), Effects of osmolality and ionic strength on the mechanism of Ca^{2+} release in skinned skeletal muscle fibres of the toad, *J. Physiol.*, **464:** 629–648.

Lännergren, J. and Noth, J. (1973), Tension in isolated frog muscle fibers induced by hypertonic solutions, *J. Gen. Physiol.*, **61:** 158–175.

Lattanzio, F. A., Jr., Schlatterer, R. G., Nicar, M., Campbell, K. P., and Sutko, J. L. (1987), The effects of ryanodine on passive calcium fluxes across sarcoplasmic reticulum membranes, *J. Biol. Chem.*, **262:** 2711–2718.

Lea, T. J., Griffiths, P. J., Tregear, R. T., and Ashley, C. C. (1986), An examination of the ability of inositol 1,4,5-trisphosphate to induce calcium release and tension development in skinned skeletal muscle fibres of frog and crustacea, *FEBS Lett.*, **207:** 153–161.

Lee, H. C. (1993), Potentiation of calcium- and caffeine-induced calcium release by cyclic ADP-ribose, *J. Biol. Chem.*, **268:** 293–299.

Lesh, R. E., Marks, A. R., Somlyo, A. V., Fleischer, S., and Somlyo, A. P. (1993), Antiryanodine receptor antibody binding sites in vascular and endocardial endothelium, *Circ. Res.*, **72:** 481–488.

Lindsay, A. R. G. and Williams, A. J. (1991), Functional characterization of the ryanodine receptor purified from sheep cardiac muscle sarcoplasmic reticulum, *Biochim. Biophys. Acta*, **1064:** 89–102.

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

Lu, X., Xu, L., and Meissner, G. (1994), Activation of the skeletal muscle calcium release channel by a cytoplasmic loop of the dihydropyridine receptor, *J. Biol. Chem.*, **269:** 6511–6516.

Lüttgau, H. C. and Oetliker, H. (1968), The action of caffeine on the activation of the contractile mechanism in striated muscle fibres, *J. Physiol.*, **194:** 51–74.

MacKenzie, A. E., Korneluk, R. G., Zorzato, F., Fujii, J., Phillips, M., Iles, D., Wieringa, B., Leblond, S., Bailly, J., Willard, H. F., Duff, C., Worton, R. G., and MacLennan, D. H. (1990), The human ryanodine receptor gene: its mapping to 19q13.1, placement in a chromosome 19 linkage group, and exclusion as the gene causing myotonic dystrophy, *Am. J. Hum. Genet.*, **46:** 1082–1089.

MacLennan, D. H. (1990), Molecular tools to elucidate problems in excitation-contraction coupling, *Biophys. J.*, **58:** 1355–1365.

MacLennan, D. H., Duff, C., Zorzato, F., Fujii, J., Phillips, M., Korneluk, R. G., Frodis, W., Britt, B. A., and Worton, R. G. (1990), Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia, *Nature (London)*, **343:** 559–561.

MacLennan, D. H. and Phillips, M. S. (1992), Malignant hyperthermia, *Science*, **256:** 789–794.

Maeda, N., Kawasaki, T., Nakade, S., Yokota, N., Taguchi, T., Kasai, M., and Mikoshiba, K. (1991), Structural and functional characterization of inositol 1,4,5-trisphosphate receptor channel from mouse cerebellum, *J. Biol. Chem.*, **266:** 1109–1116.

Marban, E. and Wier, W. G. (1985), Ryanodine as a tool to determine the contributions of calcium entry and calcium release to the Ca transient and contraction of cardiac Purkinje fibers, *Circ. Res.*, **56:** 133–138.

Marks, A. R., Fleischer, S., and Tempst, P. (1990), Surface topography analysis of the ryanodine receptor/junctional channel complex based on proteolysis sensitivity mapping, *J. Biol. Chem.*, **265:** 13,143–13,149.

Martin, J., Geromanos, S., Tempst, P., and Hartl, F. U. (1993), Identification of nucleotide-binding regions in the chaperonin proteins GroEL and GroES, *Nature (London)*, **366:** 279–282.

Martonosi, A. N. and Beeler, T. J. (1983), Mechanism of Ca^{2+} transport by sarcoplasmic reticulum, in *Handbook of Physiology, Skeletal Muscle*, Peackey, L. D., Adrian, R. H., and Geiger, S. R., Eds., American Physiological Society, Bethesda, MD, 417–485.

McCarthy, T. V., Healy, J. M. S., Heffron, J. J. A., Lehane, M., Deufel, T., Lehmann-Horn, F., Farrall, M., and Johnson, K. (1990), Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2, *Nature (London)*, **343:** 562–564.

McGrew, S. G., Wolleben, C., Siegl, P., Inui, M., and Fleischer, S. (1989), Positive cooperativity of ryanodine binding to the calcium release channel of sarcoplasmic reticulum from heart and skeletal muscle, *Biochemistry*, **28:** 1686–1691.

McPherson, P. S. and Campbell, K. P. (1990), Solubilization and biochemical characterization of the high affinity [^{3}H]ryanodine receptor from rabbit brain membranes, *J. Biol. Chem.*, **265:** 18,454–18,460.

McPherson, P. S. and Campbell, K. P. (1993a), The ryanodine receptor/Ca^{2+} release channel, *J. Biol. Chem.*, **268:** 13,766–13,768.

McPherson, P. S. and Campbell, K. P. (1993b), Characterization of the major brain form of the ryanodine receptor/Ca^{2+} release channel, *J. Biol. Chem.*, **268:** 19,785–19,790.

McPherson, P. S., Kim, Y. K., Valdivia, H., Knudson, C. M., Takekura, H., Franzini-Armstrong, C., Coronado, R., and Campbell, K. P. (1991), The brain ryanodine receptor: a caffeine-sensitive calcium release channel, *Neuron*, **7:** 17–25.

Meissner, G. (1986), Ryanodine activation and inhibition of the Ca^{2+} release channel of sarcoplasmic reticulum, *J. Biol. Chem.*, **261:** 6300–6306.

Meissner, G. and El-Hashem, A. (1992), Ryanodine as a functional probe of the skeletal muscle sarcoplasmic reticulum Ca^{2+} release channel, *Mol. Cell. Biochem.*, **114:** 119–123.

Meissner, G. and Henderson, J. S. (1987), Rapid calcium release from cardiac sarcoplasmic reticulum vesicles is dependent on Ca^{2+} and is modulated by Mg^{2+}, adenine

nucleotide, and calmodulin, *J. Biol. Chem.*, **262:** 3065–3073.

Meissner, G. and McKinley, D. (1976), Permeability of sarcoplasmic reticulum membrane. The effect of changed ionic environments on Ca^{2+} release, *J. Membr. Biol.*, **30:** 79–98.

Meissner, G., Rousseau, E., and Lai, A. F. (1989), Structural and functional correlation of the trypsin-digested Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum, *J. Biol. Chem.*, **264:** 1715–1722.

Melzer, W., Rios, E., and Schneider, M. F. (1984), Time course of calcium release and removal in skeletal muscle fibers, *Biophys. J.*, **45:** 637–641.

Mészáros, L. G., Bak, J., and Chu, A. (1993), Cyclic ADP-ribose as an endogenous regulator of the non-skeletal type ryanodine receptor Ca^{2+} channel, *Nature (London)*, **364:** 76–79.

Mészáros, L. G. and Volpe, P. (1991), Caffeine- and ryanodine-sensitive Ca^{2+} stores of canine cerebrum and cerebellum neurons, *Am. J. Physiol.*, **261:** C1048–C1054.

Michalak, M., Dupraz, P., and Shoshan-Barmatz, V. (1988), Ryanodine binding to sarcoplasmic reticulum membrane: comparison between cardiac and skeletal muscle, *Biochim. Biophys. Acta*, **939:** 587–594.

Mickelson, J. R., Knudson, C. M., Kennedy, C. F. H., Yang, D. I., Litterer, L. A., Rempel, W. E., Campbell, K. P., and Louis, C. F. (1992), Structural and functional correlates of a mutation in the malignant hyperthermia-susceptible pig ryanodine receptor, *FEBS Lett.*, **301:** 49–52.

Mignery, G. A., Newton, C. L., Archer, B. T., III, and Südhof, T. C. (1990), Structure and expression of the rat inositol 1,4,5-trisphosphate receptor, *J. Biol. Chem.*, **265:** 12,679–12,685.

Mignery, G. A. and Südhof, T. C. (1990), The ligand binding site and transduction mechanism in the inositol-1,4,5-trisphosphate receptor, *EMBO J.*, **9:** 3893–3898.

Mikami, A., Imoto, K., Tanabe, T., Niidome, T., Mori, Y., Takeshima, H., Narumiya, S., and Numa, S., (1989), Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel, *Nature (London)*, **340:** 230–233.

Mikos, G. J. and Snow, T. R. (1987), Failure of inositol 1,4,5-trisphosphate to elicit or potentiate Ca^{2+} release from isolated skeletal muscle sarcoplasmic reticulum, *Biochim. Biophys. Acta*, **927:** 256–260.

Mikoshiba, K. (1993), Inositol 1,4,5-trisphosphate receptor, *Trends Pharmacol. Sci.*, **14:** 86–89.

Mitchell, M. R., Powell, T., Terrar, D. A., and Twist, V. W. (1984), Ryanodine prolongs Ca-currents while suppressing contraction in rat ventricular muscle cells, *Br. J. Pharmacol.*, **81:** 13–15.

Miyawaki, A., Furuichi, T., Ryou, Y., Yoshikawa, S., Nakagawa, T., Saitoh, T., and Mikoshiba, K. (1991), Structure-function relationships of the mouse inositol 1,4,5-trisphosphate receptor, *Proc. Natl. Acad. Sci. U.S.A.*, **88:** 4911–4915.

Morrissette, J., Heisermann, G., Cleary, J., Ruoho, A., and Coronado, R. (1993), Cyclic ADP-ribose induced Ca^{2+} release in rabbit skeletal muscle sarcoplasmic reticulum, *FEBS Lett.*, **330:** 270–274.

Movsesian, M. A., Thomas, A. P., Selak, M., and Williamson, J. R. (1985), Inositol trisphosphate does not release Ca^{2+} from permeabilized cardiac myocytes and sarcoplasmic reticulum, *FEBS Lett.*, **185:** 328–332.

Murayama, T. and Ogawa, Y. (1992), Purification and characterization of two ryanodine-binding protein isoforms from sarcoplasmic reticulum of bullfrog skeletal muscle, *J. Biochem.*, **112:** 514–522.

Murayama, T. and Ogawa, Y. (1993), Potential factors which affect the Ca^{2+}-sensitivity of ryanodine receptors, *Biomed. Res.*, **14 (Suppl. 2):** 71–72.

Nagasaki, K. and Kasai, M. (1980), Magnesium permeability of sarcoplasmic reticulum vesicles monitored in terms of chlortetracycline fluorescence, *J. Biochem.*, **87:** 709–716.

Nakai, J., Imagawa, T., Hakamata, Y., Shigekawa, M., Takeshima, H., and Numa, S. (1990), Primary structure and functional expression from cDNA of the cardiac ryanodine receptor/calcium release channel, *FEBS Lett.*, **271:** 169–177.

Nakanishi, S., Kuwajima, G., and Mikoshiba, K. (1992), Immunohistochemical localization of ryanodine receptors in mouse central nervous system, *Neurosci. Res.*, **15:** 130–142.

Natori, R. (1954), The property and contraction process of isolated myofibrils, *Jikeikai Med. J.*, **1:** 119–126.

Nosek, T. M., Williams, M. F., Zeigler, S. T., and Godt, R. E. (1986), Inositol trisphosphate enhances calcium release in skinned cardiac and skeletal muscle, *Am. J. Physiol.*, **250:** C807–C811.

Novella, I. S., Borrego, B., Mateu, M. G., Domingo, E., Giralt, E., and Andreu, D. (1993), Use of substituted and tandem-repeated peptides to probe the relevance of the highly conserved RGD tripeptide in the immune response against foot-and-mouth disease virus, *FEBS Lett.*, **330:** 253–259.

O'Brien, J., Meissner, G., and Block, B. A. (1993), The fastest contracting muscles of nonmammalian vertebrates express only one isoform of the ryanodine receptor, *Biophys. J.*, **65:** 2418–2427.

Ogawa, Y. (1970), Some properties of fragmented frog sarcoplasmic reticulum with particular reference to its response to caffeine, *J. Biochem.*, **67:** 667–683.

Ogawa, Y. and Ebashi, S. (1973), Ca^{2+} uptake and release by fragmented sarcoplasmic reticulum with special reference to the effect of β,γ-methylene adenosine triphosphate, in *Organization of Energy Transducing Membranes*, Nakao, M. and Packer, L., Eds., University of Tokyo Press, Tokyo, 127–140.

Ogawa, Y. and Ebashi, S. (1976), Ca-releasing action of β,γ-methylene adenosine triphosphate on fragmented sarcoplasmic reticulum, *J. Biochem.*, **80:** 1149–1157.

Ogawa, Y. and Harafuji, H. (1989), Ca-release by phosphoinositides from sarcoplasmic reticulum of frog skeletal muscle, *J. Biochem.* **106:** 864–867.

Ogawa, Y. and Harafuji, H. (1990a), Effect of temperature on [^{3}H]ryanodine binding to sarcoplasmic reticulum

from bullfrog skeletal muscle, *J. Biochem.*, **107:** 887–893.

Ogawa, Y. and Harafuji, H. (1990b), Osmolarity-dependent characteristics of [^{3}H]ryanodine binding to sarcoplasmic reticulum, *J. Biochem.*, **107:** 894–898.

Ogawa, Y., Harafuji, H., and Kurebayashi, N. (1985), Mechanism of calcium release from sarcoplasmic reticulum: effect of caffeine on magnesium binding to fragmented sarcoplasmic reticulum from bullfrog skeletal muscle, in *Structure and Function of Sarcoplasmic Reticulum,* Fleischer, S. and Tonomura, Y., Eds., Academic Press, Orlando, FL, 411–427.

Ogawa, Y., Harigaya, S., Ebashi, S., and Lee, K. S. (1971), Sarcoplasmic reticulum: calcium uptake and release systems in muscle, in *Methods in Pharmacology,* Vol. 1, Schwartz, A., Ed., Appleton-Century-Crofts, New York, 327–346.

Ogawa, Y. and Kurebayashi, N. (1982), The Ca-releasing action of halothane on fragmented sarcoplasmic reticulum, *J. Biochem.*, **92:** 899–905.

Ogawa, Y., Kurebayashi, N., Irimajiri, A., and Hanai, T. (1981), Transient kinetics for Ca uptake by fragmented sarcoplasmic reticulum from bullfrog skeletal muscle with reference to the rate of relaxation of living muscle, in *Molecular and Cellular Aspects of Muscle Function,* Varga, E., Kövér, A., Kovács, T., and Kovács, L., Eds., Pergamon Press and Akademiai Kiado, Budapest, 417–435.

Ohta, T. and Endo, M. (1986), Inhibition of calcium-induced calcium release by dantrolene at mammalian body temperature, *Proc. Jpn. Acad.*, **62:** 329–332.

Olivares, E. B., Tanksley, S. J., Airey, J. A., Beck, C. F., Ouyang, Y., Deerinck, T. J., Ellisman, M. H., and Sutko, J. L. (1991), Nonmammalian vertebrate skeletal muscles express two triad junctional foot protein isoforms, *Biophys. J.*, **59:** 1153–1163.

Otsu, K., Fujii, J., Periasamy, M., Difilippantonio, M., Uppender, M., Ward, D. C., and MacLennan, D. H. (1993), Chromosome mapping of five human cardiac and skeletal muscle sarcoplasmic reticulum protein genes, *Genomics,* **17:** 507–509.

Otsu, K., Khanna, V. K., Archibald, A. L., and MacLennan, D. H. (1991), Cosegregation of porcine malignant hyperthermia and a probable causal mutation in the skeletal muscle ryanodine receptor gene in backcross families, *Genomics,* **11:** 744–750.

Otsu, K., Willard, H. F., Khanna, V. K., Zorzato, F., Green, N. M., and MacLennan, D. H. (1990), Molecular cloning of cDNA encoding the Ca^{2+} release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum, *J. Biol. Chem.*, **265:** 13,472–13,483.

Ouyang, Y., Deerinck, T. J., Walton, P. D., Airey, J. A., Sutko, J. L., and Ellisman, M. H. (1993), Distribution of ryanodine receptors in the chicken central nervous system, *Brain Res.*, **620:** 269–280.

Oyamada, H., Iino, M., and Endo, M. (1993), Effects of ryanodine on the properties of Ca^{2+} release from the sarcoplasmic reticulum in skinned skeletal muscle fibres of the frog, *J. Physiol.*, **470:** 335–348.

Oyamada, H., Murayama, T., Takagi, T., Iino, M., Iwabe, N., Miyata, T., Ogawa, Y., and Endo, M. (1994), Primary structure and distribution of ryanodine-binding protein isoforms of the bullfrog skeletal muscle, *J. Biol. Chem.*, **269:** 17,206–17,214.

Ozawa, E., Hosoi, K., and Ebashi, S. (1967), Reversible stimulation of muscle phosphorylase *b* kinase by low concentration of calcium ions, *J. Biochem.*, **61:** 531–533.

Padua, R. A., Wan, W., Nagy, J. I., and Geiger, J. D. (1991), [^{3}H]Ryanodine binding sites in rat brain demonstrated by membrane binding and autoradiography, *Brain Res.*, **542:** 135–140.

Padua, R. A., Yamamoto, T., Fyda, D., Sawchuk, M. A., Geiger, J. D., and Nagy, J. I. (1992), Autoradiographic analysis of [^{3}H]ryanodine binding sites in rat brain: regional distribution and the effects of lesions on sites in the hippocampus, *J. Chem. Neuroanat.*, **5:** 63–73.

Page, E. and Upshaw-Earley, J. (1977), Volume changes in sarcoplasmic reticulum of rat hearts perfused with hypertonic solutions, *Circ. Res.*, **40:** 355–366.

Palade, P. (1987), Drug-induced Ca^{2+} release from isolated sarcoplasmic reticulum. I. Use of phyrophosphate to study caffeine-induced Ca^{2+} release, *J. Biol. Chem.*, **262:** 6135–6141.

Pape, P. C., Jong, D.-S., Chandler, W. K., and Baylor, S. M. (1993), Effect of fura-2 on action potential-stimulated calcium release in cut twitch fibers from frog muscle, *J. Gen. Physiol.*, **102:** 295–332.

Parker, I. and Zhu, P. H. (1987), Effect of hypertonic solutions on calcium transients in frog twitch muscle fibres, *J. Physiol.*, **383:** 615–627.

Pessah, I. N., Francini, A. O., Scales, D. J., Waterhouse, A. L., and Casida, J. E. (1986), Calcium-ryanodine receptor complex. Solubilization and partial characterization from skeletal muscle junctional sarcoplasmic reticulum vesicles, *J. Biol. Chem.*, **261:** 8643–8648.

Pessah, I. N., Stambuk, R. A., and Casida, J. E. (1987), Ca^{2+}-activated ryanodine binding: mechanisms of sensitivity and intensity modulation by Mg^{2+}, caffeine, and adenine nucleotides, *Mol. Pharmacol.*, **31:** 232–238.

Pessah, I. N., Waterhouse, A. L., and Casida, J. E. (1985), The calcium-ryanodine receptor complex of skeletal and cardiac muscle, *Biochem. Biophys. Res. Commun.*, **128:** 449–456.

Putney, J. W., Jr. (1993), Excitement about calcium signaling in inexcitable cells, *Science,* **262:** 676–678.

Putney, J. W. and Bird, G. S. (1993), The inositol phosphate-calcium signaling system in nonexcitable cells, *Endocr. Rev.*, **14:** 610–631.

Quane, K. A., Healy, J. M. S., Keating, K. E., Manning, B. M., Couch, F. J., Palmucci, L. M., Doriguzzi, C., Fagerlund, T. H., Berg, K., Ording, H., Bendixen, D., Mortier, W., Linz, U., Muller, C. R., and McCarty, T. V. (1993), Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia, *Nature Genet.*, **5:** 51–55.

Radermacher, M., Wagenknecht, T., Grassucci, R., Frank, J., Inui, M., Chadwick, C., and Fleischer, S. (1992), Cryo-

EM of the native structure of the calcium release channel/ryanodine receptor from sarcoplasmic reticulum, *Biophys. J.*, **61:** 936–940.

Rios, E. and Brum, G. (1987), Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle, *Nature (London)*, **325:** 717–720.

Rios, E., Ma, J., and González, A. (1991), The mechanical hypothesis of excitation-contraction (EC) coupling in skeletal muscle, *J. Muscle Res. Cell Motil.*, **12:** 127–135.

Rios, E. and Pizarro, G. (1991), Voltage sensor of excitation-contraction coupling in skeletal muscle, *Physiol. Rev.*, **71:** 849–908.

Rios, E., Pizarro, G., and Stefani, E. (1992), Charge movement and the nature of signal transduction in skeletal muscle excitation-contraction coupling, *Annu. Rev. Physiol.*, **54:** 109–133.

Rogers, E. F., Koniuszy, F. R., Shavel, J., Jr., and Folkers, K. (1948), Plant insecticides. I. Ryanodine, a new alkaloid from *Ryania speciosa* Vahl, *J. Am. Chem. Soc.*, **70:** 3086–3088.

Ross, E. M. (1990), Pharmacodynamics: mechanisms of drug action and the relationship between drug concentration and effect, in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed., Gilman, A. G., Rall, T. W., Nies, A. S., and Taylor, P., Eds., Pergamon Press, New York, 33–40.

Rousseau, E., Smith, J. S., Henderson, J. S., and Meissner, G. (1986), Single channel and $^{45}Ca^{2+}$ flux measurements of the cardiac sarcoplasmic reticulum calcium channel, *Biophys. J.*, **50:** 1009–1014.

Rousseau, E., Smith, J. S., and Meissner, G. (1987), Ryanodine modifies conductance and gating behavior of single Ca^{2+} release channel, *Am. J. Physiol.*, **253:** C364–C368.

Ruest, L., Taylor, D. R., and Deslongchamps, P. (1985), Investigation of the constituents of *Ryania Speciosa*, *Can. J. Chem.*, **63:** 2840–2843.

Ruoslahti, E. and Pierschbacher, M. D. (1986), Arg-Gly-Asp: a versatile cell recognition signal, *Cell*, **44:** 517–518.

Saito, A., Inui, M., Radermacher, M., Frank, J., and Fleischer, S. (1988), Ultrastructure of the calcium release channel of sarcoplasmic reticulum, *J. Cell. Biol.*, **107:** 211–219.

Sakai, T. (1963), Action of the local anesthetics on the mechanical response of the caffeinized muscle by rapid cooling, *Jikeikai Med. J.*, **10:** 113–120.

Sakai, T. (1965), The effects of temperature and caffeine on activation of the contractile mechanism in the striated muscle fibres, *Jikeikai Med. J.*, **12:** 88–102.

Scherer, N. M. and Ferguson, J. E. (1985), Inositol 1,4,5-trisphosphate is not effective in releasing calcium from skeletal sarcoplasmic reticulum microsomes, *Biochem. Biophys. Res. Commun.*, **128:** 1064–1070.

Schneider, M. F. and Chandler, W. K. (1973), Voltage dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling, *Nature (London)*, **242:** 244–246.

Schwartz, L. M., McCleskey, E. W., and Almers, W. (1985), Dihydropyridine receptors in muscle are voltage-dependent but most are not functional calcium channels, *Nature (London)*, **314:** 747–751.

Seok, J. H., Xu, L., Kramarcy, N. R., Sealock, R., and Meissner, G. (1992), The 30 S lobster skeletal muscle Ca^{2+} release channel (ryanodine receptor) has functional properties distinct from the mammalian channel proteins, *J. Biol. Chem.*, **267:** 15,893–15,901.

Simon, B. J., Klein, M. G., and Schneider, M. F. (1991), Calcium dependence of inactivation of calcium release from the sarcoplasmic reticulum in skeletal muscle fibers, *J. Gen. Physiol.*, **97:** 437–471.

Smith, J., Coronado, R., and Meissner, G. (1986), Single channel measurements of the calcium release channel from skeletal muscle sarcoplasmic reticulum: activation by Ca^{2+} and ATP and modulation by Mg^{2+}, *J. Gen. Physiol.*, **88:** 573–588.

Smith, J. S., Imagawa, T., Ma, J., Fill, M., Campbell, K. P., and Coronado, R. (1988), Purified ryanodine receptor from rabbit skeletal muscle is the calcium-release channel of sarcoplasmic reticulum, *J. Gen. Physiol.*, **92:** 1–26.

Somlyo, A. V., Gonzalez-Serratos, H., Shuman, H., McClellan, G., and Somlyo, A. P. (1981), Calcium release and ionic changes in the sarcoplasmic reticulum of tetanized muscle: an electron-probe study, *J. Cell Biol.*, **90:** 577–594.

Somlyo, A. V., Shuman, H., and Somlyo, A. P. (1977a), Composition of sarcoplasmic reticulum *in situ* by electron probe X-ray microanalysis, *Nature (London)*, **268:** 556–558.

Somlyo, A. V., Shuman, H., and Somlyo, A. P. (1977b), Elemental distribution in striated muscle and the effects of hypertonicity: electron probe analysis of cryo sections, *J. Cell Biol.*, **74:** 828–857.

Sommer, J. R. and Johnson, E. A. (1979), Ultrastructure of cardiac muscle, in *Handbook of Physiology*, Vol. 1, *The Cardiovascular System*, Berne, R. M., Sperelakis, N., and Geiger, S., Eds., American Physiological Society, Bethesda, MD, 113–186.

Sorrentino, V., Giannini, G., Malzac, P., and Mattei, M. G. (1993), Localization of a novel ryanodine receptor gene (RYR3) to human chromosome 15q14-q15 by *in situ* hybridization, *Genomics*, **18:** 163–165.

Sorrentino, V. and Volpe, P. (1993), Ryanodine receptors: how many, where and why?, *Trends Pharmacol. Sci.*, **14:** 98–103.

Spedding, M. and Paoletti, R. (1992), Classification of calcium channels and the sites of action of drugs modifying channel function, *Pharmacol. Rev.*, **44:** 363–376.

Sperelakis, N. and Rubio, R. (1971), Ultrastructural changes produced by hypertonicity in cat cardiac muscle, *J. Mol. Cell. Cardiol.*, **3:** 139–156.

Stein, M. B., Padua, R. A., Nagy, J. I., and Geiger, J. D. (1992), High affinity [^{3}H]ryanodine binding sites in postmortem human brain: regional distribution and effects of calcium, magnesium and caffeine, *Brain Res.*, **585:** 349–354.

Su, J. Y. (1987), Effects of ryanodine on skinned skeletal muscle fibers of the rabbit, *Pflugers Arch.*, **410:** 510–516.

Su, J. Y. (1988), Effects of ryanodine on skinned myocardial fibers of the rabbit, *Pflugers Arch.*, **411:** 132–136.

Suárez-Isla, B. A., Irribarra, V., Oberhauser, A., Larralde, L., Bull, R., Hidalgo, C., and Jaimovich, E. (1988), Inositol (1,4,5)-trisphosphate activates a calcium channel in isolated sarcoplasmic reticulum membranes, *Biophys. J.*, **54:** 737–741.

Sutko, J. L., Airey, J. A., Murakami, K., Takeda, M., Beck, C., Deerinck, T., and Ellisman, M. H. (1991), Foot protein isoforms are expressed at different times during embryonic chicken skeletal muscle development, *J. Cell Biol.*, **113:** 793–803.

Sutko, J. L., Ito, K., and Kenyon, J. L. (1985a), Ryanodine: a modifier of sarcoplasmic reticulum calcium release in striated muscle, *Fed. Proc.*, **44:** 2984–2988.

Sutko, J. L., Thompson, L. J., Schlatterer, R. G., Lattanzio, F. A., Fairhurst, A. S., Campbell, C., Martin, S. F., Deslongchamps, P., Ruest, L., and Taylor, D. R. (1985b), Separation and formation of ryanodine from dehydroryanodine. Preparation of tritium-labeled ryanodine, *J. Labeled Cmpd. Radiopharmacol.*, **23:** 215–222.

Sutko, J. L. and Kenyon, J. L. (1983), Ryanodine modification of cardiac muscle responses to potassium-free solutions: evidence for inhibition of sarcoplasmic reticulum calcium release, *J. Gen. Physiol.*, **82:** 385–404.

Sutko, J. L., Robinson, E., Lattanzio, F. A., Jr., Schlatterer, R. G., Deslongchamps, P., and Ruest, L. (1990), Pharmacology of the *Ryania* alkaloids: the ester A, a ryanodine analog that only increases sarcoplasmic reticulum calcium permeability, in *Transduction in Biological Systems*, Hidalgo, C., Bacigalupo, J., Jaimovich, E., and Vergara, J., Eds., Plenum Press, New York, 465–473.

Sutko, J. L. and Willerson, J. T. (1980), Ryanodine alteration of the contractile state of rat ventricular myocardium: comparison with dog, cat, and rabbit ventricular tissues, *Circ. Res.*, **46:** 332–343.

Takagi, A., Sugita, H., Toyokura, Y., and Endo, M. (1976), Malignant hyperpyrexia. Effect of halothane on single skinned muscle fibers, *Proc. Jpn. Acad.* **52:** 603–606.

Takasawa, S., Nata, K., Yonekura, H., and Okamoto, H. (1993), Cyclic ADP-ribose in insulin secretion from pancreatic β cells, *Science*, **259:** 370–373.

Takeshima, H., Nishi, M., Iwabe, N., Miyata, T., Hosoya, T., Masai, I., and Hotta, Y. (1994), Isolation and characterization of a gene for a ryanodine receptor-calcium release channel in *Drosophila melanogaster*, *FEBS Lett.*, **337:** 81–87.

Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., Minamino, N., Matsuo, H., Ueda, M., Hanaoka, M., Hirose, T., and Numa, S., (1989), Primary structure and expression from complementary DNA of skeletal muscle ryanodine receptor, *Nature (London)*, **339:** 439–445.

Takeshima, H., Nishimura, S., Nishi, M., Ikeda, M., and Sugimoto, T. (1993), A brain-specific transcript from the 3′-terminal region of the skeletal muscle ryanodine receptor gene, *FEBS Lett.*, **322:** 105–110.

Tanabe, T., Beam, K. G., Powell, J. A., and Numa, S. (1988), Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA, *Nature (London)*, **336:** 134–139.

Tanabe, T., Beam, K. G., Adams, B. A., Niidome, T., and Numa, S. (1990a), Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling, *Nature (London)*, **346:** 567–569.

Tanabe, T., Mikami, A., Numa, S., and Beam, K. G. (1990b), Cardiac-type excitation-contraction coupling in dysgenic skeletal muscle injected with cardiac dihydropyridine receptor cDNA, *Nature (London)*, **344:** 451–453.

Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T., and Numa, S. (1987), Primary structure of the receptor for calcium channel blockers from skeletal muscle, *Nature (London)*, **328:** 313–318.

Taylor, S. R. Rüdel, R., and Blinks, J. R. (1975), Calcium transients in amphibian muscle, *Fed. Proc.*, **34:** 1379–1381.

Thastrup, O., Cullen, P. J., Drøbak, B. K., Hanley, M. R., and Dawson, A. P. (1990), Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase, *Proc. Natl. Acad. Sci. U.S.A.*, **87:** 2466–2470.

Thorens, S. and Endo, M. (1975), Calcium-induced calcium release and "depolarization"-induced calcium release: their physiological significance, *Proc. Jpn. Acad.*, **51:** 473–478.

Timerman, A. P., Ogunbumni, E., Freund, E., Wiederrecht, G., Marks, A. R., and Fleischer, S. (1993), The calcium release channel of sarcoplasmic reticulum is modulated by FK-506-binding protein: dissociation and reconstitution of FKBP-12 to the calcium release channel of skeletal muscle sarcoplasmic reticulum, *J. Biol. Chem.*, **268:** 22,992–22,999.

Uto, A., Arai, H., and Ogawa, Y. (1991), Reassessment of fura-2 and the ratio method for determination of intracellular Ca^{2+} concentrations, *Cell Calcium*, **12:** 29–37.

Valdivia, H. H. and Coronado, R. (1989), Inhibition of dihydropyridine-sensitive calcium channels by the plant alkaloid ryanodine, *FEBS Lett.*, **244:** 333–337.

Valdivia, C., Valdivia, H. H., Potter, B. V. L., and Coronado, R. (1990), Ca^{2+} release by inositol-trisphosphorothioate in isolated triads of rabbit skeletal muscle, *Biophys. J.*, **57:** 1232–1243.

Vergara, J., Tsien, R. Y., and Delay, M. (1985), Inositol 1,4,5-trisphosphate: a possible chemical link in excitation-contraction coupling in muscle, *Proc. Natl. Acad. Sci. U.S.A.*, **82:** 6352–6356.

Vilsen, B. and Andersen, J. P. (1992), Deduced amino acid sequence and E1-E2 equilibrium of the sarcoplasmic reticulum Ca^{2+}-ATPase of frog skeletal muscle: comparison with the Ca^{2+}-ATPase of rabbit fast twitch muscle, *FEBS Lett.*, **306:** 213–218.

Volpe, P., Salviati, G., DiVirgilio, F., and Pozzan, T. (1985), Inositol 1,4,5-trisphosphate induces calcium release from sarcoplasmic reticulum of skeletal muscle, *Nature (London)*, **316:** 347–349.

Wagenknecht, T., Grassucci, R., Frank, J., Saito, A., Inui, M., and Fleischer, S. (1989), Three-dimensional architecture of the calcium channel/foot structure of sarcoplasmic reticulum, *Nature (London)*, **338:** 167–170.

Wagner-Mann, C., Hu, Q., and Sturek, M. (1992), Multiple effects of ryanodine on intracellular free Ca^{2+} in smooth muscle cells from bovine and porcine coronary artery: modulation of sarcoplasmic reticulum function, *Br. J. Pharmacol.*, **105:** 903–911.

Walton, P. D., Airey, J. A., Sutko, J. L., Beck, C. F., Mignery, G. A., Südhof, T. C., Deerinck, T. J., and Ellisman, M. H. (1991), Ryanodine and inositol trisphosphate receptors coexist in avian cerebellar Purkinje neurons, *J. Cell Biol.*, **113:** 1145–1157.

Waterhouse, A. L., Holden, I., and Casida, J. E. (1984), 9,21-Didehydroryanodine: a new principal toxic constituent of the botanical insecticide *Ryania*, *J. Chem. Soc. Chem. Commun.*, 1265–1266.

Wayne, R., Staves, M. P., and Leopold, A. C. (1992), The contribution of the extracellular matrix to gravisensing in characean cells, *J. Cell Sci.*, **101:** 611–623.

Weber, A. (1968), The mechanism of the action of caffeine on sarcoplasmic reticulum, *J. Gen. Physiol.*, **52:** 760–772.

Weber, A. and Herz, R. (1968), The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum, *J. Gen. Physiol.*, **52:** 750–759.

Wibo, M., Bravo, G., and Godfraind, T. (1991), Postnatal maturation of excitation-contraction coupling in rat ventricle in relation to the subcellular localization and surface density of 1,4-dihydropyridine and ryanodine receptors, *Circ. Res.*, **68:** 662–673.

Wier, W. G. (1990), Cytoplasmic $[Ca^{2+}]$ in mammalian ventricle: dynamic control by cellular processes, *Annu. Rev. Physiol.*, **52:** 467–485.

Wier, W. G., Yue, D. T., and Marban, E. (1985), Effects of ryanodine on intracellular Ca^{2+} transients in mammalian cardiac muscle, *Fed. Proc.* **44:** 2989–2993.

Wierenga, R. K. and Hol, W. G. J. (1983), Predicted nucleotide-binding properties of p21 protein and its cancer-associated variant, *Nature (London)*, **302:** 842–844.

Williams, A. J. (1992), Ion conduction and discrimination in the sarcoplasmic reticulum ryanodine receptor/calcium-release channel, *J. Muscle Res. Cell Motil.*, **13:** 7–26.

Winegrad, S. (1979), Electromechanical coupling in heart muscle, in *Handbook of Physiology*, Vol. 1, *The Cardiovascular System*, Berne, R. M., Sperelakis, N., and Geiger, S., Eds., American Physiological Society, Bethesda, MD, 393–428.

Witcher, D. R., Kovacs, R. J., Schulman, H., Cefali, D. C., and Jones, L. R. (1991), Unique phosphorylation site on the cardiac ryanodine receptor regulates calcium channel activity, *J. Biol. Chem.*, **266:** 11,144–11,152.

Witcher, D. R., McPherson, P. S., Kahl, S. D., Lewis, T., Bentley, P., Mullinix, M. J., Windass, J. D., and Campbell, K. P. (1994), Photoaffinity labeling of the ryanodine receptor/Ca^{2+} release channel with an azido derivative of ryanodine, *J. Biol. Chem.*, **269:** 13,076–13,079.

Witcher, D. R., Strifler, B. A., and Jones, L. R. (1992), Cardiac-specific phosphorylation site for multifunctional Ca^{2+}/calmodulin-dependent protein kinase is conserved in the brain ryanodine receptor, *J. Biol. Chem.*, **267:** 4963–4967.

Yamazawa, T., Iino, M., and Endo, M. (1992), Presence of functionally different compartments of the Ca^{2+} store in single intestinal smooth muscle cells, *FEBS Lett.*, **301:** 181–184.

Zhang, Y., Chen, H. S., Khanna, V. K., DeLeon, S., Phillips, M. S., Schappert, K., Britt, B. A., Brownell, A. K. W., and MacLennan, D. H. (1993), A mutation in the human ryanodine receptor gene associated with cental core disease, *Nature Genet.*, **5:** 46–50.

Zimanyi, I. and Pessah, I. (1991), Pharmacological characterization of the specific binding of [^{3}H]ryanodine to rat brain microsomal membranes, *Brain Res.*, **561:** 181–191.

Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N. M., Lai, F. A., Meissner, G., and MacLennan, D. H. (1990), Molecular cloning of cDNA encoding human and rabbit forms of the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum, *J. Biol. Chem.*, **265:** 2244–2256.

Zupanc, G. K. H., Airey, J. A., Maler, L., Sutko, J. L., and Ellisman, M. H. (1992), Immunohistochemical localization of ryanodine binding proteins in the central nervous system of gymnotiform fish, *J. Comp. Neurol.*, **325:** 135–151.