Minireview

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 268, No. 19, Issue of July 5, pp. 13765-13768, 1993

The Ryanodine Receptor/Ca^{2+} Release Channel*

Peter S. McPherson‡§ and Kevin P. Campbell‡¶‖

From the Howard Hughes Medical Institute, ¶Department of Physiology and Biophysics and the ‡Neuroscience Program, University of Iowa College of Medicine, Iowa City, Iowa 52242

Intracellular Ca^{2+} pools play a critical role in the regulation of the free cytosolic Ca^{2+} concentration and subsequent cellular activity in a wide variety of cell types. The storage and release of Ca^{2+} from the sarcoplasmic reticulum of skeletal muscle and its role in muscle contraction is a well established example of such regulation. In the 1980s, the channel responsible for sarcoplasmic reticulum Ca^{2+} release was identified as the ryanodine receptor, but only recently has the ryanodine receptor been recognized as having a role in Ca^{2+} regulation in non-muscle cells. This review will examine the role of the ryanodine receptor as a component of excitation-contraction (E-C) coupling as well as a mediator of Ca^{2+} release from the endoplasmic reticulum of a variety of cell types.

E-C coupling in skeletal muscle represents a unique and well studied form of signal transduction (for recent reviews see Refs. 1–5) in which depolarization of the sarcolemma leads to Ca^{2+} release from the sarcoplasmic reticulum. The depolarizing potential is transmitted down invaginations of the sarcolemma (transverse tubules (T-tubules)), which form junctions with the terminal cisternae region of the sarcoplasmic reticulum. The junctions are referred to as triad junctions as each is composed of one T-tubule and two apposed terminal cisternae. Ca^{2+}, which is released at the triad junction, is resequestered into the sarcoplasmic reticulum by a Ca^{2+}-ATPase, which is present on the longitudinal tubule regions of the sarcoplasmic reticulum.

Several hypotheses exist to explain the mechanism of E-C coupling (6), the most widely accepted of which states that a "voltage sensor" located in the T-tubule responds to depolarization by gating Ca^{2+} release from the sarcoplasmic reticulum. The molecular basis of this phenomenon involves the dihydropyridine receptor (DHPR) as the T-tubule voltage sensor and the ryanodine receptor as the sarcoplasmic reticulum Ca^{2+} release channel. Although these two proteins are the major components of E-C coupling, a variety of proteins are located specifically at the triad junction. A list of the major junctional specific components that may interact with the ryanodine receptor is provided in Table I. Junctional specific proteins play various roles in organization of the triad structure and/or the storage and release of Ca^{2+}.

Dihydropyridine Receptor in E-C Coupling

In 1973, Schneider and Chandler (7) proposed that charged molecules within the T-tubule membrane move upon depolarization and that the movement of these molecules provides a direct physical link between T-tubule depolarization and sarcoplasmic reticulum Ca^{2+} release. Their hypothesis was based on the observation that a voltage-dependent charge movement, which was closely connected to muscle contraction, could be measured across the T-tubule membrane under conditions where all ionic currents were blocked (7). It was later observed that dihydropyridines, specific blockers of the DHPR, blocked charge movement and sarcoplasmic reticulum Ca^{2+} release with similar voltage and dose dependences, suggesting that the charge movement was due to gating of the DHPR and confirming its importance in the activation of sarcoplasmic reticulum Ca^{2+} release (5).

The DHPR was purified from skeletal muscle and determined to have five subunits (reviewed in Ref. 8), the primary structures of which have been elucidated (9, 10). The α_1 channel-forming subunit has four internal repeats, each of which contains the characteristic S4 segment, which probably operates as a voltage sensor. Although it has been suggested that different α_1 subunits may serve the functions of voltage sensor and Ca^{2+} channel (discussed in Ref. 3), it is now clear that a single class of α_1 performs both tasks (11). However, Ca^{2+} influx through the DHPR is unnecessary in skeletal E-C coupling, and the DHPR is more likely involved in E-C coupling through its actions as a voltage sensor (2).

The critical role of the DHPR in E-C coupling was confirmed when it was determined that both intramembrane charge movement and E-C coupling, which are absent in dysgenic myotubes lacking the DHPR α_1 subunit, could be restored by α_1 transfection (2). Electron microscopy studies revealed that the DHPR and ryanodine receptor are adjacent to each other and in fact may make physical contact (12). Further, the temporal appearance and subcellular distribution of the DHPR α_1 subunit and the ryanodine receptor are very similar or identical throughout all stages of the development of T-tubules and triads (13). Taken together, these data suggest that activation of the DHPR (charge movement) may activate the ryanodine receptor through a direct interaction between the two proteins. Although the region on the DHPR α_1 subunit that mediates such an interaction has been localized to the intracellular loop between domains II and III (2), no direct interaction between the DHPR and ryanodine receptor has yet been demonstrated.

It is possible that an interaction between the DHPR and the ryanodine receptor is mediated by a third protein. Caswell *et al.* (14) have isolated a junctional sarcoplasmic reticulum-specific 95-kDa protein (triadin) that may bind to both the ryanodine and dihydropyridine receptors and which they suggest mediates a DHPR-ryanodine receptor interaction. However, cloning of a 94-kDa glycoprotein, which appears to be identical to triadin (15), indicates that only 47 amino acids of the protein are cytoplasmic with the bulk of the protein located in the lumen of the sarcoplasmic reticulum (16) (Fig. 1). Thus, it is unlikely that triadin could interact directly with the DHPR. The lumenal region of triadin is highly positively charged (16), and it may interact with negative charges on calsequestrin, the high capacity, moderate affinity Ca^{2+}-binding protein that is localized in the lumenal region of junctional sarcoplasmic reticulum. Triadin may in fact be equivalent to the elongated protein strands that have been proposed to link calsequestrin to the junctional sarcoplasmic reticulum membrane (20), and it may provide a link between calsequestrin and the ryanodine receptor (Fig. 1). This would allow for possible calsequestrin control of ryanodine receptor activation (21) and ryanodine receptor control of the Ca^{2+} affinity state of calsequestrin (22).

Ryanodine Receptor in E-C Coupling

Although the sarcoplasmic reticulum had been implicated as the site of Ca^{2+} release in skeletal muscle E-C coupling in the early 1960s, the identification of the Ca^{2+} release channel remained elusive. The identification of the channel was greatly aided by the use of ryanodine, a neutral plant alkaloid that was known to effect Ca^{2+} release from the sarcoplasmic reticulum (23). In the mid-1980s, the presence of a large conductance Ca^{2+} channel in the junctional region of the sarcoplasmic reticulum was directly demonstrated by incorporating junctional sarcoplasmic reticulum vesicles into planar lipid bilayers (reviewed in Ref. 24). Ryanodine could affect the conductance and gating properties of this channel. Further, nanomolar concentrations of ryanodine could lock the Ca^{2+} release chan-

* This minireview will be reprinted in the 1993 Minireview Compendium, which will be available in December, 1993.

§ Present address: Dept. of Cell Biology, Yale University, New Haven, CT 06510.

‖ Investigator of the Howard Hughes Medical Research Institute. To whom correspondence should be addressed: Howard Hughes Medical Institute, 400 Eckstein Medical Research Bldg., University of Iowa College of Medicine, Iowa City, IA 52242. Tel.: 319-335-7867; Fax: 319-335-6957.

TABLE I
Major junctional specific components in skeletal muscle

Protein	Molecular mass	Function	GenBank accession no.
	kDa		
Junctional SR components			
Ryanodine receptor	~565	Ca^{2+} release channel foot structure	X15209
Calsequestrin	63	Ca^{2+} binding and storage	M15747
94-kDa glycoprotein (triadin)	94	Calsequestrin binding?	L10065
Junctional T-system components			
Dihydropyridine receptor		Voltage sensor for E-C coupling	
α_1	170		P07293
α_2/δ	175		P13806
β	52		M25817
γ	32		M32231

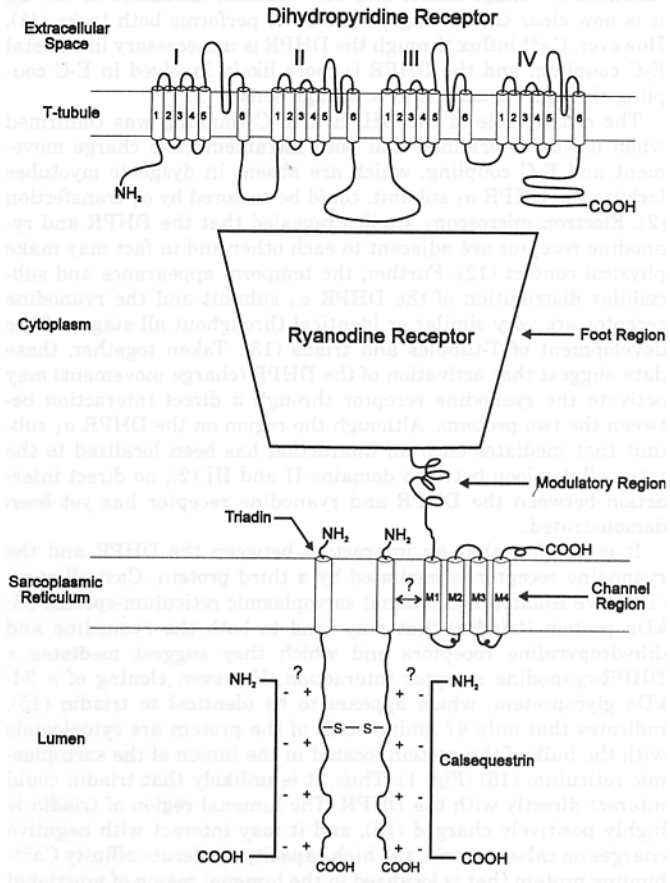

FIG. 1. **Model of the triad junction.** For simplicity, only the α_1 subunit of the dihydropyridine receptor and only one subunit of the ryanodine receptor homotetramer are shown. The ryanodine receptor is modeled after that of Takeshima *et al.* (17) although alternative models suggest as many as 12 transmembrane domains (18) and predict other regulatory domains within the foot region (19). *Question marks* indicate the speculative nature of the interaction between 94-kDa glycoprotein (triadin) and the ryanodine receptor as well as between triadin and calsequestrin.

nel into an open state, and [^{3}H]ryanodine was shown to bind specifically to junctional regions of the sarcoplasmic reticulum (24). Thus, it appeared that ryanodine was a useful probe for isolation of the junctional sarcoplasmic reticulum-specific Ca^{2+} release channel.

Several groups utilized [^{3}H]ryanodine binding assays to identify and purify the ryanodine receptor from junctional sarcoplasmic reticulum (25–27). The protein was determined to have an extremely large molecular mass and was demonstrated to form a large conductance cation channel upon incorporation into planar lipid bilayers (25). The channel had many properties similar to those of the native channel from sarcoplasmic reticulum (26). The purified ryanodine receptor also had a morphology identical to the "feet" structures (27) that had originally been observed by Franzini-Armstrong (28) to span the transverse tubule-sarcoplasmic reticulum junction. The large molecular weight of the ryanodine receptor and its association as a homotetramer give the receptor its characteristic structure and rapid migration through sucrose gradients. Thus, the ryanodine receptor appears to play a structural as well as functional role in the process of E-C coupling.

Cloning of the ryanodine receptor from rabbit (17, 18), human (18), and pig (29) has been reported. The cDNAs encode proteins of approximately 5035 amino acids from each species. The protein is predicted to have a short cytoplasmic C-terminus and between 4 and 10 transmembrane domains in the C-terminal one-fifth of the molecule (17, 18). The remainder of the protein is predicted to comprise the "foot" region (Fig. 1). When expressed in Chinese hamster ovary (CHO) or COS-1 cells, cDNAs encoding full-length ryanodine receptor generated large molecular weight receptors for [^{3}H]ryanodine (30, 31) as well as caffeine- and ryanodine-sensitive intracellular Ca^{2+} release (30) and functional channels which could be modified by physiologically relevant ligands (31). There has been some evidence to suggest that the ryanodine receptor and sarcoplasmic reticulum Ca^{2+} release channel correspond to a sulfhydryl-gated 106-kDa protein (32). While this protein may play a role in sarcoplasmic reticulum Ca^{2+} release, the expression studies strongly support the conclusion that the large molecular weight "foot" protein is the ryanodine receptor and sarcoplasmic reticulum Ca^{2+} release channel.

A large list of compounds is known to affect sarcoplasmic reticulum Ca^{2+} release. Potentiators of sarcoplasmic reticulum Ca^{2+} release include Ca^{2+}, adenine nucleotides, caffeine, halothane, ryanodine at nanomolar concentrations, sulfhydryl reagents, and bromo-eudostomin, whereas inhibitors include Mg^{2+}, Ca^{2+} at millimolar concentrations, ryanodine at micromolar concentrations, ruthenium red, procaine, calmodulin, dantrolene, and spermine (1). Recently, several peptide toxins have also been identified as activators or inhibitors of muscle ryanodine receptors (33). It is likely that many of these compounds affect Ca^{2+} release by direct actions on the ryanodine receptor/Ca^{2+} release channel as many of the compounds affect [^{3}H]ryanodine binding to the purified receptor and affect the channel conductance or open probability of purified receptor incorporated into planar lipid bilayers (reviewed in Ref. 1). Deductions from the primary structure suggest that the skeletal muscle ryanodine receptor has regulatory domains located on the N-terminal side of the first transmembrane domain (17) and/or in the sequences between amino acids 2600–3000 (19), although few of these putative regulation sites have been confirmed. Recently, Chen *et al.* (19) used a $^{45}Ca^{2+}$ overlay of fusion proteins covering the length of the skeletal muscle ryanodine receptor to determine that the region between amino acids 4478 and 4512 contains a Ca^{2+}-binding site. Antibodies against this region increase the open probability and opening time of the purified ryanodine receptor incorporated in planar lipid bilayers, suggesting that this site may be involved in Ca^{2+} regulation of the channel (19). The localization of the sites for other regulators of the ryanodine receptor will be critical to the understanding of channel functioning in E-C coupling.

Defects in the ryanodine receptor can severely affect the normal process of E-C coupling. Malignant hyperthermia (MH) is a potentially fatal genetic disease in which patients exposed to anesthesia develop muscle rigidity, hypermetabolism, and fever. In swine, the

TABLE II
Ryanodine receptor genes and expression

Gene	Tissue expressed	Identification	Ref.
RYR-1	Skeletal muscle	Purified, cloned (565 kDa)	17, 25
	Cerebellum	Immunoblot (565 kDa)	62
	Sea urchin eggs	Immunoblot (380 kDa)	65
RYR-2	Heart	Purified, cloned (565 kDa)	41, 43, 44
	Stomach	Northern blot	44
	Endothelial cells	Immunoblot, Northern blot	66
	Brain, widespread	Immunoblot, Northern blot, purification	43, 57, 62
RYR-3	Epithelial cells	Cloned	63
	Smooth muscle	Northern blot	64
	Brain	Cloned	64

corresponding condition can cause stress-induced death. The condition involves abnormal sarcoplasmic reticulum Ca^{2+} release in both humans and pigs. Ryanodine receptor involvement in the process was suggested by the findings of an altered Ca^{2+} dependence for ryanodine binding to sarcoplasmic reticulum from normal *versus* MH pigs (34) and an altered trypsin digestion pattern in the ryanodine receptor from MH swine (35). The ryanodine receptor was definitively identified as the cause of MH in swine when a single mutation in the ryanodine receptor cloned from MH pigs was discovered (29). The mutation, which caused a substitution of Cys for Arg^{615}, was linked to the MH locus with a recombination fraction of 0.0 in 338 informative meioses (36). An identical mutation appears to be responsible for MH in some human lineages, but there is genetic heterogeneity in the human disease and the search for an alternative MH locus is under way (37).

Cardiac Ryanodine Receptor

In contrast to E-C coupling in skeletal muscle, E-C coupling in heart is dependent on trans-sarcolemmal Ca^{2+} influx, which although insufficient to directly activate myofilament contraction is sufficient to activate further Ca^{2+} release from the sarcoplasmic reticulum (38). Thus, the prevailing hypothesis for the mechanism of E-C coupling in heart is that Ca^{2+} influx activates sarcoplasmic reticulum Ca^{2+} release in a process termed Ca^{2+}-induced Ca^{2+} release (CICR) (38), and the cardiac ryanodine receptor is thought to be a CICR channel (39). The recent localization of the cardiac ryanodine receptor to the corbular sarcoplasmic reticulum, well removed from the sarcolemma, provides a morphological basis for CICR (40).

Inui *et al.* (41) isolated a ryanodine receptor from cardiac sarcoplasmic reticulum and demonstrated that like the skeletal ryanodine receptor, it had a large tetrameric structure. Purified cardiac ryanodine receptor incorporated into planar lipid bilayers formed a Ca^{2+}-activatible Ca^{2+} channel, which had similar conductance properties and pharmacological regulation as the skeletal ryanodine receptor (42). cDNAs encoding the cardiac ryanodine receptor have been cloned (43, 44). The protein has an amino acid sequence that is 66% identical to the skeletal muscle receptor, with a similar membrane topology (43). Although predicted binding sites for calmodulin, ATP, and Ca^{2+} are found in both cardiac and skeletal receptors, they are not highly conserved and are located at different regions of the protein (43). Expression of cDNAs encoding the full-length cardiac ryanodine receptor in *Xenopus* oocytes (44) or COS-1 cells (45) were able to generate a caffeine-sensitive intracellular Ca^{2+} release mechanism.

Comparison of Skeletal and Cardiac Ryanodine Receptors

Although the skeletal and cardiac ryanodine receptors have sequence homology and demonstrate similar pharmacological regulation, they are clearly distinct. The two proteins are products of different genes, with the skeletal receptor on human chromosome 19 and the cardiac receptor on human chromosome 1 (43). Consistent with a mechanism of CICR for cardiac sarcoplasmic reticulum, the cardiac ryanodine receptor is more sensitive to activation by Ca^{2+} than the skeletal receptor (46). The cardiac receptor is also more sensitive to activation by caffeine and inhibition by Mg^{2+} (46) and it appears to be a better substrate for phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II (CaM-kinase) and cAMP-dependent protein kinase (A-kinase) than the skeletal receptor (47). Phosphorylation of the cardiac ryanodine receptor can be stimulated by β-adrenergic treatment in cardiac myocytes (48), and the phosphorylated channel shows alterations in [^{3}H]ryanodine binding (49) and increased channel activity (47).

Brain Ryanodine Receptor

The resting free cytosolic Ca^{2+} concentration in neurons is approximately 100 nM, and it must rise severalfold to act as a signal. In some cases, Ca^{2+} may rise several orders of magnitude above this value in isolated microdomains (50). It has become increasingly apparent that the endoplasmic reticulum plays a critical role in neuronal regulation through storage and release of Ca^{2+}. One pathway cells use to mobilize intracellular Ca^{2+} is through the generation of inositol 1,4,5-trisphosphate (IP_3) (reviewed in Ref. 51), and the IP_3 receptor is a major intracellular Ca^{2+} release channel in neurons (reviewed in Ref. 52). However, physiological and pharmacological evidence has indicated the presence of IP_3-insensitive stores in neurons that are sensitive to the ryanodine receptor ligands caffeine and ryanodine (53).

In order to understand the mechanism of Ca^{2+} release from caffeine-sensitive stores in neurons, several studies have been performed aimed at characterization of the brain ryanodine receptor. The receptor was initially identified in brain membranes utilizing [^{3}H]ryanodine binding (54–56) and was purified and shown to function as the gating mechanism for caffeine-sensitive Ca^{2+} stores in neurons as it formed a caffeine-sensitive Ca^{2+} release channel upon incorporation into planar lipid bilayers (57). The ryanodine receptor has been localized to membranes of the endoplasmic reticulum (58), and its distribution throughout the mammalian central nervous system has been studied (59, 60) and shown to be overlapping with but distinct from that of the IP_3 receptor (60). Similar to the ryanodine receptor of cardiac muscle, the brain ryanodine receptor is also a substrate for phosphorylation by CaM-kinase and A-kinase (61). Recently, it has been demonstrated that several forms of ryanodine receptor are expressed in brain. Anti-peptide antibodies specific to the skeletal muscle ryanodine receptor recognize a large molecular weight protein in cerebellum, whereas an antibody specific to the cardiac form of ryanodine receptor recognizes a protein expressed throughout the nervous system (62). Further, a protein that has approximately 70% homology to the skeletal and cardiac muscle ryanodine receptors and is likely identical to the TGF-β-inducible ryanodine receptor cloned from mink epithelial cells (63) has been cloned from brain (64). Thus, it appears that at least three distinct genes encoding for ryanodine receptor are expressed in mammalian brain.

Since the skeletal and cardiac ryanodine receptor genes have been referred to as *RYR-1* and *RYR-2*, the third gene is here called *RYR-3* (Table II). *RYR-1* appears to be expressed predominantly in skeletal muscle but is also present in mouse cerebellum and may be the gene expressed in sea urchin eggs (65). Interestingly, an alternative start site near the 3′ -terminal region of *RYR-1* appears to generate a brain-specific transcript encoding a 75-kDa protein (67). *RYR-2* is expressed predominantly in heart, but expression is also detectable in brain (widespread brain regions), stomach, and endothelial cells. *RYR-3* is expressed in brain (striatum, thalamus, hippocampus), smooth muscle (*e.g.* aorta, esophagus), and mink lung epithelial cells, and may be TGF-β-inducible.

It appears that the *RYR-2* gene is the major form of the ryanodine receptor expressed in brain. Purification of the major [^{3}H]ry-

TABLE II
Ryanodine receptor genes and expression

Gene	Tissue expressed	Identification	Ref.
RYR-1	Skeletal muscle	Purified, cloned (565 kDa)	17, 25
	Cerebellum	Immunoblot (565 kDa)	62
	Sea urchin eggs	Immunoblot (380 kDa)	65
RYR-2	Heart	Purified, cloned (565 kDa)	41, 43, 44
	Stomach	Northern blot	44
	Endothelial cells	Immunoblot, Northern blot	66
	Brain, widespread	Immunoblot, Northern blot, purification	43, 57, 62
RYR-3	Epithelial cells	Cloned	63
	Smooth muscle	Northern blot	64
	Brain	Cloned	64

corresponding condition can cause stress-induced death. The condition involves abnormal sarcoplasmic reticulum Ca^{2+} release in both humans and pigs. Ryanodine receptor involvement in the process was suggested by the findings of an altered Ca^{2+} dependence for ryanodine binding to sarcoplasmic reticulum from normal *versus* MH pigs (34) and an altered trypsin digestion pattern in the ryanodine receptor from MH swine (35). The ryanodine receptor was definitively identified as the cause of MH in swine when a single mutation in the ryanodine receptor cloned from MH pigs was discovered (29). The mutation, which caused a substitution of Cys for Arg^{615}, was linked to the MH locus with a recombination fraction of 0.0 in 338 informative meioses (36). An identical mutation appears to be responsible for MH in some human lineages, but there is genetic heterogeneity in the human disease and the search for an alternative MH locus is under way (37).

Cardiac Ryanodine Receptor

In contrast to E-C coupling in skeletal muscle, E-C coupling in heart is dependent on trans-sarcolemmal Ca^{2+} influx, which although insufficient to directly activate myofilament contraction is sufficient to activate further Ca^{2+} release from the sarcoplasmic reticulum (38). Thus, the prevailing hypothesis for the mechanism of E-C coupling in heart is that Ca^{2+} influx activates sarcoplasmic reticulum Ca^{2+} release in a process termed Ca^{2+}-induced Ca^{2+} release (CICR) (38), and the cardiac ryanodine receptor is thought to be a CICR channel (39). The recent localization of the cardiac ryanodine receptor to the corbular sarcoplasmic reticulum, well removed from the sarcolemma, provides a morphological basis for CICR (40).

Inui *et al.* (41) isolated a ryanodine receptor from cardiac sarcoplasmic reticulum and demonstrated that like the skeletal ryanodine receptor, it had a large tetrameric structure. Purified cardiac ryanodine receptor incorporated into planar lipid bilayers formed a Ca^{2+}-activatible Ca^{2+} channel, which had similar conductance properties and pharmacological regulation as the skeletal ryanodine receptor (42). cDNAs encoding the cardiac ryanodine receptor have been cloned (43, 44). The protein has an amino acid sequence that is 66% identical to the skeletal muscle receptor, with a similar membrane topology (43). Although predicted binding sites for calmodulin, ATP, and Ca^{2+} are found in both cardiac and skeletal receptors, they are not highly conserved and are located at different regions of the protein (43). Expression of cDNAs encoding the full-length cardiac ryanodine receptor in *Xenopus* oocytes (44) or COS-1 cells (45) were able to generate a caffeine-sensitive intracellular Ca^{2+} release mechanism.

Comparison of Skeletal and Cardiac Ryanodine Receptors

Although the skeletal and cardiac ryanodine receptors have sequence homology and demonstrate similar pharmacological regulation, they are clearly distinct. The two proteins are products of different genes, with the skeletal receptor on human chromosome 19 and the cardiac receptor on human chromosome 1 (43). Consistent with a mechanism of CICR for cardiac sarcoplasmic reticulum, the cardiac ryanodine receptor is more sensitive to activation by Ca^{2+} than the skeletal receptor (46). The cardiac receptor is also more sensitive to activation by caffeine and inhibition by Mg^{2+} (46) and it appears to be a better substrate for phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II (CaM-kinase) and cAMP-dependent protein kinase (A-kinase) than the skeletal receptor (47). Phosphorylation of the cardiac ryanodine receptor can be stimulated by β-adrenergic treatment in cardiac myocytes (48), and the phosphorylated channel shows alterations in [^{3}H]ryanodine binding (49) and increased channel activity (47).

Brain Ryanodine Receptor

The resting free cytosolic Ca^{2+} concentration in neurons is approximately 100 nM, and it must rise severalfold to act as a signal. In some cases, Ca^{2+} may rise several orders of magnitude above this value in isolated microdomains (50). It has become increasingly apparent that the endoplasmic reticulum plays a critical role in neuronal regulation through storage and release of Ca^{2+}. One pathway cells use to mobilize intracellular Ca^{2+} is through the generation of inositol 1,4,5-trisphosphate (IP_3) (reviewed in Ref. 51), and the IP_3 receptor is a major intracellular Ca^{2+} release channel in neurons (reviewed in Ref. 52). However, physiological and pharmacological evidence has indicated the presence of IP_3-insensitive stores in neurons that are sensitive to the ryanodine receptor ligands caffeine and ryanodine (53).

In order to understand the mechanism of Ca^{2+} release from caffeine-sensitive stores in neurons, several studies have been performed aimed at characterization of the brain ryanodine receptor. The receptor was initially identified in brain membranes utilizing [^{3}H]ryanodine binding (54–56) and was purified and shown to function as the gating mechanism for caffeine-sensitive Ca^{2+} stores in neurons as it formed a caffeine-sensitive Ca^{2+} release channel upon incorporation into planar lipid bilayers (57). The ryanodine receptor has been localized to membranes of the endoplasmic reticulum (58), and its distribution throughout the mammalian central nervous system has been studied (59, 60) and shown to be overlapping with but distinct from that of the IP_3 receptor (60). Similar to the ryanodine receptor of cardiac muscle, the brain ryanodine receptor is also a substrate for phosphorylation by CaM-kinase and A-kinase (61). Recently, it has been demonstrated that several forms of ryanodine receptor are expressed in brain. Anti-peptide antibodies specific to the skeletal muscle ryanodine receptor recognize a large molecular weight protein in cerebellum, whereas an antibody specific to the cardiac form of ryanodine receptor recognizes a protein expressed throughout the nervous system (62). Further, a protein that has approximately 70% homology to the skeletal and cardiac muscle ryanodine receptors and is likely identical to the TGF-β-inducible ryanodine receptor cloned from mink epithelial cells (63) has been cloned from brain (64). Thus, it appears that at least three distinct genes encoding for ryanodine receptor are expressed in mammalian brain.

Since the skeletal and cardiac ryanodine receptor genes have been referred to as *RYR-1* and *RYR-2*, the third gene is here called *RYR-3* (Table II). *RYR-1* appears to be expressed predominantly in skeletal muscle but is also present in mouse cerebellum and may be the gene expressed in sea urchin eggs (65). Interestingly, an alternative start site near the 3′ -terminal region of *RYR-1* appears to generate a brain-specific transcript encoding a 75-kDa protein (67). *RYR-2* is expressed predominantly in heart, but expression is also detectable in brain (widespread brain regions), stomach, and endothelial cells. *RYR-3* is expressed in brain (striatum, thalamus, hippocampus), smooth muscle (*e.g.* aorta, esophagus), and mink lung epithelial cells, and may be TGF-β-inducible.

It appears that the *RYR-2* gene is the major form of the ryanodine receptor expressed in brain. Purification of the major [^{3}H]ry-