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QUARTERLY

Regulation of Ca^{2+} release from internal stores in cardiac and skeletal muscles★✪

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It is widely accepted that Ca^{2+} is released from the sarcoplasmic reticulum by a specialized type of calcium channel, i.e., ryanodine receptor, by the process of Ca^{2+}-induced Ca^{2+} release. This process is triggered mainly by dihydropyridine receptors, i.e., L-type (long lasting) calcium channels, directly or indirectly interacting with ryanodine receptor. In addition, multiple endogenous and exogenous compounds were found to modulate the activity of both types of calcium channels, ryanodine and dihydropyridine receptors. These compounds, by changing the Ca^{2+} transport activity of these channels, are able to influence intracellular Ca^{2+} homeostasis. As a result not only the overall Ca^{2+} concentration becomes affected but also spatial distribution of this ion in the cell. In cardiac and skeletal muscles the release of Ca^{2+} from internal stores is triggered by the same transport proteins, although by their specific isoforms. Concomitantly, heart and skeletal muscle specific regulatory mechanisms are different.

Depolarization of the plasma membrane, manifested as the action potential of an excited cell, such as a muscle cell, is responsible for initiation of a cascade of processes that leads to an increase in concentration of Ca^{2+} in the cytosol. Elevated Ca^{2+} concentration, accompanied by binding of Ca^{2+} to troponin C, triggers conformational changes in pro-

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Abbreviations: RyR, ryanodine receptor; DHPR, dihydropyridine receptor; SR, sarcoplasmic reticulum; E-C, excitation-contraction coupling; CaM, calmodulin; CSQ, calsequestrin; VDCC, voltage-dependent calcium channel; $[Ca^{2+}]_i$, intracellular calcium concentration; PKA, cyclic AMP-dependent protein kinase; cADPR, cyclic ADP-ribose; CICR, Ca^{2+}-induced Ca^{2+}release; FKBP, immunosuppressant FK506 binding protein.

teins of the contractile apparatus, leading finally to muscle contraction (Zot & Potter, 1987). The functional link between electrical stimulation of the muscle and its contraction is called the excitation-contraction (E-C) coupling. First experiments related to muscle contraction revealed that heart muscle preparations, in contrast to skeletal muscle, could not contract for a long time if Ca^{2+} was absent from the bath solution (Ringer, 1883; Rich *et al.*, 1988; Bers, 1991). These observations suggest that the contraction-relaxation cycles of these two muscle types are regulated by different E-C coupling mechanisms. Additional differences between the two types of muscle were revealed by experiments in which the voltage (E_m) dependence of Ca^{2+} current (I_{Ca}), changes in $[Ca^{2+}]_i$ transient, charge movement and muscle shortening were determined. Briefly, in cardiac muscle cells the E_m-dependence of $[Ca^{2+}]_i$ transient and contraction are very similar to the E_m-dependence of I_{Ca} and are bell-shaped, in contrast to a sigmoidal shape of the curve reflecting the charge movement in skeletal muscle cells (Schneider & Chandler, 1973; Bers, 1991). These observations clearly indicated that, in skeletal muscle cells the intramembrane charge movement, is the factor triggering the increase in $[Ca^{2+}]_i$, while in cardiac muscle cells this increase is due to the influx of Ca^{2+} through the plasma membrane. Considering the mechanisms of E-C coupling in skeletal and cardiac muscle cells, it is surprising that such very homologous proteins developed different mechanisms of Ca^{2+} release from intracellular stores.

SPATIAL RELATIONSHIP BETWEEN DIHYDROPYRIDINE AND RYANODINE RECEPTORS

Muscle cell membrane depolarization induces conformational changes in dihydropyridine receptor (DHPR), i.e., L-type (long lasting) Ca^{2+} channels located in the plasma membrane. These conformational changes are translated into signal amplification by direct interactions with sarcoplasmic reticulum (SR) membrane receptors for the plant alkaloid ryanodine receptor (RyR), as in skeletal muscle cell, or through the Ca^{2+} influx, as in cardiac muscle cells (Sutko *et al.*, 1997). The spatial relationship between DHPR and RyR skeletal muscle differs from that in cardiac or smooth muscles as well as in neuronal cells. In different cell types, DHPR and RyR are localized in junctions of membranes, called triads, dyads or peripheral couplings; the latter representing the junctions between the plasma and SR membranes (Flucher & Franzini-Armstrong, 1996). The characteristic features of these junctions are the "feet", i.e., electron-dense structures spanning the narrow junctional gap (about 16 nm) separating SR membranes and transverse tubules (Langer & Peskoff, 1997). In the case of skeletal muscles, the foot structures form a semicrystalline array in the plane of the membrane, consisting of RyR connected with tetrads of DHPR (Fig. 1) (Flucher & Franzini-Armstrong, 1996). In cardiac muscles, these structures are less organized (Fig. 1) and their number differs significantly from that of RyR channels in skeletal muscles (Sun *et al.*, 1995). In skeletal muscles, the ratio of DHPR to RyR ranges from 0.6 to 2.1, while in cardiac muscles, from 0.1 to 0.3 (Sutko & Airey, 1996). It is worth stressing that these two channels are not the major components responsible for the formation of junctions between SR membranes and transverse tubules which can be formed even in the absence of DHPR and RyR in the cell (Flucher *et al.*, 1992; Takekura *et al.*, 1995a). On the other hand, co-expression of DHPR and RyR in CHO cells failed to induce the formation of the junction between SR and plasma membranes (Takekura *et al.*, 1995b). This suggests that other "docking" proteins are required for formation of the junctional connections.

The observations cited above suggest that differences in E-C coupling between skeletal and cardiac muscles are related to different

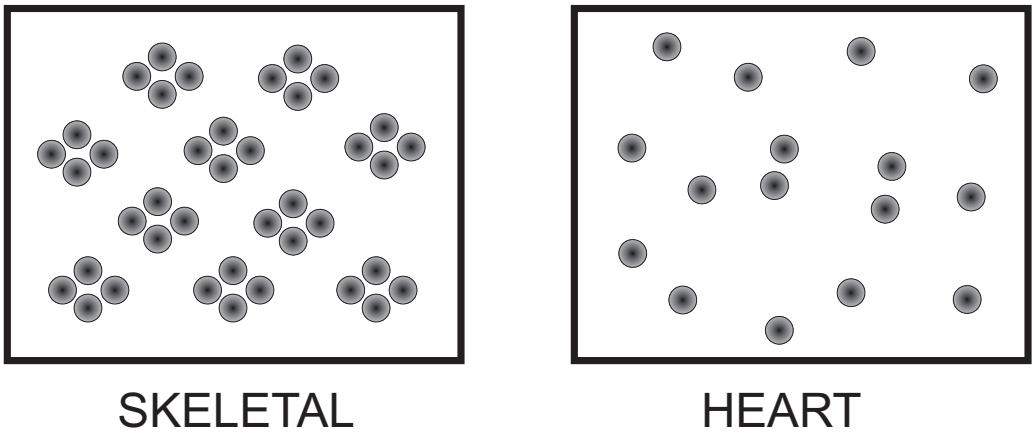

Figure 1. Different distribution of dihydropyridine receptor (DHPR) in plasma membrane of skeletal and cardiac muscle cells.

spatial organization of their respective Ca^{2+} transport systems. In skeletal muscles direct interaction between DHPR and RyR is responsible for releasing Ca^{2+} from internal stores while in cardiac muscles a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism exists under the local control of Ca^{2+} current through the L-type channels (Franzini-Armstrong & Protasi, 1997).

CALCIUM SPARKS

A small increase in $[Ca^{2+}]_i$ in cardiac muscle cells causes opening of the calcium channels located in SR membrane in a process called calcium-induced calcium release, which was observed in skinned skeletal and cardiac muscle preparations (Endo *et al.*, 1970; Fabiato, 1985a, 1985b). The release of Ca^{2+} from SR is triggered by the opening of a small number of DHPR channels and opening of a similar number of RyR channels (Fig. 2), as theoretically foreseen by Stern & Lakatta (1992), and experimentally confirmed by Cheng *et al.* (1993). The cardiomyocytes loaded with Fluo-3 showed characteristic points of brightness, called "sparks". These sparks, as it was later shown, arise at the sites adjacent to transverse tubules and provide both analog and digital gain for E-C coupling (Shacklock *et al.*, 1995; Cheng *et al.*, 1996; Isenberg *et al.*, 1996).

DHPR CALCIUM CHANNELS

Biochemical, molecular and immunoprecipitation studies revealed that DHPR present in excitable cells consist of complexes of five protein subunits. The main part of the channel is formed by the α_1 subunit (200 kDa) composed of four motifs, each containing six putative transmembrane segments. At least six genes encoding different isoforms of α_1 subunit have been identified. This variety is responsible for characteristic electrophysiological parameters of voltage-dependent calcium channel (VDCC) (Jones, 1998). In addition to α_1 subunit, VDCC consists of β (60 kDa), γ (33 kDa), and α_2 and δ subunits (170 kDa) which are connected by disulfide bonds (Jones, 1998; Mitterdorfer *et al.*, 1998). Recently, a novel VDCC-associated protein of 700 kDa has been identified in mammalian cardiomyocytes as a target protein for protein kinase A (PKA)-mediated phosphorylation. This novel protein exhibits some homology to *AHNAK*-encoded protein, which interacts with phospholipase C. It has been hypothesized that this protein plays a physiological role in transduction of cardiac β-adrenergic signal (Haase *et al.*, 1999).

The most abundant isoforms of DHPR in skeletal and cardiac muscles are L-type calcium channels consisting of α_{1s} and α_{1c} subunits, respectively. The α_1 subunit is the receptor for Ca^{2+} antagonists and can function

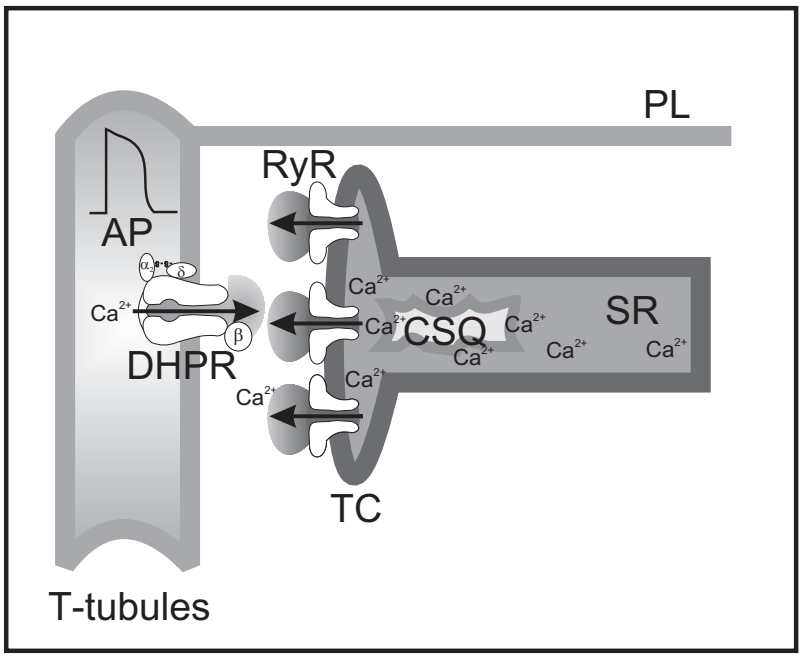

Figure 2. Schematic representation of cellular interrelation between dihydropyridine receptor (DHPR) and ryanodine receptor (RyR).

AP, action potential; CSQ, calsequestrin; PL, plasma membrane; SR, sarcoplasmic reticulum; TC, terminal cisterne; T-tubules, transversal tubules (modified from Wrzosek, 1999)

as a voltage-gated channel. It was observed that α_{1s} subunit was able to interact with RyR channels, influencing their activity (Leong & MacLennan, 1998b). Additionally, in skeletal muscle, a short amino-acid sequence of RyR has been identified, which binds to loops I–III of DHPR channel (Leong & MacLennan, 1998a; Grabner *et al.*, 1999; Zhu *et al.*, 1999; Avila & Dirksen, 2000). The DHPR channel activity is also regulated by protein kinase-mediated phosphorylation (McDonald *et al.*, 1994; Strauss *et al.*, 1997; He *et al.*, 2000; Boixel *et al.*, 2000). Moreover, it was shown that, in addition to phosphorylation of α_1 subunit, phosphorylation of DHPR β subunit mediated by PKA may be also partially responsible for modifying the channel activity (Bunemann *et al.*, 1999). Among other protein kinases involved in regulation of DHPR channel activity cyclic GMP-dependent protein kinase was found to decrease the L-type Ca^{2+} channel current (Schulz *et al.*, 1994; Haddad *et al.*, 1995), by phosphorylating the serine residue at position 533 in α_{1c} subunit (Jiang *et al.*, 2000). Involvement of cyclic GMP in regulation of DHPR channel activity is probably related to the effect of nitric oxide which activates guanylate cyclase (McDonald & Murad, 1996).

RYANODINE RECEPTOR

Molecular cloning

The functional RyR exists as a homotetramer and has a quatrefoil shape about 50 nm in diameter. The center of the quatrefoil contains a pore with an internal diameter of 1 to 2 nm, connected to four radial channels in the peripheral portion of each monomer (Wagenknecht *et al.*, 1989). The use of cryo-electron microscopy of purified RyR, in combination with three-dimensional image reconstructions, allowed creation of a three-dimensional model of the RyR channels (Radermacher *et al.*, 1994; Serysheva *et al.*, 1995; Sharma *et al.*, 1998; Sharma *et al.*, 2000b). It was also possible with the aid of mass displacement in the image analysis to distinguish between two functional states of RyR: open and closed. These states were related to measured conductance of RyR incorporated into lipid bilayers (Murayama & Ogawa, 1997; Murayama *et al.*, 1999).

In mammalian tissues three different genes encode three isoforms of RyR: RyR1, RyR2, and RyR3. cDNA corresponding to the *ryr1* gene encoding the RyR1 isoform characteristic for skeletal muscle has been cloned

(Takeshima *et al.*, 1989; Zorzato *et al.*, 1990). Rabbit isoform at a protein level has a predicted of 565 223 Da and only slightly differs from its human counterpart with predicted of 563 584 Da (97% identity). The *ryr2* gene encoding the RyR2 isoform was cloned from rabbit heart (Otsu *et al.*, 1990; Nakai *et al.*, 1990) and its predicted amino-acid sequence was published later (Tunwell *et al.*, 1996). Much less is known about the RyR3 isoform because it is found at lower concentrations than RyR1 or RyR2 (Murayama & Ogawa, 1997). Partial cDNA corresponding to RyR3 form and then full-length homolog from rabbit brain were cloned (Giannini *et al.*, 1992; Hakamata *et al.*, 1992).

Although three isoforms of RyR are highly homologous proteins, with 67–70% identity, there are three regions in their molecules, D1, D2, and D3, where the amino-acid sequences significantly diverge (Takeshima *et al.*, 1989; Zorzato *et al.*, 1990; Otsu *et al.*, 1990; Hakamata *et al.*, 1992; Sorrentino & Volpe, 1993). In skeletal muscles, region D2 of RyR1 (amino acids 1303–1406), containing charged amino-acid residues, is thought to be essential for E-C coupling (Yamazawa *et al.*, 1997). Recently, region D2 has been overexpressed as a glutathione *S*-transferase fusion protein and crystallized (Kim *et al.*, 1999). Moreover, molecular imaging of RyR1 and RyR3 isoforms clearly revealed that region D2 is missing in RyR3 isoform (Sharma *et al.*, 2000b). Comparison of reconstructed images of the three RyR isoforms showed that all isoforms are highly conserved with respect to the three-dimensional organization, in which a large part of protein mass (about 80%) forms a cytoplasmic assembly. Despite of similarities in structure, all the three isoforms of RyR differ, however, in the way they are regulated by endogenous and exogenous factors.

Regulation of RyR by endogenous factors

The most important regulator of RyR is Ca^{2+} which not only is transported by the channel but also modulates its activity. Interestingly, in the absence of Ca^{2+}, RyR conductance for monovalent ions is even higher than for Ca^{2+} (Tinker & Williams, 1992; Coronado *et al.*, 1994; Liu *et al.*, 1998). Ca^{2+} has both activating and inhibiting effects on Ca^{2+} release from SR vesicles, the activity of a single channel, and ryanodine binding to RyR (Meissner, 1986; Michalak *et al.*, 1988; Ashley & Williams, 1990). Ryanodine binding to RyR is absolutely Ca^{2+}dependent. RyR channels contain two different Ca^{2+} binding sites, with high and low affinity for the cation. Differences in binding constants for these sites were determined using trivalent cations, La^{3+} and Tb^{3+}. Tb^{3+}, each of which, when it binds to proteins, changes its luminescence, thus they were found to be sensitive probes for displacing Ca^{2+} from calcium-binding sites. RyR has two binding sites for Tb^{3+}, one at low concentrations, Tb^{3+} activated, and the second, which at high concentration, inhibited the channel activity of RyR reconstituted in planar lipid bilayer. Part of Tb^{3+} was found occluded and it was impossible to remove it from protein using EGTA or high concentration of Ca^{2+} (Hadad *et al.*, 1994).

Several putative binding sites for Ca^{2+} in RyR have been proposed on the basis of analysis of primary structure of RyR, point directed mutagenesis, modification with dicyclohexylcarbodiimide, and by using specific antibodies directed to the Ca^{2+}-binding domains (Chen *et al.*, 1998; Takeshima *et al.*, 1989; Chen *et al.*, 1992; 1993; Martinez-Azorin *et al.*, 1993). Changes in sensitivity of RyR to Ca^{2+} were also observed in pathological states, such as malignant hyperthermia (Loke & MacLennan, 1998; Jurkat-Rott *et al.*, 2000).

The bell-shaped dependence of RyR activity on Ca^{2+} concentration is affected by increased concentrations of Mg^{2+}, which is another divalent cation influencing the RyR channel activity (Lamb & Stephenson, 1994; Murayama *et al.*, 2000; Lamb, 2000). Mg^{2+} competes with Ca^{2+} for both activatory and inhibitory binding sites (Meissner *et al.*, 1997; Murayama *et*

al., 2000). RyR isoforms are affected by Mg^{2+} in a different manner. This could be explained by the differences between RyR1 and RyR2 in the mechanism of Ca^{2+} release from SR stores (Lamb, 2000). Zn^{2+}, which is known to be a catalytic and structural element of many proteins involved in the metabolic regulation and gene expression in mammalian cells, was found to modulate also RyR activity (Berg & Shi, 1996) by binding to activatory and inhibitory sites for Ca^{2+} (Xia *et al.*, 2000).

It is well documented that not only divalent but also monovalent cations modulate RyR function (Meissner, 1994; Zucchi & Ronca-Testoni, 1997). For example, H^+ exerts a profound effect on RyR activity and CICR processes (Meissner & Henderson, 1987; Michalak, 1988; Kentish & Xiang, 1997). Changes in pH in skeletal muscle cells are observed during prolonged activity and fatigue (Fitts, 1994), while in cardiac muscle cells, during ischemia (Mohabir *et al.*, 1991; Xu *et al.*, 1996). The effects of H^+ on cytoplasmic and luminal domains of RyR from rabbit skeletal muscle incorporated into planar lipid bilayer were also investigated (Harrison & Bers, 1987; Rousseau & Pinkos, 1990; Ma & Zhao, 1994; Laver *et al.*, 2000), revealing a difference in time dependence of recovery of the channels (Ma & Zhao, 1994; Laver *et al.*, 2000). Moreover, it was found that ionic composition and ionic strength could affect competitive binding of Mg^{2+} to the Ca^{2+} activatory and inhibitory binding sites, as well as binding of anions to specific regulatory sites on RyR molecules (Ma *et al.*, 1993; Sukhareva *et al.*, 1994; Meissner *et al.*, 1997; Zucchi & Ronca-Testoni, 1997).

During intensive exercise the concentration of lactate in skeletal muscle cells increases and can reach up to 30 mM. It has been shown that this metabolite may affect RyR activity independently of H^+, although acidification of cytoplasm is a feature concomitant with lactate production (Favero *et al.*, 1995). Recently, the results illustrating the effects of lactate on depolarization-induced Ca^{2+} release in mechanically skinned skeletal muscle fibers were published. These data revealed, however, that lactate had an only minimal effect on the contractile apparatus and depolarization-induced Ca^{2+} release, and was not a major factor in muscle fatigue (Dutka & Lamb, 2000).

It has been observed using SR vesicles that ATP enhances the release of Ca^{2+} even in nanomolar Ca^{2+} concentration range, and increases ryanodine binding to RyR (Meissner, 1984; Campbell *et al.*, 1987; Shoshan-Barmatz & Ashley, 1998; Zarka & Shoshan-Barmatz, 1993). Moreover, ATP was found to activate RyR incorporated into planar lipid bilayer (Ashley, 1989; Zucchi & Ronca-Testoni, 1997; Kermode *et al.*, 1998). The interaction of ATP with RyR1 and RyR2 is relatively well characterized, but some controversy still exists as concerns RyR3 (Chen *et al.*, 1997; Jeyakumar *et al.*, 1998; Murayama *et al.*, 1999). Recently, ATP-induced activation of RyR3 expressed in HEK293 cells was studied in planar lipid bilayer (Manunta *et al.*, 2000) and it has been shown that RyR3 is not active at resting levels of Ca^{2+}. Addition of ATP increased the open time of the channel and RyR3 was not inactivated at millimolar Ca^{2+} concentrations. Amino-acid sequence analysis identified putative nucleotide-binding motifs (GXGXXG) in all isoforms of RyR (Takeshima *et al.*, 1989; Zorzato *et al.*, 1990; Hakamata *et al.*, 1992). These motifs were also identified using a photoreactive ATP analog and V8 protease digestion, and were suggested to play a role in gating behavior of the channel (Zarka & Shoshan-Barmatz, 1993). In working muscle under normal conditions the overall ATP level is relatively stable, but during extensive exercise or in some pathological states the intracellular level of ATP can significantly drop down, leading to changes not only in energetic state of a muscle but also in concentration of ATP metabolites and Mg^{2+}. These changes may influence RyR channels and E-C coupling (Blazev & Lamb, 1999a; 1999b). Therefore, it has been suggested that an in-

crease in adenosine concentration may be at least partially responsible for fatigue of the muscle (Blazev & Lamb, 1999a).

Inorganic phosphate (P_i), one of the important metabolites in a muscle cell, is able to influence the contraction-relaxation cycle of cardiac and skeletal muscles. It has been proposed that P_i exerts its effects by interaction with the actin–myosin binding function or by modulating earlier events of E-C coupling, namely Ca^{2+} release from SR stores. In addition P_i could be a factor contributing to the muscle fatigue (Fitts, 1994). It is known from previous studies that P_i exerts a stimulatory effect on RyR channels in cardiac and skeletal muscles (Fruen *et al.*, 1994; Xiang & Kentish, 1995; Kentish & Xiang, 1997; Posterino & Fryer, 1998). However, the mechanism of action of P_i remains elusive. It was proposed that ADP and P_i could mimic the binding of ATP to RyR (Xiang & Kentish, 1995). Recently, it has been found that the addition of P_i changes a single channel open probability and decreases mean channel closed time of a skeletal muscle RyR; mean channel open times remained, however, unaffected (Balog *et al.*, 2000). Moreover, in skinned skeletal muscle fibers, P_i enhanced CICR (Balog *et al.*, 2000). On the basis of these data it has been suggested that P_i acts on RyR channels *via* a mechanism distinct from that exerted by adenine nucleotides, and can be an endogenous modulator of the skeletal muscles under fatigue conditions (Balog *et al.*, 2000).

In addition to adenine nucleotides and P_i, cADP-ribose (cADPR), a metabolite of β-NAD^+, has been also recognized as an endogenous regulator of RyR2 and RyR3 channels in many cell types (Galione *et al.*, 1991; Galione, 1992; Hua *et al.*, 1994; Rakovic *et al.*, 1999). RyR1 isoform of the channel was not activated by cADPR (Sonnleitner *et al.*, 1998). Studies on intact cardiac cells revealed that endogenous cADPR plays a role in regulation of E-C coupling (Iino *et al.*, 1997). It was shown that in guinea pig ventricular cells antagonists of cADPR inhibited arrhythmogenic oscillations of intracellular Ca^{2+} and $[Ca^{2+}]_i$ oscillations evoked by overloading of SR stores by Ca^{2+} in the presence of isoproterenol or ouabain (Rakovic *et al.*, 1999). As was shown by Noguchi *et al.* (1997), cADPR binds to immunosuppressant FK506 binding protein 12.6 (FKBP) (see next paragraph) within a RyR molecule. Recently, it has been observed that, in neuronal cells NG108-15, cADPR can act as a direct agonist of RyR channels and, in addition, can indirectly interact with L-type voltage-activated Ca^{2+} channels (Hashii *et al.*, 2000).

RyRs are integral proteins of SR membrane. About 20% of their molecule is buried in membrane but, surprisingly, the effects of lipids and lipid derivatives on RyR activity are not well documented. Only controversial data related to the action of fatty acids on Ca^{2+} release from SR stores are available (Cheah, 1981; Messineo *et al.*, 1984; Zucchi & Ronca-Testoni, 1997). Arachidonic acid, for example, has been found to induce Ca^{2+} release from skeletal and cardiac muscles and to inhibit binding of ryanodine to SR membranes, however, a single channel open probability of RyR remained unaffected (Damron & Bond, 1993; el-Hayek *et al.*, 1993; Zucchi & Ronca-Testoni, 1997). The observations of Uehara *et al.* (1996) suggest that arachidonic acid directly modifies the structure of the ryanodine binding site in RyRs. Recently sphingosine, a long-chain amino-alcohol which is a component of sphingolipids, was found to modulate, in a noncompetitive manner, ryanodine binding to RyRs from skeletal and cardiac muscles, by interacting with a site between Arg4475 and the C-terminus of RyR channels (Needleman *et al.*, 1997; Sharma *et al.*, 2000a).

Protein–protein interaction in regulation of RyR activity

There are many cellular proteins exerting various direct or indirect effects on the activity of RyR channels. Some of them are regu-

lated by Ca^{2+} or by phosphorylation-dephosphorylation processes (Shoshan-Barmatz & Ashley, 1998; MacKrill, 1999). Some of these proteins interact only with specific isoforms of RyR (Leong & MacLennan, 1998b; MacKrill, 1999). These proteins were divided into a few categories, depending on their cellular location, role and interactions, and were characterized not only as regulators of RyRs but also as other Ca^{2+} release channels, the activity of which is regulated by inositol 1,4,5,-trisphosphate (MacKrill, 1999).

Calsequestrin (CSQ), a Ca^{2+} storage protein located in the lumen of SR membranes, especially in their terminal cisterns, was overexpressed in mouse cardiac muscles (Jones *et al.*, 1998). Cardiomyocytes from these hearts were by 50–100% larger than in normal animals and had significant electrophysiological and electromechanical defects. In addition, transgenic mice overexpressing cardiac CSQ developed with age extensive electrical remodeling of ionic current, and upregulation of Ca^{2+}/Na^{+} exchanger; it has been found that Ca^{2+} signaling pathways are the primary site of the defect (Knollmann *et al.*, 2000). The transgenic animals overexpressing CSQ, were characterized by impaired Ca^{2+} signaling, resulting from reduced co-ordination and decreased frequency of Ca^{2+} sparks; the latter could not be restored by increased calcium current, but with caffeine, which was found to alter the sensitivity of RyR channels to Ca^{2+} (Wang *et al.*, 2000). The transgenic mice overexpressing CQS and characterized by heart failure and premature death exhibited also alterations in β-adrenergic receptor signaling preceding the development of heart failure (Cho *et al.*, 1999). Recently, it has been shown that not only the amount of CSQ, but also phosphorylation-dephosphorylation processes, can influence Ca^{2+} homeostasis in striated muscles (Szegedi *et al.*, 1999).

Annexin VI, a Ca^{2+} and phospholipid binding protein, was shown to regulate RyR2 activity, at the nanomolar concentration range. Annexin VI increased the mean open time and open probability of RyR2 channels (Diaz-Munoz *et al.*, 1990). Moreover, targeted overexpression of annexin VI in transgenic mice caused altered signaling, attributed to the probable effects of annexin VI on plasma membrane Ca^{2+}-ATPase or Na^{+}/Ca^{2+} exchanger (Gunteski-Hamblin *et al.*, 1996).

Sarcalumenin (150 kDa) and a histidine-rich Ca^{2+} binding protein (160 kDa) were identified in the lumen of SR from skeletal muscle cells (Orr & Shoshan-Barmatz, 1996; Shoshan-Barmatz *et al.*, 1996b), and were found to be phosphorylated by SR casein kinase II. ATP required for phosphorylation of these two proteins in the lumen of SR is probably transported *via* a voltage-dependent anion channel (Shoshan-Barmatz *et al.*, 1996a). Recently, a cardiac isoform of sarcalumenin of 130 kDa, was also identified, and found to be phosphorylated by casein kinase II. Phosphorylation of sarcalumenin resulted in modification of its interaction with RyR (Hadad *et al.*, 1999).

Triadin and junctin are examples of integral membrane proteins that interact with RyR channels and with CSQ (MacKrill, 1999). Triadin from rabbit skeletal muscles was shown, using the ligand overlay and crosslinking analysis, to bind RyR and α1 subunit of DHPR (Caswell *et al.*, 1991). Triadin is localized in the junctional terminal cisterns of SR in rabbit skeletal muscles (immunofluorescence and microscopy analysis). This protein consists of 706 amino acids, mostly basic residues (pI 10.18) (Knudson *et al.*, 1993a; 1993b). Using surface plasmon resonance spectroscopy and overlay assay, a functional interaction of cytoplasmic part of triadin with skeletal muscle RyR was demonstrated. This interaction can be important in the control of E-C coupling in skeletal muscles (Groh *et al.*, 1999), since it has been shown that C-terminus of triadin interacts with both CSQ and the luminal portion of RyR (Guo *et al.*, 1996a). Using triadin antibodies that did not discriminate between isoforms, three triadin

isoforms of 35 kDa, 40 kDa and 92 kDa, respectively, were identified in rabbit cardiac SR vesicles, as also predicted by cDNA cloning (Guo *et al.*, 1996b). Recently triadin 1 was identified as the predominant isoform expressed in mammalian myocardium (Kobayashi & Jones, 1999).

By using iodide labeled CSQ overlay assays, three major CSQ binding proteins were identified in cardiac SR vesicles (Mitchell *et al.*, 1988). The 26 kDa protein was purified, cloned and named junctin. An identical protein was identified in skeletal muscles (Jones *et al.*, 1995). It has been suggested that interactions between triadins, junctin and CSQ work as a link, regulator and sensor of Ca^{2+} in the lumen of SR, and also as a link between RyR and DHPR (Jones *et al.*, 1995).

The larger part of RyR channel which is exposed to the cytoplasmic site of the cell interacts with many accessory proteins; these proteins modulate the RyR activity directly or through the phosphorylation-dephosphorylation processes (Shoshan-Barmatz & Ashley, 1998; MacKrill, 1999). One of among them is calmodulin (CaM), which is a ubiquitous Ca^{2+} binding protein for which three binding sites on RyR have been identified: PM1, PM2 and PM3 (Menegazzi *et al.*, 1994; Wagenknecht *et al.*, 1997). The modulation of RyR activity by CaM was shown to be biphasic; at the nanomolar Ca^{2+} concentration range CaM stimulates RyR activity; the biphasic profile of stimulation by CaM is probably a consequence of different binding of CaM at different Ca^{2+} concentrations (Fuentes *et al.*, 1994; Tripathy *et al.*, 1995; Ikemoto *et al.*, 1995).

Sorcin is a 22 kDa EF-hand Ca^{2+} binding protein (Zamparelli *et al.*, 2000), which undergoes Ca^{2+}-dependent translocation from cytoplasm to membrane. Sorcin is associated with RyR channels and was determined to be present in cardiac myocytes by co-immunoprecipitation (Meyers *et al.*, 1995). Recently, it has been observed that sorcin, at the nanomolar concentration range, may inhibit RyR2 activity, while phosphorylation of sorcin, mediated by PKA, reduces this inhibition. It was also shown that sorcin interacted with the C-terminus of DHPR from both skeletal and cardiac muscles. It has been proposed that interactions between sorcin and RyR and DHPR can form a platform of interchannel communication during E-C coupling (Lokuta *et al.*, 1997; Meyers *et al.*, 1998).

The S100 family of EF-hand proteins, localized predominantly in the cytosol of muscle cells, was recognized as potent modulators of RyR1 activity in skeletal muscles. It was shown that S100 interacted directly with RyR1 in three putative binding sites. One of them overlaps with the CaM binding site (Schafer & Heizmann, 1996; Treves *et al.*, 1997), leading to speculations about the role of S100 proteins in the cardiac muscle contraction-relaxation cycle (Remppis *et al.*, 1996).

FKBP belong to a family of proteins which bind immunosuppressant drugs, FK506 and rapamycin. Among several existing isoforms, the predominant FKBP12 of 12 kDa is engaged in mediating the immunosuppressive actions of the drug. FKBP12 is also involved in catalysis of peptidylpropyl-*cis-trans*-isomerization, and probably is involved in protein folding. Binding of FK506 and/or rapamycin to FKBP12 inhibits its isomerase activity and alters its association with other proteins (Marks, 1996). In addition, it has been shown that FKBP12 binds to cardiac RyR isoform and modulates its channel activity (Timerman *et al.*, 1993), with a stoichiometry of one FKBP12 molecule per one monomer of functional tetramer of RyR (Wagenknecht *et al.*, 1997). RyR2 channels from heart muscle interact with a specific isoform of FKPB12, FKBP12.6, while the skeletal isoform (RyR1), interacts with both FKBP12 and FKBP12.6 (Timerman *et al.*, 1996). The selectivity of FKBP12.6 for RyR2 isoform is related to differences in three amino acids between FKBP12 and FKBP12.6 (Xin *et al.*, 1999). Phosphorylation of FKPB12.6 by PKA dissociated this protein from RyR2. This dissociation

regulates the channel open probability of RyR. Using cosedimentation and coimmunoprecipitation methods a macromolecular complex composed of RyR2, FKBP12.6, PKA, the protein phosphatases PP1 and PP2A, and anchoring protein mAKAP, has been identified (Marx *et al.*, 2000). This complex is probably involved in regulation of RyR channel activity by PKA; in failing heart this complex is hyperphosphorylated by PKA leading to abnormal activity of RyR (Marx *et al.*, 2000).

REACTIVE OXYGEN SPECIES

RyR channels are under the constant influence of reactive oxygen species, such as superoxide radical anion, singlet oxygen, hydrogen peroxide, and hydroxyl radicals that are by-products of oxidative metabolism, and nitric oxide as a product of nitric oxide synthase. Production of reactive oxygen species is increased during inflammation, aging, endotoxic shock, and ischemia-reperfusion of heart and other tissues (Kourie, 1998). Recently, oxidative and nitrosilation processes were shown to exert a modulatory role on RyR channel activity (Zable *et al.*, 1997; Xu *et al.*, 1998; Zhang *et al.*, 1999; Haarmann *et al.*, 1999; Suko *et al.*, 1999; Hart & Dulhunty, 2000). In addition, it has been found that oxidation of sulfhydryl groups overrides Mg^{2+} inhibition of CICR in skeletal muscles (Donoso *et al.*, 2000). Oxidative stress may affect the binding of CaM to RyR, changing in this way the activity of the channel (Porter Moore *et al.*, 1999; Suko *et al.*, 2000).

CONCLUDING REMARKS

Ca^{2+} liberated from internal cellular stores (SR) regulates the contraction of mammalian cardiac and skeletal muscles. The systems involved in Ca^{2+} regulation in these muscles consist of proteins that form of similar pattern. The only differences are in tissue-specific isoforms, their distribution, and spatial organization. Endogenous compounds which regulate the activity of RyR and DHPR channels by direct or indirect interactions, display different regulatory abilities. For example, Mg^{2+}, which in the case of skeletal muscles inhibits RyR activity at the micromolar concentration range, has no effect on RyR activity in cardiac muscles. The differences in amino-acid composition between DHPR and RyR from cardiac and skeletal muscle form a basis for differences in regulation of opening of RyR channels. As a consequence, the release of Ca^{2+} from SR is fully responsible for the contraction of skeletal muscles, but only in part in heart muscle (Chiesi *et al.*, 1994; Wrzosek, 1999). However, as suggested by Eisner *et al.* (1998), the temporary inhibition or activation of RyR channels produces only a transient effect on systolic Ca^{2+}. It seems that more important for E-C coupling are changes in Ca^{2+} compartmentalization within the cell. The disturbances of these processes may lead to development of pathological states related to impaired Ca^{2+} homeostasis.

REFERENCES

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.

Ashley, R.H. & Williams, A.J. (1990) Divalent cation activation and inhibition of single calcium release channels from sheep cardiac sarcoplasmic reticulum. *J. Gen. Physiol.* **95**, 981–1005.

Avila, G. & Dirksen, R.T. (2000) Functional impact of the ryanodine receptor on the skeletal muscle L-type Ca^{2+} channel. *J. Gen. Physiol.* **115**, 467–480.

Balog, E.M., Fruen, B.R., Kane, P.K. & Louis, C.F. (2000) Mechanisms of P_i regulation of the skel-

etal muscle SR Ca^{2+} release channel. *Am. J. Physiol. Cell Physiol.* **278**, C601–C611.

Berg, J.M. & Shi, Y. (1996) The galvanization of biology: A growing appreciation for the roles of zinc. *Science* **271**, 1081–1085.

Bers, D.M. (1991) *Excitation-Contraction coupling and Cardiac Contractile Force*; pp. 119–148. Kluwer Academic Publishers, Dordrecht, Boston, London.

Blazev, R. & Lamb, G.D. (1999a) Adenosine inhibits depolarization-induced Ca^{2+} release in mammalian skeletal muscle. *Muscle Nerve* **22**, 1674–1683.

Blazev, R. & Lamb, G.D. (1999b) Low [ATP] and elevated [Mg^{2+}] reduce depolarization-induced Ca^{2+} release in rat skinned skeletal muscle fibres. *J. Physiol.* (*London*) **520**, 203–215.

Boixel, C., Tessier, S., Pansard, Y., Lang- Lazdunski, L., Mercadier, J.J. & Hatem, S.N. (2000) Tyrosine kinase and protein kinase C regulate L-type Ca^{2+} current cooperatively in human atrial myocytes. *Am. J. Physiol. Heart Circ. Physiol.* **278**, H670–H676.

Bunemann, M., Gerhardstein, B.L., Gao, T. & Hosey, M.M. (1999) Functional regulation of L-type calcium channels *via* protein kinase A-mediated phosphorylation of the β_2 subunit. *J. Biol. Chem.* **274**, 33851–33854.

Campbell, K.P., Knudson, C.M., Imagawa, T., Leung, A.T., Sutko, J.L., Kahl, S.D., Raab, C.R. & Madson, L. (1987) Identification and characterization of the high affinity [^{3}H]ryanodine receptor of the junctional sarcoplasmic reticulum Ca^{2+} release channel. *J. Biol. Chem.* **262**, 6460–6463.

Caswell, A.H., Brandt, N.R., Brunschwig, J.P. & Purkerson, S. (1991) Localization and partial characterization of the oligomeric disulfide-linked molecular weight 95000 protein (triadin) which binds the ryanodine and dihydropyridine receptors in skeletal muscle triadic vesicles. *Biochemistry* **30**, 7507–7513.

Cheah, A.M. (1981) Effect of long chain unsaturated fatty acids on the calcium transport of sarcoplasmic reticulum. *Biochim. Biophys. Acta* **648**, 113–119.

Chen, S.R., Ebisawa, K., Li, X. & Zhang, L. (1998) Molecular identification of the ryanodine receptor Ca^{2+} sensor. *J. Biol. Chem.* **273**, 14675–14678.

Chen, S.R., Zhang, L. & 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**, 23318–23326.

Chen, S.R., Zhang, L. & MacLennan, D.H. (1993) 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**, 13414–13421.

Chen, S.R.W., Li, X., Ebisawa, K. & Zhang, L. (1997) Functional characterization of the recombinant type 3 Ca^{2+} release channel (ryanodine receptor) expressed in HEK293 cells. *J. Biol. Chem.* **272**, 24234–24246.

Cheng, H., Lederer, W.J. & Cannell, M.B. (1993) Calcium sparks: Elementary events underlying excitation-contraction coupling in heart muscle. *Science* **262**, 740–744.

Cheng, H., Lederer, M.R., Xiao, R.P., Gomez, A.M., Zhou, Y.Y., Ziman, B., Spurgeon, H., Lakatta, E.G. & Lederer, W.J. (1996) Excitation-contraction coupling in heart: New insights from Ca^{2+} sparks. *Cell Calcium* **20**, 129–140.

Chiesi, M., Wrzosek, A. & Grueninger, S. (1994) The role of the sarcoplasmic reticulum in various types of cardiomyocytes. *Mol. Cell. Biochem.* **130**, 159–171.

Cho, M.C., Rapacciuolo, A., Koch, W.J., Kobayashi, Y., Jones, L.R. & Rockman, H.A. (1999) Defective beta-adrenergic receptor signaling precedes the development of dilated cardiomyopathy in transgenic mice with calsequestrin overexpression. *J. Biol. Chem.* **274**, 22251–22256.

Coronado, R., Morrissette, J., Sukhareva, M. & Vaughan, D.M. (1994) Structure and function of ryanodine receptors. *Am. J. Physiol.* **266**, C1485–C1504.

Damron, D.S. & Bond, M. (1993) Modulation of Ca^{2+} cycling in cardiac myocytes by arachidonic acid. *Circ. Res.* **72**, 376–386.

Diaz-Munoz, M., Hamilton, S.L., Kaetzel, M.A., Hazarika, P. & Dedman, J.R. (1990) Modulation of Ca^{2+} release channel activity from sarcoplasmic reticulum by annexin VI (67-kDa calcimedin). *J. Biol. Chem.* **265**, 15894–15899.

Donoso, P., Aracena, P. & Hidalgo, C. (2000) Sulfhydryl oxidation overrides Mg^{2+} inhibition of calcium-induced calcium release in skeletal muscle triads. *Biophys. J.* **79**, 279–286.

Dutka, T.L. & Lamb, G.D. (2000) Effect of lactate on depolarization-induced Ca^{2+} release in mechanically skinned skeletal muscle fibers. *Am. J. Physiol.* **278**, C517–C525.

Eisner, D.A., Trafford, A.W., Diaz, M.E., Overend, C.L. & O'Neil, S.C. (1998) The control of Ca release from the cardiac sarcoplasmic reticulum: Regulation *versus* autoregulation. *Cardiovasc. Res.* **38**, 589–604.

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

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

Fabiato, A. (1985a) Rapid ionic modifications during the aequorin-detected calcium transient in a skinned canine cardiac Purkinje cell. *J. Gen. Physiol.* **85**, 189–246.

Fabiato, A. (1985b) Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J. Gen. Physiol.* **85**, 247–289.

Favero, T.G., Zable, A.C., Bowman, M.B., Thompson, A. & Abramson, J.J. (1995) Metabolic end products inhibit sarcoplasmic reticulum Ca^{2+} release and [^{3}H]ryanodine binding. *J. Appl. Physiol.* **78**, 1665–1672.

Fitts, R.H. (1994) Cellular mechanisms of muscle fatigue. *Physiol. Rev.* **74**, 49–94.

Flucher, B.E. & Franzini-Armstrong, C. (1996) Formation of junctions involved in excitation-contraction coupling in skeletal and cardiac muscle. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8101–8106.

Flucher, B.E., Phillips, J.L., Powell, J.A., Andrews, S.B. & Daniels, M.P. (1992) Coordinated development of myofibrils, sarcoplasmic reticulum and transverse tubules in normal and dysgenic mouse skeletal muscle, *in vivo* and *in vitro*. *Dev. Biol.* **150**, 266–280.

Franzini-Armstrong, C. & Protasi, F. (1997) Ryanodine receptors of striated muscles: A complex channel capable of multiple interactions. *Physiol. Rev.* **77**, 699–729.

Fruen, B.R., Mickelson, J.R., Shomer, N.H., Roghair, T.J. & Louis, C.F. (1994) Regulation of the sarcoplasmic reticulum ryanodine receptor by inorganic phosphate. *J. Biol. Chem.* **269**, 192–198.

Fuentes, O., Valdivia, C., Vaughan, D., Coronado, R. & Valdivia, H.H. (1994) Calcium-dependent block of ryanodine receptor channel of swine skeletal muscle by direct binding of calmodulin. *Cell Calcium* **15**, 305–316.

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

Galione, A., Lee, H.C. & Busa, W.B. (1991) Ca^{2+}-induced Ca^{2+} release in sea urchin egg homogenates: Modulation by cyclic ADP-ribose. *Science* **253**, 1143–1146.

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

Grabner, M., Dirksen, R.T., Suda, N. & Beam, K.G. (1999) The II-III loop of the skeletal muscle dihydropyridine receptor is responsible for the bi-directional coupling with the ryanodine receptor. *J. Biol. Chem.* **274**, 21913–21919.

Groh, S., Marty, I., Ottolia, M., Prestipino, G., Chapel, A., Villaz, M. & Ronjat, M. (1999) Functional interaction of the cytoplasmic domain of triadin with the skeletal ryanodine receptor. *J. Biol. Chem.* **274**, 12278–12283.

Gunteski-Hamblin, A.M., Song, G., Walsh, R.A., Frenzke, M., Boivin, G.P., Dorn, 2nd, G.W., Kaetzel, M.A., Horseman, N.D. & Dedman, J.R. (1996) Annexin VI overexpression targeted to heart alters cardiomyocyte function in transgenic mice. *Am. J. Physiol.* **270**, H1091–H1100.

Guo, W., Jorgensen, A.O. & Campbell, K.P. (1996a) Triadin, a linker for calsequestrin and the ryanodine receptor. *Soc. Gen. Physiol. Ser.* **51**, 19–28.

Guo, W., Jorgensen, A.O., Jones, L.R. & Campbell, K.P. (1996b) Biochemical characterization and molecular cloning of cardiac triadin. *J. Biol. Chem.* **271**, 458–465.

Haarmann, C.S., Fink, R.H. & Dulhunty, A.F. (1999) Oxidation and reduction of pig skeletal muscle ryanodine receptors. *Biophys. J.* **77**, 3010–3022.

Haase, H., Podzuweit, T., Lutsch, G., Hohaus, A., Kostka, S., Lindschau, C., Kott, M., Kraft, R. & Morano, I. (1999) Signaling from beta-adrenoceptor to L-type calcium channel: Identification of a novel cardiac protein kinase A target possessing similarities to *AHNAK*. *FASEB J.* **13**, 2161–2172.

Hadad, N., Meyer, H.E., Varsanyi, M., Fleischer, S. & Shoshan-Barmatz, V. (1999) Cardiac sarcalumenin: Phosphorylation, comparison with the skeletal muscle sarcalumenin and modulation of ryanodine receptor. *J. Membr. Biol.* **170**, 39–49.

Hadad, N., Zable, A.C., Abramson, J.J. & Shoshan-Barmatz, V. (1994) Ca^{2+} binding sites of the ryanodine receptor/ Ca^{2+} release channel of sarcoplasmic reticulum. Low affinity binding site(s) as probed by terbium fluorescence. *J. Biol. Chem.* **269**, 24864–24869.

Haddad, G.E., Sperelakis, N. & Bkaily, G. (1995) Regulation of the calcium slow channel by cyclic GMP dependent protein kinase in chick heart cells. *Mol. Cell. Biochem.* **148**, 89–94.

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

Harrison, S.M. & Bers, D.M. (1987) The effect of temperature and ionic strength on the apparent Ca-affinity of EGTA and the analogous Ca-chelators BAPTA and dibromo-BAPTA. *Biochim. Biophys. Acta* **925**, 133–143.

Hart, J.D. & Dulhunty, A.F. (2000) Nitric oxide activates or inhibits skeletal muscle ryanodine receptors depending on its concentration, membrane potential and ligand binding. *J. Membr. Biol.* **173**, 227–236.

Hashii, M., Minabe, Y. & Higashida, H. (2000) cADP-ribose potentiates cytosolic Ca^{2+} elevation and Ca^{2+} entry *via* L-type voltage-activated Ca^{2+} channels in NG108-15 neuronal cells. *Biochem. J.* **345**, 207–215.

He, J.Q., Pi, Y., Walker, J.W. & Kamp, T.J. (2000) Endothelin-1 and photoreleased diacylglycerol increase L-type Ca^{2+} current by activation of protein kinase C in rat ventricular myocytes. *J. Physiol.* (*London*) **524**, 807–820.

Hua, S.Y., Tokimasa, T., Takasawa, S., Furuya, Y., Nohmi, M., Okamoto, H. & Kuba, K. (1994) Cyclic ADP-ribose modulates Ca^{2+} release channels for activation by physiological Ca^{2+} entry in bullfrog sympathetic neurons. *Neuron* **12**, 1073–1079.

Iino, S., Cui, Y., Galione, A. & Terrar, D.A. (1997) Actions of cADP-ribose and its antagonists on contraction in guinea pig isolated ventricular myocytes. Influence of temperature. *Circ. Res.* **81**, 879–884.

Ikemoto, T., Iino, M. & Endo, M. (1995) Enhancing effect of calmodulin on Ca^{2+}-induced Ca^{2+} release in the sarcoplasmic reticulum of rabbit skeletal muscle fibres. *J. Physiol.* (*London*) **487**, 573–582.

Isenberg, G., Etter, E.F., Wendt-Gallitelli, M.F., Schiefer, A., Carrington, W.A., Tuft, R.A. & Fay, F.S. (1996) Intrasarcomere [Ca^{2+}] gradients in ventricular myocytes revealed by high speed digital imaging microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5413–5418.

Jeyakumar, L.H., Copello, J.A., O'Malley, A.M., Wu, G.M., Grassucci, R., Wagenknecht, T. & Fleischer, S. (1998) Purification and characterization of ryanodine receptor 3 from mammalian tissue. *J. Biol. Chem.* **273**, 16011–16020.

Jiang, L.H., Gawler, D.J., Hodson, N., Milligan, C.J., Pearson, H.A., Porter, V. & Wray, D. (2000) Regulation of cloned cardiac L-type calcium channels by cGMP-dependent protein kinase. *J. Biol. Chem.* **275**, 6135–6143.

Jones, S.W. (1998) Overview of voltage-dependent calcium channels. *J. Bioenerg. Biomembr.* **30**, 299–312.

Jones, L.R., Zhang, L., Sanborn, K., Jorgensen, A.O. & Kelley, J. (1995) Purification, primary structure, and immunological characterization of the 26-kDa calsequestrin binding protein (junctin) from cardiac junctional sarcoplasmic reticulum. *J. Biol. Chem.* **270**, 30787–30796.

Jones, L.R., Suzuki, Y.J., Wang, W., Kobayashi, Y.M., Ramesh, V., Franzini-Armstrong, C., Cleemann, L. & Morad, M. (1998) Regulation of Ca^{2+} signaling in transgenic mouse cardiac myocytes overexpressing calsequestrin. *J. Clin. Invest.* **101**, 1385–1393.

Jurkat-Rott, K., McCarthy, T. & Lehmann-Horn, F. (2000) Genetics and pathogenesis of malignant hyperthermia. *Muscle Nerve* **23**, 4–17.

Kentish, J.C. & Xiang, J.Z. (1997) Ca^{2+}- and caffeine-induced Ca^{2+} release from the sarcoplasmic reticulum in rat skinned trabeculae: effects of pH and Pi. *Cardiovasc. Res.* **33**, 314–323.

Kermode, H., Williams, A.J. & Sitsapesan, R. (1998) The interactions of ATP, ADP, and inorganic phosphate with the sheep cardiac ryanodine receptor. *Biophys. J.* **74**, 1296–1304.

Kim, S., Shin, D.W., Kim, D.H. & Eom, S.H. (1999) Crystallization and preliminary X-ray crystallographic studies of the D2 region of the skeletal muscle ryanodine receptor. *Acta Crystallogr. D. Biol. Crystallogr.* **55**, 1601–1603.

Knollmann, B.C., Knollmann-Ritschel, B.E., Weissman, N.J., Jones, L.R. & Morad, M. (2000) Remodelling of ionic currents in hypertrophied and failing hearts of transgenic mice overexpressing calsequestrin. *J. Physiol.* (*London*) **525**, 483–498.

Knudson, C.M., Stang, K.K., Jorgensen, A.O. & Campbell, K.P. (1993a) Biochemical characterization of ultrastructural localization of a major junctional sarcoplasmic reticulum glycoprotein (triadin). *J. Biol. Chem.* **268**, 12637–12645.

Knudson, C.M., Stang, K.K., Moomaw, C.R., Slaughter, C.A. & Campbell, K. (1993b) Primary structure and topological analysis of a skeletal muscle-specific junctional sarcoplasmic reticulum glycoprotein (triadin). *J. Biol. Chem.* **268**, 12646–12654.

Kobayashi, Y.M. & Jones, L.R. (1999) Identification of triadin 1 as the predominant triadin isoform expressed in mammalian myocardium. *J. Biol. Chem.* **274**, 28660–28668.

Kourie, J.I. (1998) Interaction of reactive oxygen species with ion transport mechanisms. *Am. J. Physiol.* **275**, C1–C24.

Lamb, G.D. (2000) Excitation-contraction coupling in skeletal muscle: Comparisons with cardiac muscle. *Clin. Exp. Pharmacol. Physiol.* **27**, 216–224.

Lamb, G.D. & Stephenson, D.G. (1994) Effects of intracellular pH and $[Mg^{2+}]$ on excitation-contraction coupling in skeletal muscle fibres of the rat. *J. Physiol.* (*London*) **478**, 331–339.

Langer, G.A. & Peskoff, A. (1997) Role of the diadic cleft in myocardial contractile control. *Circulation* **96**, 3761–3765.

Laver, D.R., Eager, K.R., Taoube, L. & Lamb, G.D. (2000) Effects of cytoplasmic and luminal pH on Ca^{2+} release channels from rabbit skeletal muscle. *Biophys. J.* **78**, 1835–1851.

Leong, P. & MacLennan, D.H. (1998a) A 37-amino acid sequence in the skeletal muscle ryanodine receptor interacts with the cytoplasmic loop between domains II and III in the skeletal muscle dihydropyridine receptor. *J. Biol. Chem.* **273**, 7791–7794.

Leong, P. & MacLennan, D.H. (1998b) Complex interactions between skeletal muscle ryanodine receptor and dihydropyridine receptor proteins. *Biochem. Cell Biol.* **76**, 681–694.

Liu, W., Pasek, D.A. & Meissner, G. (1998) Modulation of Ca^{2+}-gated cardiac muscle Ca^{2+}-release channel (ryanodine receptor) by mono-

and divalent ions. *Am. J. Physiol.* **274**, C120–C128.

Loke, J. & MacLennan, D.H. (1998) Malignant hyperthermia and central core disease: Disorders of Ca^{2+} release channels. *Am. J. Med.* **104**, 470–486.

Lokuta, A.J., Meyers, M.B., Sander, P.R., Fishman, G.I. & Valdivia, H.H. (1997) Modulation of cardiac ryanodine receptors by sorcin. *J. Biol. Chem.* **272**, 25333–25338.

Ma, J., Anderson, K., Shirokov, R., Levis, R., Gonzalez, A., Karhanek, M., Hosey, M.M., Meissner, G. & Rios, E. (1993) Effects of perchlorate on the molecules of excitation-contraction coupling of skeletal and cardiac muscle. *J. Gen. Physiol.* **102**, 423–448.

Ma, J. & Zhao, J. (1994) Highly cooperative and hysteretic response of the skeletal muscle ryanodine receptor to changes in proton concentrations. *Biophys. J.* **67**, 626–633.

MacKrill, J.J. (1999) Protein-protein interactions in intracellular Ca^{2+}-release channel function. *Biochem. J.* **337**, 345–361.

Manunta, M., Rossi, D., Simeoni, I., Butelli, E., Romanin, C., Sorrentino, V. & Schindler, H. (2000) ATP-induced activation of expressed RyR3 at low free calcium. *FEBS Lett.* **471**, 256–260.

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

Martinez-Azorin, F., Gomez-Fernandez, J.C. & Fernandez-Belda, F. (1993) Limited carbodiimide derivatization modifies some functional properties of the sarcoplasmic reticulum Ca^{2+} release channel. *Biochemistry* **32**, 8553–8559.

Marx, S.O., Reiken, S., Hisamatsu, Y., Jayaraman, T., Burkhoff, D., Rosemblit, N. & Marks, A.R. (2000) PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): Defective regulation in failing hearts. *Cell* **101**, 365–376.

McDonald, L.J. & Murad, F. (1996) Nitric oxide and cyclic GMP signaling. *Proc. Soc. Exp. Biol. Med.* **211**, 1–6.

McDonald, T.F., Pelzer, S., Trautwein, W. & Pelzer, D.J. (1994) Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. *Physiol. Rev.* **74**, 365–507.

Meissner, G. (1984) Adenine nucleotide stimulation of Ca^{2+}-induced Ca^{2+} release in sarcoplasmic reticulum. *J. Biol. Chem.* **259**, 2365–2374.

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

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

Meissner, G. & 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., Rios, E., Tripathy, A. & Pasek, D.A. (1997) Regulation of skeletal muscle Ca^{2+} release channel (ryanodine receptor) by Ca^{2+} and monovalent cations and anions. *J. Biol. Chem.* **272**, 1628–1638.

Menegazzi, P., Larini, F., Treves, S., Guerrini, R., Quadroni, M. & Zorzato, F. (1994) Identification and characterization of three calmodulin binding sites of the skeletal muscle ryanodine receptor. *Biochemistry* **33**, 9078–9084.

Messineo, F.C., Rathier, M., Favreau, C., Watras, J. & Takenaka, H. (1984) Mechanisms of fatty acid effects on sarcoplasmic reticulum. III. The effects of palmitic and oleic acids on sarcoplasmic reticulum function — a model for fatty acid membrane interactions. *J. Biol. Chem.* **259**, 1336–1343.

Meyers, M.B., Pickel, V.M., Sheu, S.S., Sharma, V.K., Scotto, K.W. & Fishman, G.I. (1995) Association of sorcin with the cardiac ryanodine receptor. *J. Biol. Chem.* **270**, 26411–26418.

Meyers, M.B., Puri, T.S., Chien, A.J., Gao, T., Hsu, P.H., Hosey, M.M. & Fishman, G.I. (1998) Sorcin associates with the pore-forming subunit of voltage-dependent L-type Ca^{2+} channels. *J. Biol. Chem.* **273**, 18930–18935.

Michalak, M. (1988) Identification of the Ca^{2+}-release activity and ryanodine receptor in sarcoplasmic-reticulum membranes during cardiac myogenesis. *Biochem. J.* **253**, 631–636.

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

Mitchell, R.D., Simmerman, H.K. & Jones, L.R. (1988) Ca^{2+} binding effects on protein conformation and protein interactions of canine cardiac calsequestrin. *J. Biol. Chem.* **263**, 1376–1381.

Mitterdorfer, J., Grabner, M., Kraus, R.L., Hering, S., Prinz, H., Glossmann, H. & Striessnig, J. (1998) Molecular basis of drug interaction with L-type Ca^{2+} channels. *J. Bioenerg. Biomembr.* **30**, 319–334.

Mohabir, R., Lee, H.C., Kurz, R.W. & Clusin, W.T. (1991) Effects of ischemia and hypercarbic acidosis on myocyte calcium transients, contraction, and pH_i in perfused rabbit hearts. *Circ. Res.* **69**, 1525–1537.

Murayama, T., Kurebayashi, N. & Ogawa, Y. (2000) Role of Mg^{2+} in Ca^{2+}-induced Ca^{2+} release through ryanodine receptors of frog skeletal muscle: Modulations by adenine nucleotides and caffeine. *Biophys. J.* **78**, 1810–1824.

Murayama, T., Oba, T., Katayama, E., Oyamada, H., Oguchi, K., Kobayashi, M., Otsuka, K. & Ogawa, Y. (1999) Further characterization of the type 3 ryanodine receptor (RyR3) purified from rabbit diaphragm. *J. Biol. Chem.* **274**, 17297–17308.

Murayama, T. & Ogawa, Y. (1997) Characterization of type 3 ryanodine receptor (RyR3) of sarcoplasmic reticulum from rabbit skeletal muscles. *J. Biol. Chem.* **272**, 24030–24037.

Nakai, J., Imagawa, T., Hakamat, Y., Shigekawa, M., Takeshima, H. & Numa, S. (1990) Primary structure and functional expression from cDNA of the cardiac ryanodine receptor/calcium release channel. *FEBS Lett.* **271**, 169–777.

Needleman, D.H., Aghdasi, B., Seryshev, A.B., Schroepfer, Jr., G.J. & Hamilton, S.L. (1997) Modulation of skeletal muscle Ca^{2+}-release channel activity by sphingosine. *Am. J. Physiol.* **272**, C1465–C1474.

Noguchi, N., Takasawa, S., Nata, K., Tohgo, A., Kato, I., Ikehata, F., Yonekura, H. & Okamoto, H. (1997) Cyclic ADP-ribose binds to FK506-binding protein 12.6 to release Ca^{2+} from islet microsomes. *J. Biol. Chem.* **272**, 3133–3136.

Orr, I. & Shoshan-Barmatz, V. (1996) Modulation of the skeletal muscle ryanodine receptor by endogenous phosphorylation of 160/150-kDa proteins of the sarcoplasmic reticulum. *Biochim. Biophys. Acta* **1283**, 80–88.

Otsu, K., Willard, H.F., Khanna, V.K., Zorzato, F., Green, N.M. & 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**, 13472–13483.

Porter Moore, C., Zhang, J.Z. & Hamilton, S.L. (1999) A role for cysteine 3635 of RYR1 in redox modulation and calmodulin binding. *J. Biol. Chem.* **274**, 36831–36834.

Posterino, G.S. & Fryer, M.W. (1998) Mechanisms underlying phosphate-induced failure of Ca^{2+} release in single skinned skeletal muscle fibres of the rat. *J. Physiol.* (*London*) **512**, 97–108.

Radermacher, M., Rao, V., Grassucci, R., Frank, J., Timerman, A.P., Fleischer, S. & Wagenknecht, T. (1994) Cryo-electron microscopy and three-dimensional reconstruction of the calcium release channel/ryanodine receptor from skeletal muscle. *J. Cell Biol.* **127**, 411–423.

Rakovic, S., Cui, Y., Iino, S., Galione, A., Ashamu, G.A., Potter, B.V. & Terrar, D.A. (1999) An antagonist of cADP-ribose inhibits arrhythmogenic oscillations of intracellular Ca^{2+} in heart cells. *J. Biol. Chem.* **274**, 17820–17827.

Remppis, A., Greten, T., Schafer, B.W., Hunziker, P., Erne, P., Katus, H.A. & Heizmann, C.W. (1996) Altered expression of the Ca^{2+}-binding protein S100A1 in human cardiomyopathy. *Biochim. Biophys. Acta* **1313**, 253–257.

Rich, T.L., Langer, G.A. & Klassen, M.G. (1988) Two components of coupling calcium in single

ventricular cell of rabbits and rats. *Am. J. Physiol.* **254**, H937–H946.

Ringer, S. (1883) A further contribution regarding the influence of the different constituents of the blood on the contractions of the heart. *J. Physiol.* (*London*) **4**, 29–42.

Rousseau, E. & Pinkos, J. (1990) pH modulates conducting and gating behaviour of single calcium release channels. *Pflugers Arch.* **415**, 645–647.

Schafer, B.W. & Heizmann, C.W. (1996) The S100 family of EF-hand calcium-binding proteins: Functions and pathology. *Trends Biochem. Sci.* **21**, 134–140.

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

Schulz, R., Rose, J. & Heusch, G. (1994) Involvement of activation of ATP-dependent potassium channels in ischemic preconditioning in swine. *Am. J. Physiol.* **267**, H1341–H1352.

Serysheva, II, Orlova, E.V., Chiu, W., Sherman, M.B., Hamilton, S.L. & van Heel, M. (1995) Electron cryomicroscopy and angular reconstitution used to visualize the skeletal muscle calcium release channel. *Nature Struct. Biol.* **2**, 18–24.

Shacklock, P.S., Wier, W.G.C. & Balke, W. (1995) Local Ca^{2+} transients (Ca^{2+} sparks) originate at transverse tubules in rat heart cells. *J. Physiol.* (*London*) **487**, 601–608.

Sharma, C., Smith, T., Li, S., Schroepfer, Jr., G.J. & Needleman, D.H. (2000a) Inhibition of Ca^{2+} release channel (ryanodine receptor) activity by sphingolipid bases: Mechanism of action. *Chem. Phys. Lipids* **104**, 1–11.

Sharma, M.R., Jeyakumar, L.H., Fleischer, S. & Wagenknecht, T. (2000b) Three-dimensional structure of ryanodine receptor isoform three in two conformational states as visualized by cryo-electron microscopy. *J. Biol. Chem.* **275**, 9485–9491.

Sharma, M.R., Penczek, P., Grassucci, R., Xin, H.B., Fleischer, S. & Wagenknecht, T. (1998) Cryoelectron microscopy and image analysis of the cardiac ryanodine receptor. *J. Biol. Chem.* **273**, 18429–18434.

Shoshan-Barmatz, V. & Ashley, R.H. (1998) The structure, function, and cellular regulation of ryanodine-sensitive Ca^{2+} release channels. *Int. Rev. Cytol.* **183**, 185–270.

Shoshan-Barmatz, V., Hadad, N., Feng, W., Shafir, I., Orr, I., Varsanyi, M. & Heilmeyer, L.M. (1996a) VDAC/porin is present in sarcoplasmic reticulum from skeletal muscle. *FEBS Lett.* **386**, 205–210.

Shoshan-Barmatz, V., Orr, I., Weil, S., Meyer, H., Varsanyi, M. & Heilmeyer, L.M. (1996b) The identification of the phosphorylated 150/160-kDa proteins of sarcoplasmic reticulum, their kinase and their association with the ryanodine receptor. *Biochim. Biophys. Acta* **1283**, 89–100.

Sonnleitner, A., Conti, A., Bertocchini, F., Schindler, H. & Sorrentino, V. (1998) Functional properties of the ryanodine receptor type 3 (RyR3) Ca^{2+} release channel. *EMBO J.* **17**, 2790–2798.

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

Stern, M.D. & Lakatta, E.G. (1992) Excitation-contraction coupling in the heart: The state of the question. *FASEB J.* **6**, 3092–3100.

Strauss, O., Mergler, S. & Wiederholt, M. (1997) Regulation of L-type calcium channels by protein tyrosine kinase and protein kinase C in cultured rat and human retinal pigment epithelial cells. *FASEB J.* **11**, 859–867.

Sukhareva, M., Morrissette, J. & Coronado, R. (1994) Mechanism of chloride-dependent release of Ca^{2+} in the sarcoplasmic reticulum of rabbit skeletal muscle. *Biophys. J.* **67**, 751–765.

Suko, J., Drobny, H. & Hellmann, G. (1999) Activation and inhibition of purified skeletal muscle calcium release channel by NO donors in single channel current recordings. *Biochim. Biophys. Acta* **1451**, 271–287.

Suko, J., Hellmann, G. & Drobny, H. (2000) Modulation of the calmodulin-induced inhibition of sarcoplasmic reticulum calcium release chan-

nel (Ryanodine receptor) by sulfhydryl oxidation in single channel current recordings and [^{3}H]Ryanodine binding. *J. Membr. Biol.* **174**, 105–120.

Sun, X.H., Protasi, F., Takahashi, M., Takeshima, H., Ferguson, D.G. & Franzini-Armstrong, C. (1995) Molecular architecture of membranes involved in excitation-contraction coupling of cardiac muscle. *J. Cell Biol.* **129**, 659–671.

Sutko, J.L. & Airey, J.A. (1996) Ryanodine receptor Ca^{2+} release channels: Does diversity in form equal diversity in function? *Physiol. Rev.* **76**, 1027–1071.

Sutko, J.L., Airey, J.A., Welch, W. & Ruest, L. (1997) The pharmacology of ryanodine and related compounds. *Pharmacol. Rev.* **49**, 53–98.

Szegedi, C., Sarkozi, S., Herzog, A., Jona, I. & Varsanyi, M. (1999) Calsequestrin: More than «only» a luminal Ca^{2+} buffer inside the sarcoplasmic reticulum. *Biochem. J.* **337**, 19–22.

Takekura, H., Nishi, M., Noda, T., Takeshima, H. & Franzini-Armstrong, C. (1995a) Abnormal junctions between surface membrane and sarcoplasmic reticulum in skeletal muscle with a mutation targeted to the ryanodine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3381–3385.

Takekura, H., Takeshima, H., Nishimura, S., Takahashi, M., Tanabe, T., Flockerzi, V., Hofmann, F. & Franzini-Armstrong, C. (1995b) Co-expression in CHO cells of two muscle proteins involved in excitation-contraction coupling. *J. Muscle Res. Cell Motil.* **16**, 465–480.

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

Timerman, A.P., Ogunbumni, E., Freund, E., Wiederrecht, G., Marks, A.R. & 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**, 22992–22999.

Timerman, A.P., Onoue, H., Xin, H.B., Barg, S., Copello, J., Wiederrecht, G. & Fleischer, S. (1996) Selective binding of FKBP12.6 by the cardiac ryanodine receptor. *J. Biol. Chem.* **271**, 20385–20391.

Tinker, A. & Williams, A.J. (1992) Divalent cation conduction in the ryanodine receptor channel of sheep cardiac muscle sarcoplasmic reticulum. *J. Gen. Physiol.* **100**, 479–493.

Treves, S., Scutari, E., Robert, M., Groh, S., Ottolia, M., Prestipino, G., Ronjat, M. & Zorzato, F. (1997) Interaction of S100A1 with the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle. *Biochemistry* **36**, 11496–11503.

Tripathy, A., Xu, L., Mann, G. & Meissner, G. (1995) Calmodulin activation and inhibition of skeletal muscle Ca^{2+} release channel (ryanodine receptor). *Biophys. J.* **69**, 106–119.

Tunwell, R.E., Wickenden, C., Bertrand, B.M., Shevchenko, V.I., Walsh, M.B., Allen, P.D. & Lai, F.A. (1996) The human cardiac muscle ryanodine receptor-calcium release channel: Identification, primary structure and topological analysis. *Biochem. J.* **318**, 477–487.

Uehara, A., Yasukochi, M. & Imanaga, I. (1996) Modulation of ryanodine binding to the cardiac Ca^{2+} release channel by arachidonic acid. *J. Mol. Cell. Cardiol.* **28**, 43–51.

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

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

Wang, W., Cleemann, L., Jones, L.R. & Morad, M. (2000) Modulation of focal and global Ca^{2+} release in calsequestrin- overexpressing mouse cardiomyocytes. *J. Physiol.* (*London*) **524**, 399–414.

Wrzosek, A. (1999) Main systems involved in calcium regulation in cardiac muscle cells and their functional relationship. *Pol. J. Pharmacol.* **51**, 187–200.

Xia, R.H., Cheng, X.Y., Wang, H., Chen, K.Y., Wei, Q.Q., Zhang, X.H. & Zhu, P.H. (2000) Biphasic modulation of ryanodine binding to sarcoplasmic reticulum vesicles of skeletal muscle by Zn^{2+} ions. *Biochem. J.* **345**, 279–286.

Xiang, J.Z. & Kentish, J.C. (1995) Effects of inorganic phosphate and ADP on calcium handling by the sarcoplasmic reticulum in rat skinned cardiac muscles. *Cardiovasc. Res.* **29**, 391–400.

Xin, H.B., Rogers, K., Qi, Y., Kanematsu, T. & Fleischer, S. (1999) Three amino acid residues determine selective binding of FK506-binding protein 12.6 to the cardiac ryanodine receptor. *J. Biol. Chem.* **274**, 15315–15319.

Xu, L., Eu, J.P., Meissner, G. & Stamler, J.S. (1998) Activation of the cardiac calcium release channel (ryanodine receptor) by poly-*S*-nitrosylation. *Science* **279**, 234–237.

Xu, L., Mann, G. & Meissner, G. (1996) Regulation of cardiac Ca^{2+} release channel (ryanodine receptor) by Ca^{2+}, H^{+}, Mg^{2+}, and adenine nucleotides under normal and simulated ischemic conditions. *Circ. Res.* **79**, 1100–1109.

Yamazawa, T., Takeshima, H., Shimuta, M. & Iino, M. (1997) A region of the ryanodine receptor critical for excitation-contraction coupling in skeletal muscle. *J. Biol. Chem.* **272**, 8161–8164.

Zable, A.C., Favero, T.G. & Abramson, J.J. (1997) Glutathione modulates ryanodine receptor from skeletal muscle sarcoplasmic reticulum. Evidence for redox regulation of the Ca^{2+} release mechanism. *J. Biol. Chem.* **272**, 7069–7077.

Zamparelli, C., Ilari, A., Verzili, D., Giangiacomo, L., Colotti, G., Pascarella, S. & Chiancone, E. (2000) Structure-function relationships in sorcin, a member of the penta EF- hand family. Interaction of sorcin fragments with the ryanodine receptor and an *Escherichia coli* model system. *Biochemistry* **39**, 658–666.

Zarka, A. & Shoshan-Barmatz, V. (1993) Characterization and photoaffinity labeling of the ATP binding site of the ryanodine receptor from skeletal muscle. *Eur. J. Biochem.* **213**, 147–154.

Zhang, J.Z., Wu, Y., Williams, B.Y., Rodney, G., Mandel, F., Strasburg, G.M. & Hamilton, S.L. (1999) Oxidation of the skeletal muscle Ca^{2+} release channel alters calmodulin binding. *Am. J. Physiol.* **276**, C46–C53.

Zhu, X., Gurrola, G., Jiang, M.T., Walker, J.W. & Valdivia, H.H. (1999) Conversion of an inactive cardiac dihydropyridine receptor II-III loop segment into forms that activate skeletal ryanodine receptors. *FEBS Lett.* **450**, 221–226.

Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N.M., Lai, F.A., Meissner, G. & 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.

Zot, A.S. & Potter, J.D. (1987) Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. *Annu. Rev. Biophys. Biophys. Chem.* **16**, 535–559.

Zucchi, R. & Ronca-Testoni, S. (1997) The sarcoplasmic reticulum Ca^{2+} channel/ryanodine receptor: Modulation by endogenous effectors, drugs and disease states. *Pharmacol. Rev.* **49**, 1–51.