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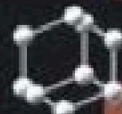

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Essential Roles of Intracellular Calcium Release Channels in Muscle, Brain, Metabolism, and Aging

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Summary: Calcium (Ca^{2+}) release from intracellular stores controls numerous cellular processes, including cardiac and skeletal muscle contraction, synaptic transmission and metabolism. The ryanodine receptors (RyRs: RyR1, RyR2, RyR3) and inositol 1,4,5-trisphosphate receptors (IP3Rs: IP3R1, IP3R2, IP3R3) are the major Ca^{2+} release channels (CRCs) on the endo/sarcoplasmic reticulum (ER/SR). RyRs and IP3Rs comprise macromolecular signaling complexes that include modulatory proteins which regulate channel activity in response to extracellular signals resulting in intracellular Ca^{2+} release. Here we focus on the roles of CRCs in heart, skeletal muscle, brain, metabolism, and aging.

Keywords: Aging, arrhythmias, cognitive dysfunction, diabetes, excitation-contraction coupling, heart failure, IP3 receptors, metabolism, mitochondria, neurodegenerative disorders, RyR, skeletal muscle.

INTRODUCTION

Calcium (Ca^{2+}) is an essential signaling molecule in all cells. Indeed, Ca^{2+} is involved in numerous fundamental functions, including cell life and death [1-4]. Between these two events, Ca^{2+} finely regulates countless events as gene transcription, secretion, muscle contraction, and generation of fuels in various metabolic pathways, to name but a few [5-8]. Cytosolic Ca^{2+} signals are produced by rapidly increasing the concentration of free Ca^{2+} ions [9] by opening channels permeable to Ca^{2+} either in the surface cell membrane or in the membranes of intracellular organelles containing high Ca^{2+} concentrations, namely the endoplasmic and sarcoplasmic reticula (ER, SR). Amplification of external stimuli by triggering the release of intracellular Ca^{2+} stores is a common signaling mechanism in the cell. The ER/SR represents a heterogeneous compartment constituted by interconnected network of tubules [10, 11]. Besides constituting the major Ca^{2+} reservoir within the cell, being thereby crucial in the fine regulation of intracellular Ca^{2+} concentration, the ER/SR is involved in a plethora of functional processes [12], including protein synthesis and protein transport, stress signaling, lipid synthesis, carbohydrate metabolism and detoxification reactions [5, 13-15].

The ER/SR of most cell types presents two types of intracellular Ca^{2+} release channels (CRC): the ryanodine receptors (RyRs) and the inositol 1,4,5-trisphosphate receptors (IP3Rs) [16]. There is ~40% homology between the RyR and lP3R in the putative transmembrane regions, a sequence similarity sufficient to indicate that these two channels evolved from a common ancestral cation release channel in unicellular species.

The evaluation of the phylogeny of these two channels suggests that RyRs arose from ancestral IP3R-like channels by incorporating promiscuous 'RyR' and 'SPRY' domains (so named because these sequence repeats were identified in both *Dictyostelium discoideum* tyrosine kinase Spore lysis A, splA and the mammalian RyR) via horizontal gene transfer. Indeed, the structure of the mammalian RyR1 has recently been solved showing that it is a member of the six transmembrane ion channel family [17]. Putative RyRs and IP3Rs have been also identified in unicellular organisms including *Capsaspora owczarzaki, Salpingoeca rosetta, and Monosiga brevicollis* as well as in pathogenic unicellular parasites including *Leishmania major, Leishmania infantum, Tryponosoma cruzi, and Tryponosoma brucei* [18, 19]. Though structurally related, RyRs and IP3Rs have distinct physiologic and pharmacologic profiles [20].

RyRs

RyR is a member of the same gene family as IP3R, but has evolved specialized functions relevant to excitation-contraction (E-C) coupling in striated muscles [21] and is developmentally regulated [2, 22].

RyR is a homotetramer comprised of four 565 kDa monomers [23], of approximately 5000 amino acids each [17]. There are three subtypes of RyRs in mammalian tissues: RyR1 and RyR2 are required for skeletal muscle and cardiac E-C coupling, respectively [21, 24], and are also expressed in various non-muscle tissues [5, 25]; RyR3 was

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originally identified in the brain [26] but exhibits an ubiquitous expression, as it is widely expressed in other non-neuronal tissues [27].

RyR was named based on its purification using the high affinity plant alkaloid ryanodine [28], an agent known to profoundly alter SR Ca^{2+} release events [29]. When bound to RyR at low concentrations ryanodine locks the channel in a half open state, thereby resulting in depletion of Ca^{2+} from the SR and subsequent interruption of E-C coupling. This explains the historical use of extracts from the *Ryania* plant family by natives of South and Central America as poison for arrowheads: indeed the release of SR Ca^{2+} via the locked open RyRs causes tetany, and at high concentrations ryanodine blocks the channel [28]. RyR is normally closed at low cytosolic [Ca^{2+}] (~100–200 nM); at submicromolar cytosolic [Ca^{2+}], Ca^{2+} binds to high-affinity binding sites on RyR increasing its open probability (P_o). Channel activity is maximal at cytosolic [Ca^{2+}] ~10 μM while elevating cytosolic [Ca^{2+}] beyond this point leads to a reduction in P_o [30, 31].

We recently solved by single-particle cryo-electron microscopy the structure of RyR1 [17], demonstrating that it adopts a four-fold symmetric mushroom-like superstructure, with the large 'cap' (about 80% of the mass) located in the cytosol and the 'stalk' embedded in the ER/SR membrane, with six transmembrane helices (S1-S6) per protomer surrounding the central pore [17]. Each protomer is built around an extended scaffold of alpha-solenoid repeats which include an aminoterminal, a bridging, and a core solenoid. At the extreme outer corners of the tetramer there are three SPRY domains and two pairs of RyR repeats, RY12 and RY34, the latter containing a regulatory protein kinase A (PKA) phosphorylation site. The RyR1 pore domain most closely resembles that of the voltage-gated sodium channel (NavAB) and presents a single cytosolic constriction in the ion conduction pathway, at the S6 bundle crossing [17]. Glycine residues in the pore-lining helices may operate as "hinges" to facilitate the orientation of the cytoplasmatic extension of S6 in order to modulate the aperture of the channel. Notably, Gly^{4934} is conserved in all RyR isoforms and in IP3R.

RyR Macromolecular Complex

The large and complex structure of RyR contains function-modifying phosphorylation sites and protein-binding domains, providing an attractive target for disease intervention. The high-resolution structure of native RyR1 revealed the complete domain structure of the channel in unprecedented detail [17]. RyRs are macromolecular signaling complexes, in which multiple proteins bind to a domain of the channel modulating its function [21, 32].

The Ca^{2+} stabilizing proteins calstabin1 (calcium channel stabilizing binding protein1, previously known as FKBP12) and calstabin2 (FKBP12.6) are peptidyl-propyl-*cis-trans* isomerases that associate via amphiphilic β-sheet structures with RyR1 and RyR2, respectively, such that one calstabin protein is bound to each RyR monomer [33-35], in order to regulate the channel gating through protein-protein interactions [36] and prevent pathological intracellular Ca^{2+} leak [37, 38]. Calstabin1 and calstabin2 differ at only 18 positions out of 108 residues.

We identified the calstabin-binding loop as part of the aminoterminal subdomain of the bridging solenoid [17]. Calstabin binding may rigidify the interface between such subdomain with SPRY1-2, thereby stabilizing the connection with the cytosolic regulatory domains and eventually altering the relative orientation of these domains [17]. Highly conserved leucine-isoleucine zipper motifs in RyR2 form binding sites for adaptor proteins that mediate binding of other proteins [32, 39], including kinases (e.g. PKA [40, 41], CaMKIIdelta [42]) and phosphatases (e.g. PP1 and PP2A). Specifically, the adaptor protein mAKAP mediates the binding of PKA and phosphodiesterase PDE43, whereas PP1 and PP2A are targeted to RyR2 via spinophilin and PR130, respectively [43, 44]. All of the above mentioned proteins regulate the phosphorylation-dephosphorylation of RyR2 in Ser^{2809} (Ser^{2808} in mouse) [45] in response to stress [45-48]. Other channels are also regulated by stress signals, including the voltage-gated Ca^{2+} channels [49]. RyR function is also affected by oxidation, nitrosylation, and other post-translational modifications [5, 41, 45, 47, 50]. Many other modulatory proteins complex directly and indirectly with RyR, including sorcin [51], calmodulin [52], homer [53], histidine-rich Ca^{2+} binding protein [54], S100A1 [55], triadin [56], junctin [57], and calsequestrin [58].

IP3Rs

IP3R channels are homo or heterotetramers composed of four subunits (approximately 300,000 Da each). The structure of the IP3R monomer comprises a ligand-ligand pocket at the amino terminus, a central cytosolic regulatory domain, and a pore region containing six transmembrane helices forming the Ca^{2+} channel region located at the carboxy terminus [57, 59].

Three forms of IP3Rs (types 1, 2 and 3) have been characterized [60-63]. Virtually all cell types have at least one form of IP3R, and many express all three types. Additionally, the subcellular distribution of the three isoforms is cell-type dependent [64]. IP3R1 has a broad tissue distribution but is especially abundant in the cerebellum, where it was initially purified and characterized [65]. It is also expressed in vascular smooth muscle cells, thyroid, uterus, and lymphocytes [20, 66, 67]. IP3R2 is expressed in cardiac muscle, as well as liver, kidney, and other epithelial tissues [68], while IP3R3 is expressed in endothelial cells, testis, endocrine and exocrine pancreas, spleen, gastrointestinal tract, and thymus [69].

IP3R function is regulated by at least two major cellular signaling pathways: the second messenger inositol 1,4,5-trisphosphate (IP3) [70], produced primarily by phospholipase C (PLC) metabolism of phosphoinositol-4,5-bisphosphate (PIP2) in response to the stimulation of G-protein-coupled receptors (GPCRs) or receptor tyrosine kinases [1, 71, 72], and phosphorylation by non-receptor tyrosine kinases [73].

IP3R Macromolecular Complex

Numerous signaling molecules bind to IP3R regulating its function, including Bcl-2, calmodulin [74], caldendrin

[75], carbonic anhydrase-related protein (CARP) [76], ankyrin [77], homer [78], and IP3R-binding protein released by IP3 (IRBIT) [79]. Studies in murine T-cells demonstrated that the BH4 domain of Bcl-2, which distinguishes anti- from pro-apoptotic members in the Bcl-2 family, is necessary and sufficient in binding to IP3Rs, inhibiting apoptosis [80]. The binding of Bcl-2 to IP3R promotes low-amplitude oscillatory Ca^{2+} signals and inhibits high-amplitude sustained elevations that are instead associated with cell death [81]. Intriguingly, a Bcl-2 binding site has been recently identified also in RyR2 [82]. CARP has been shown to bind IP3R1 causing conformational changes of the channel that reduce its affinity towards IP3 [76]. IRBIT suppresses the activation of IP3R by competing with IP3, acting as an endogenous pseudoligand whose inhibitory activity can be modulated by its phosphorylation status [83]. Moreover, the IP_3R structure undergoes major conformational changes under influence of Ca^{2+} [84].

Various kinases, including CaMKII, Fyn, PKA PKG, and PKC, have been shown to phosphorylate IP3R [73, 85, 86]. We demonstrated that PKA and the phosphatases PP1 and PP2A are active components of the IP3R1 macromolecular complex [86]. A physical association between IP3R1 and calstabin1 had also been proposed [87] but subsequent studies have not been able to observe the formation of such interaction, despite detecting RyR1/calstabin1 complexes in parallel experiments [88, 89].

HEART

Ca^{2+} is vital for the proper functioning of a healthy heart, as it is mechanistically involved in both myocardial contractile performance [90] and regulation of rhythmic beating [91]. Abnormalities in the regulation of Ca^{2+} homeostasis are associated with cardiovascular disorders including arrhythmias, myocardial hypertrophy, and heart failure. Cardiac contraction is a tightly regulated process beginning with depolarization of the sinoatrial node, situated at the junction between the right atrium and the superior vena cava. This generates a wave of depolarization that travels throughout the atria, converging onto the atrioventricular node, and from there spreading throughout the ventricles. Ca^{2+} finely orchestrates these processes, linking myocardial depolarization to myocardial contraction through E-C coupling [92]. Indeed, the rise in cytoplasmic $[Ca^{2+}]$ is critical for activation of actin-myosin cross-bridging, shortening of the sarcomere, and muscle contraction.

Cardiac Excitation-Contraction Coupling

An Italian physician, Luigi Galvani, established in the 18th century the relationship between electrical signaling and mechanical contraction conducting seminal experiments in frog legs, pioneering bioelectricity [93]. In 1883 Sidney Ringer was the first to discover that Ca^{2+} was a key component of cardiac contractility [6]. His observations were confirmed in 1913 by George Mines, who elegantly demonstrated that this specific ion was responsible for linking excitation and contraction [94]. Subsequent studies confirmed that cardiac contractility is directly proportional to systolic cytosolic $[Ca^{2+}]$ [95, 96].

RyR2 and Heart Failure

Despite tremendous advances in the treatment of myocardial infarction, heart failure (HF), and cardiac arrhythmias [97], cardiovascular disease remains the leading cause of death worldwide, causing ~600.000 deaths per year in the US alone [98-100]. HF is associated with significant impairment in myocardial contractility, such that rates of both contraction and relaxation are slowed [101, 102].

RyR2 is responsible for Ca^{2+} release from the SR, where it is stored at high concentration (in the millimolar range), thereby raising the cytosolic $[Ca^{2+}]$ about ten-fold (from ~100 nM to ~1 μM) with each heartbeat in order to activate cardiac muscle contraction. Abnormal intracellular Ca^{2+} handling represents a major cause of decreased muscle contraction (systolic dysfunction) and defective relaxation (diastolic dysfunction) in patients with HF, as confirmed by reports of reduced Ca^{2+} transient amplitude, increased Ca^{2+} transient duration, prolonged Ca^{2+} transient decay time, and decreased SR Ca^{2+} load, leading to impaired contractility [44, 103]. The mechanistic role of diastolic Ca^{2+} leak through dysfunctional RyR2 in the pathophysiology of HF has been experimentally established for the first time in 2000 [44].

RyR2 leak in HF is caused by stress-induced remodeling of the RyR2 macromolecular complex due to PKA hyperphosphorylation, oxidation, and nitrosylation of the channel, eventually resulting in an altered stoichiometry of the RyR2 complex, with displacement of calstabin2 [41], phosphatases [104], and PDE4D3 [43] from the channel. Depletion of PDE4D3 and phosphatases results in elevated levels of cyclic adenosine mono-phosphate (cAMP) [43] and a decreased rate of dephosphorylation, thereby promoting further PKA hyperphosphorylation. The term PKA hyperphosphorylation describes RyR2 in which 3–4 of the four RyR2 monomers are chronically PKA phosphorylated. Hyperphosphorylated/calstabin2 depleted channels are sensitized to cytosolic Ca^{2+}, leading to inappropriate Ca^{2+} release during diastole, referred to as a diastolic SR Ca^{2+} leak. Most recently, we demonstrated that intracellular Ca^{2+} leak via RyR2 plays a key role in mitochondrial Ca^{2+} overload and dysfunction in HF [105].

As part of the classical 'fight-or-flight' response stress pathway [45], sympathetic nervous system activation causes catecholamine release and β-adrenergic receptor (βAR) stimulation, increasing the gain of E-C coupling system and enhancing contractility [106]. Binding of catecholamines to βARs activates a G protein-coupled intracellular signaling cascade [107-110], leading to the activation of adenylylcyclase with subsequent increased intracellular levels of cAMP, which in turn triggers PKA. RyR2 phosphorylation by PKA increases the P_o of the channel by causing dissociation of the stabilizing protein calstabin2, resulting in increased sensitivity of RyR2 to Ca^{2+}-dependent activation [44, 104].

Hyperactivation of sympathetic nervous system in HF is initially compensatory, but eventually accelerates the progression of the disease, posing severe toxicity on the chronically failing heart [107, 108, 111]. In this sense, the beneficial effects of βAR blockers and other therapeutic approaches that mitigate or protect the heart against this sympathetic overdrive are well documented [108, 112, 113]. In-

triguingly, the discovery of leaky RyR2 in HF [44] provides a compelling mechanism to explain the therapeutic efficacy of βAR blockers in HF. Despite βAR blockers represent a standard therapy in HF [108, 109], there is no accepted understanding of their exact mechanism of action in such a disease. In fact, multiple explanations have been suggested, including altered myocardial gene expression and increased βAR density [109, 114].

We demonstrated that systemic administration of βAR-blockers reduces PKA hyperphosphorylation of RyR2 and restores calstabin2 binding to RyR2 channels both in animal models of HF [115] and in cardiac samples from human heart transplant recipients [116]. We interpreted these findings as indicating that in failing hearts βAR-blockers can indirectly fix the leak in RyR2 channels by inhibiting PKA hyperphosphorylation of the channel. This hypothesis was confirmed by proving that βAR-blockers can improve cardiac function after MI in WT but not in RyR2-S2808D mice, in which Ser^{2808} of RyR2 is substituted with an aspartic acid residue, in order to mimic a constitutive PKA phosphorylation of the channel [41].

Alternative mechanisms underlying SR Ca^{2+} leak in HF have been proposed, including phosphorylation of RyR2 by Ca^{2+}/Calmodulin kinase II (CaMKII) at Ser^{2815} (Ser^{2814} in mouse) [117]. However, we developed genetically altered mice that harboring RyR2 channels that cannot be phosphorylated by CaMKII (RyR2-S2814A), demonstrating *in vivo* that they were not protected against post-myocardial infarction HF progression [42]. Moreover, CaMKII phosphorylation of RyR2 is required for the rate-related increase in contractility [42]. Indeed, CaMKII is activated by Ca^{2+} itself and is exposed to more Ca^{2+} at higher heart rates, thereby resulting in increased CaMKII phosphorylation of RyR2, augmented SR Ca^{2+} release, and increased contractility (Bowditch phenomenon, *frequency*-dependent inotropy, or treppe [118]). Thus, CaMKII activation during HF is related to HR, which in turn increases the integral of cytosolic [Ca^{2+}] [42]. Additionally, CaMKII has been demonstrated to be also activated by oxidation [119-121], and the role of oxidative stress in cardiovascular disease is well established [105].

RyR2 and Cardiac Arrhythmias

Intracellular CRCs play a major role in arrhythmogenesis [43, 48, 122, 123]. Indeed, increased RyR2 activity causes atrial and ventricular arrhythmias, particularly associated with increased catecholaminergic stimulation [123-126]. Altered Ca^{2+} handling, as seen in HF, contributes to triggered activity arising from early after-depolarization (EAD) or delayed after-depolarization (DAD). EAD occurs in the setting of increasingly labile repolarization observed in HF, during phase 2 and phase 3 of the action potential. DAD occurs during phase 4 and results from elevated intracellular Ca^{2+} load and spontaneous SR Ca^{2+} release, which leads to activation of transient inward current.

Besides the acknowledged role in providing a substrate for arrhythmias in patients with HF or cardiac hypertrophy [43, 127], RyR2 has also been linked to at least two forms of sudden cardiac death (SCD): catecholaminergic polymorphic ventricular tachycardia (CPVT) [5, 128] and arrhythmogenic right ventricular dysplasia type 2 (ARVD2) [129]. Moreover, exon 3 deletion of RyR2 has been associated with left ventricular noncompaction and CPVT [130].

CPVT is a rare inherited form of exercise-induced SCD that occurs in typically young individuals with structural normal hearts [5]. Several mutations in RyR2 have been linked to CPVT [128]. RyR2s with most of CPVT mutations exhibit reduced affinity for calstabin2, which results in leaky channels during exercise [131]. Calstabin2 deficient and haploinsufficient mice have CPVT and their RyR2 channels display slightly increased open probability at baseline that increases substantially following exercise [131].

ARVD is another inherited cardiac disorder characterized by substitution of the right ventricular myocardium with fibrofatty tissue, which appears to be a result of progressive death of cardiomyocytes resulting from a combination of inflammation and apoptosis [132, 133]. Twelve different types of ARVD have been identified hitherto, and type 2 is caused by mutations in *RyR2* [134]. Clinical presentation of ARVD is characterized by arrhythmias of right ventricular origin, ranging from premature beats to ventricular fibrillation, often leading to SDC [135]. Similar to CPVT, ARVD patients also exhibit exercise- and adrenergic-induced fatal arrhythmias. Available therapy for both CPVT and ARVD remain limited, and implantable cardioverter/defibrillator remains the best choice [133, 136]. We have shown that pharmacological stabilization of RyR2 prevents intracellular Ca^{2+} leak and arrhythmias [137], providing a promising potential therapeutic option for these disorders.

Most recently, we and others have also demonstrated that leaky RyR2s are key players in the pathophysiology of atrial fibrillation (AF) [123, 124, 138]. We proved that atrial RyR2s from patients with chronic AF were oxidized, phosphorylated and depleted of calstabin2 [123]. Likewise, mice with intracellular Ca^{2+} leak exhibited increased atrial RyR2 oxidation, mitochondrial dysfunction, ROS production and AF susceptibility. Interestingly, AF could be prevented by inhibition of mitochondrial ROS generation and by pharmacological treatment of RyR2 leak [123], indicating that mitochondrial oxidative stress and alterations in RyR2 may form a vicious cycle in the development of AF.

IP3Rs and Cardiac Disease

The role of IP3Rs in the heart remains quite controversial [105, 139]. Within cardiac myocytes, IP3R mRNA levels are ~50-fold lower than that of the cardiac RyR mRNA [140]. The relative amount of IP3Rs is too low and the kinetics of Ca^{2+} release from IP3Rs is too slow compared with RyRs to contribute to the Ca^{2+} transient during E-C coupling. Besides, Ca^{2+} flux via RyR has been shown to be markedly larger than the flux through IP3R [141, 142].

The study of IP3-induced Ca^{2+} release in cardiomyocytes has been difficult because Ca^{2+} release from RyR2, required for E-C coupling, dominates in the heart [105]. Indeed, direct studies of Ca^{2+} release in response to IP3 in cardiac preparations have revealed Ca^{2+} responses that are of much lower magnitude than those seen with the RyR system [143]. Likewise, direct generation of IP3 in intact non-beating neonatal cardiomyocytes does not alter cytosolic free Ca^{2+} [144].

IP_3Rs are more abundant in atrial than in ventricular cardiomyocytes and even more abundant in conduction tissue cells [145, 146]. Atrial and ventricular tissue express a similar proportion of IP3R1 and IP3R2 [145]; however, studies in isolated cardiomyocytes revealed that IP3R2 is clearly the predominant isoform in contractile myocardial cells and in sinoatrial node [147], whereas IP3R1 is the predominant isoform in other cell types, including endothelial cells and Purkinje fibers [145, 146]. In adult rabbit ventricular myocytes, IP_3Rs were implicated in the regulation of gene expression, based on the finding that the endothelin 1–induced mobilization of Ca^{2+} from the nuclear envelope was blocked by 2-aminoethoxydiphenyl borate (2-APB) [148], which is though considered a reliable blocker of store-operated Ca^{2+} entry but an inconsistent inhibitor of IP3-induced Ca^{2+} release [149].

In summary, most studies are in agreement that activation of IP3Rs does not significantly contribute to cardiac E-C coupling. A functional role for these channels has been also proposed in the pathophysiology of cardiac arrhythmias as well as myocardial hypertrophy [150]. Indeed, a significant upregulation of IP3Rs mirrored by enhanced channel activity [151] has been observed in patients with chronic atrial fibrillation [152], which remains the most common sustained arrhythmias, affecting 2.3 million adults in the US [98, 153, 154]. A reduction of sustained arrhythmias after application of 2-APB has also been reported [155]. Moreover, increased IP3 generation in the heart has been associated with the induction of ventricular arrhythmias [156]. A marked increase in IP3 levels in the heart occurs during early post-ischemic reperfusion following activation of α_1AR or thrombin receptor and correlates temporally with the development of ventricular tachycardia and fibrillation [156]. Of interest, inhibition of PLC-induced IP3 generation prevents reperfusion-related arrhythmias [157].

IP3Rs have been indirectly implicated in the development of left ventricular hypertrophy via upstream signaling pathways coupled to IP3, including α_1AR, angiotensin II, and endothelin-1. However, several studies have demonstrated a main role for diacylglycerol (the other product resulting from PLC hydrolysis) and subsequent PKC activation, more than a direct IP3R activation, in the induction of cardiac hypertrophy [158-160].

SKELETAL MUSCLE

Depolarization of skeletal myocytes involves a protein-protein interaction [161] across the junctional cleft between the dihydropyridine receptor ($Ca_v1.1$) on specialized invaginations of the sarcolemma (transverse tubules) and RyR1 on the SR membrane (terminal cisternae) [162]. Ergo, $Ca_v1.1$ acts as a marionettist on RyR1, eventually leading to Ca^{2+} release from the SR.

Remarkably, RyRs were initially observed in skeletal muscle, visualized in electron micrographs as large electron-dense masses located along the face of the SR terminal cisternae, which is closely apposed to transverse tubule membranes to form a structure named triad junction [163]. Therefore, the RyRs were initially termed triad junctional foot proteins [164, 165]. Noda and colleagues provided the *in vivo* evidence for a functional role of RyR1 in E-C coupling, engineering a mouse lacking exon 2 of *RyR1* and demonstrating that such a model exhibits severe skeletal muscle abnormalities and dies perinatally due to respiratory failure [166]. Subsequent ultrastructural studies of hindlimb and diaphragm muscles demonstrated the absence of RyR1-Cav1.1 complexes [167], which are essential for a proper E-C coupling in the skeletal muscle [168].

RyR1 dysfunction has been described in both inherited and acquired muscle disorders [169, 170]. Malignant hyperthermia (MH) and central core disease (CCD) represent the best examples of RyR1 channelopathies in the skeletal muscle [171].

Role of RyR1 in Malignant Hyperthermia and Central Core Disease

MH is a pharmacogenetic disorder, inherited in an autosomal dominant fashion and continues to be of major concern for anesthetic-induced deaths in otherwise healthy individuals [172]. MH episodes are typically rapid and severe, reaching core body temperatures of 43°C, leading to organ failure and death if not rapidly treated. The exact prevalence of MH susceptibility is difficult to determine since the syndrome only becomes apparent after exposure to triggering agents including volatile anesthetic agents such as halothane, isoflurane, sevoflurane, desflurane, enflurane and the neuromuscular blocking agent succinylcholine [173]. Susceptibility can be determined *in vitro* by measuring the contractile response to caffeine or halothane in biopsied muscle fibers. A related syndrome referred to as porcine stress syndrome is found in certain lines of domestic swine where stressed pigs undergo stress-induced hyperthermia [174].

These disorders are thought to develop following extreme skeletal muscle contraction that results from excess Ca^{2+} in the myoplasm following anesthesia in humans or during stress in pigs; this Ca^{2+} overload causes sustained contractions, which accounts for the rapid onset of muscle rigidity, leading to an augmented metabolic demand on myocytes, which depletes ATP levels. Myocytes respond by increasing ATP production via oxidative phosphorylation and glycolysis, and such a prolonged hypermetabolic state generates heat. Alterations in ^{3}H-ryanodine binding properties in porcine MH samples provided evidence linking RyR1 dysfunction to the disease [175], which was later confirmed by biophysical experiments [176].

CCD is a dominantly inherited congenital myopathy, first described in 1956 [177]. The name derives from the typical appearance of the biopsy on light microscopy, where the muscle cells have metabolically inactive tissue in the center of type I myofibers, appearing as cores that are devoid of mitochondria and specific oxidative enzyme stains. Common symptoms include hypotonia, delayed motor milestones, and skeletal abnormalities including congenital hip dislocation and scoliosis.

There is no clear division between MH and CCD and some *RyR1* mutations have been linked to a combined MH and CCD phenotype [178]. Notably, most MH mutations in *RyR1* gene lead to a gain-of-function effect, with increased sensitivity to channel agonists, causing a leak in RyR1 channels, whereas most CCD-related mutations have been associated with channels displaying reduced conductance [179].

Importantly, the mutated codons giving rise to MH and CCD tend to cluster in three specific regions of the *RyR1* gene, that correspond to the following domains in the amino-acidic sequence: regions 1 (C35–R614) and 2 (D2129–R2458) reside in the myoplasmic foot domain of the protein, whereas region 3 (I3916–G4942) is located in the transmembrane/luminal region of the highly conserved carboxy-terminal domain, important for allowing Ca^{2+} flux through the channel [17].

Mutations in *RyR1* are also associated with other rare CCD-related congenital myopathies including centronuclear myopathy, multi-minicore disease, Samaritan myopathy, congenital fiber-type disproportion, and nemaline myopathy [180-182].

Role of RyRs1 in Muscular Dystrophy

We established that intracellular Ca^{2+} leak via RyR1 represents an essential feature of different forms of muscular dystrophy (MD), including Duchenne MD [183] and limb-girdle (or Erb's) MD [169]. Specifically, RyR1 from a Duchenne MD murine model (*mdx* mouse) was excessively cysteine nitrosylated and depleted of calstabin1, leading to increased spontaneous RyR1 openings and overall reduced specific muscle force [183]. Similar findings were obtained when evaluating RyR1 in β-sarcoglycan-deficient mice, an established model of limb-girdle DM [169].

Thus, we demonstrated common mechanisms of maladaptation of RyR1, including post-translational modifications of the channel and dissociation of the stabilizing subunit calstabin1, in two major disorders that weaken the muscular system hampering locomotion and that remain virtually without effective pharmacological treatment. We proved in both cases that stabilizing the RyR1-calstabin1 association ameliorated muscle function [169, 183], thereby providing an innovative therapeutic target and potential options for the treatment of DM.

In conditions of strenuous muscular stress or in a disease such as HF, both of which characterized by chronic activation of the sympathetic nervous system and increased production of reactive oxygen and nitrogen species [108, 184, 185], skeletal muscle function is impaired, possibly due to altered E-C coupling. Indeed, the amount of Ca^{2+} released from the SR during each contraction of the muscle is reduced, Ca^{2+} reuptake is slowed, and aberrant Ca^{2+} release events can occur [40, 186].

We have shown in both an animal model as well as in exercising humans that chronic βAR stimulation and depletion of calstabin1 from RyR1 plays a mechanistic role in the contractile failure and muscle fatigue, defined as decline in ability of a muscle to generate force, observed during sustained exercise [187]. Consistent with these observations, we have demonstrated that the remodeling of RyR1 plays a pivotal role also in sarcopenia and muscle aging [47] and we were able to prevent RyR1 dysfunction and ameliorate skeletal muscle function in aging by genetically enhancing mitochondrial antioxidant activity [188], as extensively discussed in the dedicated section of this overview.

Since skeletal muscle dysfunction, as seen in HF or muscular disorders, remains practically without effective treatment, drugs that restore RyR Ca^{2+} release function, represent promising candidates. In this sense, these drugs could be ideal in conditions with co-morbidity between cardiac and skeletal muscles. Indeed, as muscular RyR1 undergoes post-translational modifications in HF [40, 186], remodeling of the cardiac RyR2 has been also reported in mouse models of Duchenne MD, triggering ventricular arrhythmias [50].

IP3Rs and Muscle Pathophysiology

In contrast to the acknowledged prominent role of RyR1, there is no strong experimental evidence for a significant functional action of IP3R-mediated Ca^{2+} release in adult muscle fibers. Immunodetectable IP3Rs and specific [^{3}H]IP3–binding sites were reported to be preferentially expressed in slow oxidative (type I) and fast oxidative glycolytic (type IIA) fibers [189]. The presence of IP_3Rs has been later confirmed in both rabbit and mouse skeletal muscle [190]. Most recently, the expression of the three isoforms has been investigated in adult murine skeletal muscle, revealing that IP3R1 is absent in slow-type muscle fibers and is expressed preferentially in fast, mixed metabolism (type IIX) fibers whereas IP3R2 and IP3R3 are ubiquitously expressed in all muscle fiber types [191].

Although IP3Rs have been shown to be present and functional in cultured muscle cells [192, 193], no significant change in Ca^{2+} signal has been detected in adult muscle fibers after IP3 microinjection or photolysis of membrane-permeant IP3 [194]. Available data seem to converge towards a potential role of IP3Rs in neuromuscular junctions and in satellite cells [195].

CENTRAL NERVOUS SYSTEM

Ca^{2+} signaling regulates numerous neurological processes including synaptic transmission, secretion, excitability, learning, and memory. Emerging evidence suggests that several types of neurological disorders may share a common underlying mechanism, i.e. altered Ca^{2+} release from the ER [196-199]. The involvement of IP3Rs and RyRs in neurodegenerative disease relies at least in part on abnormal Ca^{2+} signaling that ultimately leads to excitotoxicity in specific areas of the central nervous system [200].

Intracellular CRCs and Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a rapidly progressing disease characterized by upper and lower motor neuron degeneration, eventually resulting in muscle atrophy and weakness throughout the body [201-203]. The current knowledge of its causes is very limited and it seems to derive from multiple and complex environmental factors and genetic predisposition [204, 205].

The most studied gene associated with ALS susceptibility is the copper-zinc superoxide dismutase [206, 207]. Several investigators have proposed an autoimmune origin for ALS [208, 209]. Supporting this view, Pagani and colleagues demonstrated that IgG isolated from ~50% of ALS patients can increase Ca^{2+} levels in motor nerve terminals in murine tissue cultures, enhancing spontaneous neurotransmitter release at neuromuscular junctions [210]. Of interest, both RyR and IP3R were necessary to maintain the IgG-dependent en-

IP3R were necessary to maintain the IgG-dependent enhancement of neurotransmitter release [210].

Most recently, a genome-wide association study unveiled *IP3R2* gene as a potential player in sporadic ALS, identifying a specific mutation (rs2306677) in intron 42 of *IP3R2* gene [211]. *IP3R2* is a very plausible susceptibility gene for ALS since it is involved in glutamate-mediated neurotransmission, is one of the main regulators of intracellular Ca^{2+} concentrations, and is an important player in apoptosis [211].

The role of RyRs in ALS pathophysiology has been less studied. Intriguingly, an imbalance between RyR and calstabin in spinal cords of neurological controls and patients with motor neuron disease has been reported in a Japanese study, revealing a significantly decreased expression of calstabin in neurons from patients with ALS [212]. Moreover, dantrolene sodium, a myorelaxant currently used to treat malignant hyperthermia, neuroleptic malignant syndrome, spasticity and Ecstasy (3,4-methylenedioxy-metamphetamine, MDMA) intoxication, had been proposed as potential therapy for ALS [213]. Of note, even though dantrolene is generally labeled as 'RyR inhibitor', a precise mechanism of action has not been identified and several investigators have demonstrated that the RyR2 isoform is not a target of such a drug [214, 215]. Recently, Laver and colleagues reported that calmodulin is essential for dantrolene action [216]: its absence in single RyR assays might therefore explain why this drug does not inhibit RyR in lipid bilayer preparations.

Intracellular CRCs and Alzheimer's Disease

Alzheimer's disease (AD) is the most common type of dementia, characterized clinically by progressive deterioration of cognitive functions including memory, reasoning, and language. Ca^{2+} certainly plays a pivotal role in the pathogenesis of the disease. Indeed, altered Ca^{2+} signaling has been detected in brain cells from both AD patients and animal models of AD, even before the appearance of amyloid Aβ-plaques or tangles, considered pathological hallmark features of AD [217, 218]. Whether Aβ-plaques are a cause or a consequence of AD remains controversial.

According to Demuro and Parker cytotoxicity of Aβ-amyloid oligomers involves Ca^{2+} release from the ER via activation of IP3Rs, as demonstrated *in vitro* in Xenopous oocytes [219]. Supporting this view, studies in fibroblasts isolated from patients with familial AD revealed that presenilins activate IP3Rs potentiating Ca^{2+} release from the ER [220]. These results have been confirmed in other cell types. Abnormal Ca^{2+} homeostasis in AD is further amplified by the increased Aβ-amyloid production caused by IP3R activation [221], in a vicious cycle that ultimately leads to cell death.

Most recently, Foskett demonstrated that an exaggerated Ca^{2+} signaling mediated by IP3R1 contributes to AD pathogenesis [222]. On the other hand, several reports have shown alterations of expression and function of RyRs in different murine models of the disease, in human AD-affected brains, and in cells expressing familial AD-linked mutations on the β-amyloid precursor protein and presenilins [223-225]. Moreover, altered RyR2 expression has been reported in a study of the whole-genome expression profile of sporadic and monogenic early-onset AD [226]. Available data suggest that alterations in RyR-mediated Ca^{2+} handling are associated to AD etiopathogenesis through means of various mechanisms, including the regulation of Aβ-amyloid production, synaptic function, memory and learning abilities, and neuronal death [227].

Intracellular CRCs and Huntington's Disease

Huntington's disease (HD) is a dominantly inherited genetic disorder characterized by dementia, slowly progressing movement disorders and psychiatric symptoms [228]. It is caused by an exaggerated polyglutamine expansion in the aminoterminus of the huntingtin protein. Mutant huntingtin impairs both protein folding and protein degradation. Interestingly, mutant huntingtin has been reported to disrupt Ca^{2+} signaling by forming a complex with the NMDA receptor and synapse-associated protein 90 (SAP-90) [229]. Additionally, huntingtin can bind and activate IP3R1 and impair mitochondrial function [230]. Consistent with these findings, the exogenous expression of the human mutant huntingtin in Drosophila melanogaster led to retinal degeneration, which was prevented by knocking-down IP3R1 [231].

Intracellular CRCs and Post-Traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) presents with a diverse set of symptoms involving a mixture of psychological, social, and biological processes [232]. Neurobiological research did not clarify a clear mechanism underlying the disease, but overall, there is mounting evidence that symptomatology following experiences of traumatic stress may derive from effects on brain function and structure [232]. Current therapy for PTSD remains largely supportive, and not mechanism-based.

Using chronic restraint to induce stress in mice we recently demonstrated that hippocampal RyR2s of stressed mice were phosphorylated by PKA, oxidized and nitrosylated, inducing intracellular Ca^{2+} leak [46]. The mice also exhibited impaired cognitive performance in various behavioural tests, including the Morris water maze, which are known to depend on hippocampal function. Pharmacologic or genetic inhibition of the leak prevented the cognitive dysfunction [46]. Since stress-induced cognitive impairment shares many features with PTSD [233], the pathway that we elucidated could be a potential therapeutic target. The link between PTSD and ER Ca^{2+} has been also confirmed in the rat, validating a functional role for ER stress and apoptosis in the pathophysiology of the disease [234].

Intracellular CRCs and Spinocerebellar Ataxias

Spinocerebellar ataxias (SCA) are a group of genetic movement disorders characterized by progressive ataxia and cerebellar atrophy [235]. Since cerebellar Purkinje cells express high levels of IP3R1, this channel has been proposed as a key player in cerebellar long-term depression generation, which protects neuronal Purkinje cells against high glutamate stimulation [236].

Several types of SCA are caused by mutations that generate poliglutamine proteins, such as ATXN2 [237] and ATXN3 [238], which have been shown to bind IP3R1, potentiating ER Ca^{2+} release. Moreover, deletions of different

regions of *IP3R1* gene have been linked to other types of SCA, not associated with polyglutamine repeats [239, 240]. A profound derangement in IP3R1-dependent Ca^{2+} signaling has been also reported in the opisthotonos mouse, which is ataxic and convulsive [241].

METABOLISM

Mounting evidence indicates that changes in intracellular [Ca^{2+}] play a pivotal role in glucose homeostasis, modulating both insulin secretion from the pancreatic islets of Langerhans and insulin resistance in peripheral responses of liver, muscle and adipocytes [5, 242-245].

Several studies have demonstrated that altered cellular Ca^{2+} homeostasis is a key contributor to impaired beta cell function and survival in type 2 diabetes mellitus (T2DM) [246, 247]. However, according to the classical view of the glucose sensing machinery, insulin secretion largely depends on the depolarization of the plasma membrane and the subsequent voltage-activated Ca^{2+} influx from the extracellular space [248, 249].

Most recently, we established both in humans and animal models [5] the mechanistic role of Ca^{2+} mobilization from the ER via RyR2 in insulin secretion, a topic that had remained controversial for decades. In fact, while the expression of IP3R1 [250, 251] and RyR2 [252-254] had been reported in pancreatic beta cells, their functional role had not been fully dissected heretofore.

Intracellular CRCs and Glucose Homeostasis

A seminal study revealed that mice with double knockout of IP3R2 and IP3R3 are lean and hypoglycemic despite a normal caloric intake, most likely as a result of difficulty in nutrient digestion, and exhibit severe impairment in Ca^{2+} signaling and secretion in acinar cells of the exocrine tissues [255]. Moreover, variations within *IP3R3* had been identified as a risk factor for type 1 diabetes mellitus (T1DM) in humans [256]. However, a subsequent study based on a family-based approach, which is not subject to population stratification, did not confirm an obvious role of genetic variation of the *IP3R3* gene in T1DM risk [257].

A demonstration of a connection between glucose-stimulated insulin secretion and Ca^{2+} signaling through IP_3-sensitive Ca^{2+} stores comes from mice heterozygous for the *anx7* gene, which encodes for Ca^{2+}-activated GTPase supporting Ca^{2+} channel activities [258]. These mice exhibit defects in IP3R expression, and Ca^{2+} signaling in panreatic islets, and impaired insulin secretion [258]. Moreover, PKA-mediated promotion of Ca^{2+}-induced Ca^{2+} release via IP3Rs has been implicated as part of the mechanism by which cAMP amplifies insulin secretion [259].

The potential involvement of RyR2 in insulin release has been suggested by *in vitro* experimental data showing an enhanced Ca^{2+} response in beta cells following caffeine treatment [260]. RyRs had been proposed to be located on insulin granules [261] but Polonsky and colleagues subsequently demonstrated that RyRs do not co-localize with secretory granules [252]. Additionally, the finding that genetic deletion of the RyR2 subunit calstabin2 induces impaired glucose-induced insulin secretion in mice suggests a functional role for RyR2 in glucose homeostasis [262].

We exploited rare RyR2 mutations identified in patients with CPVT to assess RyR2 function in beta cell dynamics. We discovered that CPVT patients with mutant leaky RyR2 present with glucose intolerance [5]. In mice, transgenic expression of CPVT-associated mutated RyR2 resulted in impaired glucose homeostasis, and an in-depth evaluation of pancreatic islets and beta cells from these animals revealed intracellular Ca^{2+} leak via oxidized and Cys-nitrosylated RyR2 channels, depleted intracellular Ca^{2+} stores, activated ER stress response, mitochondrial malfunction, and decreased fuel-stimulated insulin release [5].

We also verified the effects of pharmacological inhibition of intracellular Ca^{2+} leak in CPVT-associated RyR2-expressing mice, in human islets from diabetic patients, and in an established murine model of T2DM [5]. Our results suggest a scenario in which the chronic ER Ca^{2+} leak via RyR2 triggers ER stress and mitochondrial dysfunction in pancreatic beta cells, causing a bioenergetic deficit with decreased ATP synthesis, thereby impairing metabolism-secretion coupling. These findings raise the possibility that the relationship between diabetes and cardiovascular disorders may be more complex than previously appreciated: indeed, diabetes, which is a critical risk factor for the development of cardiovascular disease [263], can also represent a sequela of heart disease, especially considering that RyR2 channels are chronically leaky in HF [264].

Ca^{2+} handling plays a key role in the regulation of glucose homeostasis also in the liver, as evidenced by the observations that glucagon and cAMP can increase [Ca^{2+}], promoting gluconeogenesis, while Ca^{2+} chelation blocks it [265]. We have demonstrated that IP3R1 mediates the fasting-mediated activation of CaMKII in hepatocytes [266]. Moreover, PKA-dependent phosphorylation of IP3R1 has been shown to increase cytosolic [Ca^{2+}], leading to increase in the phosphatase activity of calcineurin and the subsequent dephosphorylation of the cAMP response element-binding protein (CREB)-regulated transcription coactivator 2 (CRTC2), thereby resulting in the induction of gluconeogenic gene expression [267]. On the other side, insulin-dependent phosphorylation of IP3R1 by AKT inhibits Ca^{2+} mobilization and CRTC2 dephosphorylation, resulting in the suppression of gluconeogenesis [267]. An intriguing role for both RyR and IP3R in the regulation of insulin signaling has been reported in skeletal muscle primary cultures. Specifically, insulin promotes RyR1 *S*-glutathionylation and subsequent RyR1-mediated Ca^{2+} release; in parallel, insulin stimulates IP_3 generation and activation of IP3R-mediated Ca^{2+} release [268]. These two signaling pathways have been shown to contribute to the net gain in surface GLUT4 levels elicited by insulin [268], increasing glucose uptake [269].

AGING

A well-established hallmark of aging is the progressive decline in muscle function, characterized by loss of muscle mass and reduced force generating capacity [109]. Progressive development of muscle dysfunction has been described

not only in mammals [270, 271] but also in the nematode *Caenorhabditis elegans* [272, 273].

E-C uncoupling is considered a major factor in age-dependent decline in muscle contraction. Indeed, reduced SR Ca^{2+} release has been elegantly demonstrated in aged rat gastrocnemii [274], without any significant effect on RyR1 levels [275]. A subsequent study, while confirming no overall effect of age (2-year-old rats) on RyR1 expression in the skeletal muscle, revealed a significant decrease in calstabin1 levels [276].

We recently proved that RyR1 in muscle become progressively oxidized, nitrosylated, and depleted of calstabin1, resulting in leaky channels, reduced tetanic Ca^{2+}, decreased muscle specific force and impaired exercise capacity [47]. To confirm that leaky RyR1 causes the defects in function observed in aged muscle we generated a leaky RyR1 model (RyR1-S2844D mouse), which mimics a constitutively phosphorylated state of the channel; we also investigated muscle specific calstabin1 deficient mice. Both strains prematurely develop a skeletal muscle phenotype similar to that observed in 24-month-old WT mice [47].

We provided evidence for a direct role of mitochondrial free radicals in promoting the pathological intracellular Ca^{2+} leak that underlies age-dependent loss of muscle function [188]. To investigate in detail the effects of mitochondrial anti-oxidative capacity on age-dependent skeletal muscle dysfunction and Ca^{2+} signaling we used a genetic model with enhanced mitochondrial anti-oxidant activity, in which the human *catalase* gene is targeted to and overexpressed in mitochondria. Mitochondrial overexpression of catalase resulted in reduced mitochondrial ROS levels, improved both whole organism (exercise capacity), and skeletal muscle (specific force) performance, and prevented age-related reduction in Ca^{2+} transients, also diminishing SR Ca^{2+} leak and age-dependent biochemical modifications of RyR1 [188].

In the heart, aging causes several structural modifications, even in the absence of overt cardiovascular disease. Albeit contractility at rest does not appear to be affected by age, the ability to increase ejection fraction during exercise declines in elderly, and relaxation is impaired in aged individuals compared to younger adults suggesting a blunted cardiac reserve [107]. In particular, aging results in significant biochemical and physiological changes in the E-C machinery.

The amount of Ca^{2+} delivered to the cytoplasm and the rate of Ca^{2+} removal are two of the major factors determining the rate, intensity and duration of myocyte contraction [35]. Since cardiac contraction largely reflects the magnitude and time course of increases in cytosolic Ca^{2+} (i.e. Ca^{2+} transients), processes affecting Ca^{2+} transients have been of interest when investigating the impact of age on cardiac contractile function. Several studies have focused on the effect of aging on contraction and Ca^{2+} homeostasis at the level of the individual ventricular myocyte.

Aged cardiomyocytes typically exhibit a decrease in their ability to augment contractions and Ca^{2+} transients following βAR stimulation, and the rates of decay of contractions and Ca^{2+} transients are prolonged [35, 277]. This may be attributed at least in part to an age-related reduced catecholamine sensitivity and impaired βAR signaling [109, 113]. The ability of individual ventricular myocytes to contract declines with age. Indeed, when cardiomyocytes are paced at slow stimulation rates (<1 Hz), peak contractions appear similar in young adult and aged myocytes from rodents [278]. However at higher, more physiological stimulation frequencies, the extent of cell shortening is lower in aged mouse ventricular myocytes than in young adult cells. Furthermore, re-lengthening is prolonged in aged myocytes [278], which also produce much smaller increases in peak Ca^{2+} transients than younger cells when myocytes are paced at rapid rates [279].

We have demonstrated *in vivo* that constitutive phosphorylation of RyR2 at Ser^{2808} (RyR2-S2808D mice) leads to a characteristic age-related cardiac dysfunction [41]. Indeed, serial echocardiographic measurements over the course of a year revealed that transgenic mice exhibited progressive, age-dependent cardiac dysfunction, manifested as a reduction in ejection fraction, coupled with an increase in left ventricular diameter. Similarly, cardiac catheterization at 1, 2, 6, and 12 months of age revealed a gradual deterioration of myocardial contractility in RyR2-S2808D mice that was not evident in WT littermates [41]. We also observed significant decreases in the levels of PDE4D3, PP1, PP2A, and calstabin2 in the RyR2 macromolecular complex by 6 to 12 months of age, consistent with development of myocardial dysfunction. Furthermore, RyR2-S2808D mice displayed age-associated increases in both Cys-nitrosylation and oxidation of cardiac RyR2, which contribute to the age-dependent depletion of calstabin2 from the RyR2 complex in these mice. Similar post-translational modifications have been found in patients with end-stage HF and in mice with post-ischemic HF [41, 105].

More recently, we characterized the cardiac phenotype of calstabin2 KO mice, revealing an age-associated impaired cardiac function compared to WT littermates, accompanied by augmented cardiac fibrosis, cell death, and shorter telomeres. We also demonstrated that calstabin2 deletion resulted in activation of the AKT/mTOR pathway, upregulation of microRNA-34, and impaired autophagy in the heart [35], all established hallmarks of aging [280].

The role of IP3Rs in aging remains elusive, with reports of its involvement in the regulation of *C. elegans* lifespan [273] and other studies in mammals investigating its association with neurodegenerative disorders, as discussed in the dedicated section.

CONFLICT OF INTEREST

Dr. Andrew R. Marks, MD is a consultant and member of the board of ARMGO that is targeting RyR channels for therapeutic purposes.

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