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REVIEW PAPER

Calcium signaling phenomena in heart diseases: a perspective

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Abstract Ca^{2+} is a major intracellular messenger and nature has evolved multiple mechanisms to regulate free intracellular $(Ca^{2+})_i$ level in situ. The Ca^{2+} signal inducing contraction in cardiac muscle originates from two sources. Ca^{2+} enters the cell through voltage dependent Ca^{2+} channels. This Ca^{2+} binds to and activates Ca^{2+} release channels (ryanodine receptors) of the sarcoplasmic reticulum (SR) through a Ca^{2+} induced Ca^{2+} release (CICR) process. Entry of Ca^{2+} with each contraction requires an equal amount of Ca^{2+} extrusion within a single heartbeat to maintain Ca^{2+} homeostasis and to ensure relaxation. Cardiac Ca^{2+} extrusion mechanisms are mainly contributed by Na^+/Ca^{2+} exchanger and ATP dependent Ca^{2+} pump (Ca^{2+}-ATPase). These transport systems are important determinants of $(Ca^{2+})_i$ level and cardiac contractility. Altered intracellular Ca^{2+} handling importantly contributes to impaired contractility in heart failure. Chronic hyperactivity of the β-adrenergic signaling pathway results in PKA-hyperphosphorylation of the cardiac RyR/intracellular Ca^{2+} release channels. Numerous signaling molecules have been implicated in the development of hypertrophy and failure, including the β-adrenergic receptor, protein kinase C, Gq, and the down stream effectors such as mitogen activated protein kinases pathways, and the Ca^{2+} regulated phosphatase calcineurin. A number of signaling pathways have now been identified that may be key regulators of changes in myocardial structure and function in response to mutations in structural components of the cardiomyocytes. Myocardial structure and signal transduction are now merging into a common field of research that will lead to a more complete understanding of the molecular mechanisms that underlie heart diseases. Recent progress in molecular cardiology makes it possible to envision a new therapeutic approach to heart failure (HF), targeting key molecules involved in intracellular Ca^{2+} handling such as RyR, SERCA2a, and PLN. Controlling these molecular functions by different agents have been found to be beneficial in some experimental conditions.

Keywords Calcium · Heart diseases · Plasma membrane · Sarco(endo)plasmic reticulum · Signal transduction

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Introduction

A large number of evidence accumulated concerning the altered intracellular Ca^{2+} cycling that plays a key role in the development of HF [1–3]. In normal heart, intracellular Ca^{2+} movements critically regulate mechanical contractions. In cardiac excitation(contraction (E–C) coupling, a small amount of Ca^{2+} first enters through the L-type Ca^{2+} channel (LTCC) during membrane depolarization. This Ca^{2+} influx triggers a large-scale Ca^{2+} release through the Ca^{2+} release channel of the sarcoplasmic reticulum (SR), referred to

as ryanodine receptor (RyR). The released Ca^{2+} then binds to troponin C present within the myofilaments, which induces activation of the myofilaments and consequently muscle contraction [4–6]. Relaxation is initiated by dissociation of Ca^{2+} from troponin C, followed by its reuptake into the SR through phospholamban (PLN) regulated Ca^{2+}-ATPase (SERCA2a) and subsequently trans-sarcolemmal Ca^{2+} removal through the Na^+/Ca^{2+} exchanger (NCX) operating in its forward mode [5, 6].

The net direction of Ca^{2+} transport by Na^+/Ca^{2+} exchanger is determined by the Na^+ gradient, the Ca^{2+} gradient, and the membrane potential. Na^+ entering myocytes through Na^+ channels during the cardiac action potential has been shown to accumulate in a sub-sarcolemmal space of restricted diffusion. The elevated Na^+ would then induce a Ca^{2+} influx by reverse mode of the exchanger and triggers Ca^{2+} induced Ca^{2+} release from the SR [7]. The reverse mode of Na^+/Ca^{2+} exchanger has a causative role in the consequences of heart diseases [8, 9].

Abnormalities of cardiac rhythm are a major public health burden [10]. Numerous studies have demonstrated that inhibition of specific central signaling pathways can attenuate the hypertrophic response. Indeed, hypertrophy secondary to hypertension in humans can be partially reversed with pleiotropic drugs such as angiotensin converting enzyme inhibitors, β-AR blockers, and Ca^{2+} channel blockers [11, 12].

Ca^{2+} transport mechanisms

Sarco(endo)plasmic reticulum is the major candidate for intracellular stores of activator Ca^{2+} [13]. In cardiac cells a variety of processes contribute to regulation of $(Ca^{2+})_i$ level, which include the ATP- or Na^+-dependent accumulation of Ca^{2+} by the sarco(endo)plasmic reticulum; and ATP- or Na^+-dependent Ca^{2+} extrusion across the plasma membrane. Along with the plasma membrane, different types of ion channels and other transport mechanisms are also present in sarco(endo)plasmic reticulum and play important roles in regulating Ca^{2+} dynamics in cardiac cells (Fig. 1).

Plasma membrane in the maintenance of Ca^{2+} homeostasis

There are various mechanisms that regulate $(Ca^{2+})_i$. The molecular mechanisms that regulate the cytosolic Ca^{2+} concentrations involving the plasma membrane are the Ca^{2+} entry channels, such as voltage-operated Ca^{2+} channels (VOCCs) and store operated Ca^{2+} channels (SOCCs).

Voltage operated Ca^{2+} (Ca_v) channels

In many physiological systems, voltage operated Ca^{2+} (Ca_v) channels are the molecular link between cellular membrane potential and intracellular Ca^{2+} signaling. Voltage-gated L-type Ca^{2+} channels control depolarization-induced Ca^{2+} entry in different electrically excitable cells, including mammalian heart. Alterations in the Ca_V channel activity under pathological conditions such as in heart failure or during ischemia could provide new clues for the development of drugs to treat cardiovascular diseases [14].

Ca_v channels were broadly divided into two subgroups: low-voltage-activated (LVA) and high-voltage-activated (HVA) based on their activation threshold. Auxiliary subunits, $\alpha_2\delta$ and γ of the members of the two families were characterized. Two $\alpha_2\delta$ subunits: $\alpha_2\delta$-2 and $\alpha_2\delta$-3 regulate all classes of HVA calcium channels. While the ubiquitous $\alpha_2\delta$-2 modulates both neuronal and nonneuronal channels with similar efficiency; the $\alpha_2\delta$-3 subunit regulates $Ca_v2.3$ channels more effectively. Furthermore, $\alpha_2\delta$-2 may modulate the LVA $Ca_v3.1$ channel. Four γ subunits, γ-2, γ-3, γ-4, and γ-5 were characterized. The γ-2 subunit modulates both the nonneuronal $Ca_v1.2$ channels and the neuronal $Ca_v2.1$ channel. The γ-4 subunit affected only the $Ca_v2.1$ channel. The γ-5 subunit may be a regulatory subunit of the LVA $Ca_v3.1$ channel. The $Ca_v1.2$ channel is a major target for treatment of cardiovascular diseases [15, 16].

$Ca_v1.2$, a cardiac L-type calcium channel, plays an important role for excitation and contraction of the heart. A type of lethal arrhythmias and also a type of congenital heart disease were characterized as Timothy syndrome. Timothy syndrome results from the $Ca_v1.2$ missense mutation G406R. Functional expression revealed that G406R maintains inward Ca^{2+} currents by causing nearly complete loss of voltage-dependent channel inactivation, which induces intracellular Ca^{2+} overload in multiple cell types. In the heart, prolonged Ca^{2+} current delays cardiomyocyte repolarization and increases risk of arrhythmia, the ultimate cause of death in this disorder [14–17].

Ca^{2+} activated nonselective cation channels

A class of Ca^{2+} activated nonselective cation current was identified in inside-out membrane patches taken from cultured rat heart cells [18]. In cardiac smooth

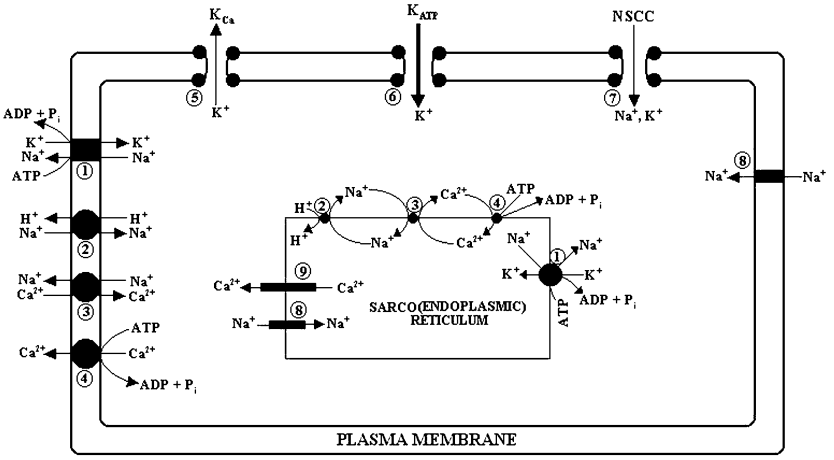

Fig. 1 Proposed scheme of different Ca^{2+} transport processes in myocardial cells. 1; Na^+/K^+ ATPase, 2; Na^+/H^+ exchanger, 3; Na^+/Ca^{2+} exchanger, 4; Ca^{2+}-ATPase, 5; Ca^{+2} activated K^+ channel, 6; ATP regulated K^+ channel, 7; nonselective cation channel (NSCC), 8; Na^+ channel, 9; Ca^{2+} channel

muscle cells, nonselective cation channels are activated by hormones and neurotransmitters, by cell stretch, and by changes in membrane potential. Activation of nonselective cation channels depolarizes the cell membrane and subsequently induces Ca^{2+} influx through voltage gated Ca^{2+} channels [19]. Ca^{2+} reversibly activated the channels in a concentration dependent manner [18]. The channel is considered to play a role in maintaining depolarization after calcium signal transduction or variables such as an arrhythmogenic transient inward current (I_{TI}) in the heart [20, 21]. Activation of nonselective cation channels may trigger contraction even when membrane depolarization is absent or when voltage gated Ca^{2+} channels are blocked, provided the Ca^{2+} permeability of these channels is sufficiently high [19].

One of the possible mechanisms by which Ca^{2+} activates the nonselective cation channels under ischemic-reperfusion injury could be due to the involvement of arachidonic acid and lysophospholipids that are generated by the activation of phospholipase A_2 [22, 23].

Ca^{2+} efflux mechanisms

Plasma membrane Ca^{2+}-ATPases (PMCA)

To maintain long-term homeostasis, all cells possess mechanisms to remove ''excess'' Ca^{2+} that has entered from the extracellular milieu. In many cells, this task is shared by the $Na^+/Ca^{2+}(K^+)$ exchangers, and the PMCA. Due to their larger capacity, PMCAs play important role when large amounts of Ca^{2+} need to be extruded in a relatively short time period. This occurs, for example, in cardiac muscle during beat-to-beat Ca^{2+} regulation [24].

PMCA form a subfamily of P-type Ca^{2+} transport ATPases that utilize the energy of the hydrolysis of ATP to drive uphill translocation of Ca^{2+} across the membrane, transiently forming a phosphorylated intermediate during the transport cycle. The main structural property discriminating PMCAs from related sub-families like the SERCAs of the internal Ca^{2+} stores are the presence of an autoinhibitory domain with a high affinity calmodulin-binding site. Binding of Ca^{2+}-calmodulin relieves the inhibition, providing a mechanism for regulation of PMCA activity by the cytosolic Ca^{2+} concentration itself from the sole ATP driven Ca^{2+} extrusion system of the plasma membrane [25].

The PMCA pump operates as an electrogenic Ca^{2+}/H^+ exchanger with a 1:1 stoichiometry [26]. The increase of its activity during Ca^{2+} signaling events may thus be involved in cellular acidosis. Such an effect has been clearly demonstrated in smooth muscle cells [27]. The PMCA pump is a substrate of intracellular proteases. Initial observation suggested that it was a substrate for the Ca^{2+}-dependent protease, calpain, and more recent work has shown that effector caspases (e.g., caspase 1 and 3) and matrix metalloprotease-2 (MMP-2) also cleave PMCA [28, 29]. Both activation and inactivation of PMCA2 and PMCA4 by caspases has been reported. Calpain attacks calmodulin-binding enzymes removing portions of the calmodulin binding sequence and leading to their ''irreversible'' activation. The protease contains regions with strong homology to calmodulin (domain IV and VI), which may be important in directing it to its target sequences. Support for this concept was provided by the binding of calpain to the calmodulin-binding domain of the erythrocyte pump [28].

Hypertension is a risk factor for thrombotic events. It has been demonstrated that platelet PMCA activity

inversely correlates with diastolic blood pressure and that inhibition of this Ca^{2+} pump could explain the elevation of cytosolic Ca^{2+} in hypertension. Interestingly, it was recently shown that platelet PMCA can be phosphorylated on tyrosine residues thereby inhibiting the activity. Tyrosine phosphorylation was increased in hypertensive humans suggesting a causal link with increased platelet $(Ca^{2+})_i$, hyperactive platelets and increased risk of stroke [25, 30].

An insensitivity of PMCA activity to ischemia was found by Dhalla et al. [31]. They investigated the effect of oxidative stress on PMCA function in a series of studies, both on perfused hearts and directly on isolated sarcolemmal membranes. Depression of sarcolemmal PMCA activity similar to that in ischemia/reperfusion was observed by cardiac perfusion with radical generating solutions. In ischemia as well as with radical generating perfusion, scavenging enzymes prevented the effects. The ATP dependent Ca^{2+} transport of purified membranes from control hearts was inhibited by oxygen radicals, and also this effect was reversed by antioxidant enzymes. Similar effects have also been observed on coronary artery smooth muscle cells [25, 32].

Regulation of PMCA by phosphorylation and dephosphorylation

Calcium pump has been shown to be regulated by protein kinase A (PKA) dependent mechanisms [33]. The serine residue phosphorylated by PKA is located in the calmodulin-binding domain of the PMCA1 isoform [34]. Although this isoform, as well as its spliced variants, are thought to be a house-keeping form of the enzyme in many cells, the transcripts encoding a potentially PKA-insensitive PMCA1 isoform has also been detected in different cells including smooth muscle cells [35]. PKC-mediated phosphorylation was demonstrated in different types of vascular cells [36, 37]. PKC phosphorylated a threonine residue, and recent studies have demonstrated that at least one serine residue located carboxy-terminally to the CaM-binding domain was also the substrate for this kinase. A study performed with PMCA2 and PMCA3 isoforms overexpressed in COS cells revealed that PKC regulated their activity in different ways [38]. Little or no phosphorylation by PKC was detected in PMCA2b and PMCA3b, whereas PMCA2a and -3a variants were phosphorylated without increasing their Ca^{2+} transport activity. Phosphorylation of PMCA4a was blocked when CaM was bound to the enzyme, but phosphorylation in the absence of CaM did not eliminate either binding or further activation of calcium pump by CaM [39, 40]. Thus, it appears that in COS cell membrane, PMCA variants 2a, 3a, 4a, and 4b are phosphorylated by PKC, but only PMCA4b is activated by the process [41].

In contrast to phosphorylation, limited data are available on specific Ca^{2+}-ATPase dephosphorylation. The protein Ser/Thr phosphatases PP1, PP2A, and PP2B account for the majority of the phosphatase activity in vivo, and are involved in multiple cellular functions. The regulation of calcium pump activity appears to be a more complex phenomenon, because protein phosphatases such as PP1 and PP2A reversibly inhibit PKC activity. These phosphatases could directly or indirectly (via PKC) regulate the calcium pump activity in the heart [42, 43].

Na^+/H^+ exchange and plasma membrane Ca^{2+} cycling

During myocardial ischemia, intracellular acidosis develops quickly, activating the exchanger to extrude H^+ into the extracellular environment and brings Na^+ into the cell. With further progression of ischemia, the cells are unable to handle Na^+ overload, causing them to use Na^+/Ca^{2+} exchanger to unload intracellular Na^+ into extracellular space. At the same time, however, Ca^{2+} is being transported into the cells, which may lead to detrimental cardiac injury, such as contracture and necrosis. During myocardial reperfusion, these events are magnified because the return of blood flow lowers the extracellular H^+ concentration, stimulating the NHE (sodium hydrogen exchanger) to extrude more intracellular H^+ ion. This leads to intracellular Na^+ excess and eventually intracellular Ca^{2+} overload and cardiac injury [44].

The NHE-1 isoform is the only significant plasma membrane isoform present in the myocardium. Intracellular acidosis is the primary stimulus for activation of the NHE-1. There are, however, many pathways, which may also lead to NHE-1 activation. Stimulation of the NHE-1 can occur via endothelin-1, angiotensin II, α1–adrenergic agonists, thrombin, and growth factors, or cardiac insult through H_2O_2 production and reduced ATP levels [27]. Activation of these pathways or ischemia and ischemia/reperfusion lead to increase in the levels of NHE-1 mRNA, which can be attenuated by the NHE-1 inhibitor, cariporide [45]. The activity of NHE-1 is elevated in animal models of myocardial infarcts and in left ventricular hypertrophy. During ischemia and reperfusion of the myocardium, NHE-1 activity catalyzes increased uptake of intracellular Na^+. This, in turn, is exchanged for extracellular Ca^{2+} by the reverse mode of Na^+/Ca^{2+} exchanger

resulting in Ca^{2+} overload and subsequently causes damage to the myocardium [46].

One interesting possibility to explain why an activation of Na^+/H^+ exchange commonly occurs is that it serves to regulate the H^+ concentration in the subdomain of the plasma membrane, $(H^+)_{sm}$ i.e., the same domain in which Ca^{2+} cycling leads to an increase in $(Ca^{2+})_{sm}$. When Ca^{2+} enters the cell through Ca^{2+} channels in the plasma membrane, these channels function as uniporters, but when Ca^{2+} is pumped out of the cell via the Ca^{2+} pump, the pump functions as a $Ca^{2+}/2H^+$ exchanger. Hence, during each turn of a cycle of Ca^{2+} in and Ca^{2+} out there is a net accumulation of $2H^+$ in the submembrane domain. Since the activities of many enzymes and transporters are H^+ sensitive and the association of Ca^{2+} with receptor protein (e.g., calmodulin) is also pH sensitive, such a change in $[H^+]_{sm}$ could have a significant effect on events occurring in this subcellular domain. However, if there is an increase in Na^+/H^+ exchanger activity along with an increase in Ca^{2+}-ATPase activity, then any change in $(H^+)_{sm}$ would be minimized and stability of plasma membrane transducer and Ca^{2+} pump functions are assured [47].

This explanation does not account, however, for the fact that the Na^+/H^+ exchanger is often activated directly by agonists, which provide inhibitory signals [48]. In some of these cases, the activation of Na^+/H^+ exchanger appears to be a direct consequence of hormone-receptor interaction. Since a common associated event is an increase in K^+ efflux which leads to hyperpolarization of the plasma membrane, it is possible that the main role of the Na^+/H^+ exchanger is to increase Na^+ influx into the cell to maintain required $(Na^+)_{sm}$ to ensure continued activity of the Na^+/K^+ ATPase or Na-pump [47].

The beneficial effects of NHE-1 inhibitors observed due to normalization of $(Na^+)_i$. Cariporide, a NHE-1 inhibitor, appeared to be insensitive to Ca^{2+} transients and SR-Ca^{2+} handling. Thus, inhibition of the $(Na^+)_i$ increase via NHE-1 inhibitors may represent a useful strategy for the treatment of heart failure [49].

Plasma membrane Na^+/Ca^{2+} exchanger

Na^+/Ca^{2+} exchanger (NCX) on the plasma membrane is thought to be the main Ca^{2+} extrusion system from the cytosol to the extracellular space in cardiac myocytes. Takimoto et al. [50] observed a reduction of NCX activity in pressure overload heart that causes contraction and the development of hypertrophy.

The Na^+/Ca^{2+} exchanger catalyzes the electrogenic exchange of $3Na^+$ for $1Ca^{2+}$ across the surface membrane. The exchange is driven by the transmembrane gradient of Na^+, which, in turn, is maintained by the Na^+/K^+ ATPase [47]. Cardiac Na^+/Ca^{2+} exchanger activity has been shown to be sensitive to external K^+ concentration. The net direction of Na^+/Ca^{2+} exchange is determined by the Na^+ and Ca^{2+} concentrations in the medium and the cytosol, and by the membrane potential. The primary role of the exchanger is the export of Ca^{2+} from the cell when the cytosolic Ca^{2+} concentration is elevated upon activation. Due to its relatively low Ca^{2+} affinity, it is likely to operate most efficiently in its Ca^{2+} efflux mode in areas of high local Ca^{2+} concentration at the peaks of Ca^{2+} transients. Under special conditions the exchanger may operate in reverse mode and catalyzes net Ca^{2+} import, but the physiological significance of this is not clearly established [51, 52].

The pathological role of NCX in hypoxic cell injury is caused by Ca^{2+} influx via ''reverse'' mode transport. During hypoxia, the intracellular Na^+ concentration rises because of its increased influx and decreased efflux. Na^+ influx increases because H^+ generation by glycolysis activates the Na^+/H^+ exchanger and also possibly because of activation of voltage dependent Na^+ channels. Furthermore, reduced ATP generation slows Na^+ efflux via the Na^+/K^+ ATPase. In addition, the cell membrane potential depolarizes during hypoxia because of a combination of direct effects on K^+ channels and run down of the transmembrane K^+ gradient. These forces combine to reverse the driving force on the Na^+/Ca^{2+} exchanger inducing ''reverse'' mode transport that brings Ca^{2+} into the cell. This raises the intracellular Ca^{2+} concentration and initiates cellular injury [51, 52].

Because reverse mode transport is the critical step in ischemia induced cytosolic Ca^{2+} elevation, inhibition of NCX, particularly inhibition of a ''reverse'' mode transport protect against ischemic injury. So far, only two specific NCX inhibitors, KB-R7943 and SEA-0400 are available. The new NCX inhibitor SN-6 is a derivative of KB-R7943 [53].

Effect of cholesterol on Na^+/Ca^{2+} exchange and K_{Ca} channel activity

Cholesterol has been shown to exert a direct influence on the structure and function of myocardial cells independent of the development of atherosclerosis. The ability of cholesterol to modify membrane structure and function of cardiomyocytes and vascular smooth muscle cells suggests that in susceptible individuals, diet related alteration of membrane cholesterol content might augment the risk of developing atherosclerosis when exposed to other pathogenic

stimuli. This view was supported by several studies in animals and humans that prolonged high plasma cholesterol increase the risk of developing atherosclerosis [54].

Na^+/Ca^{2+} exchange and Ca^{2+} dependent K^+ (K_{Ca}) channels are affected by a change in membrane cholesterol content in vascular smooth muscle cells [54]. Jeremy and McCarron [55] observed that hypercholesterolemia is associated with an altered Ca^{2+} dependent K^+ channel activity and arterial vasomotor tone, which indicated that cholesterol-mediated alterations in cellular Na^+, Ca^{2+} and K^+ contents may affect directly Na^+/Ca^{2+} exchanger and K^+ channels [55].

Role of oxidants on plasma membrane Ca^{2+} transporters

Under normoxic conditions, a number of biochemical reactions generate reactive oxygen species (ROS), which are neutralized by the antioxidant defense systems. The excessive production of ROS due to ischemic-reperfusion injuries results in enhanced oxidation of cellular biomolecules, including lipids, DNA, proteins, and amino acids [56]. Recent studies have indicated that the ROS-dependent oxidative modification of biological membranes is particularly more pronounced by oxidants that produced during ischemia-reperfusion induced cell injury [57].

Several essential proteins are known to be particularly sensitive to oxidative modifications, including ion channels, Na^+/K^+ ATPase, Na^+/Ca^{2+} exchanger, and Ca^{2+}-ATPases [58]. The oxidized proteins are often found to be functionally less active, and are more susceptible to proteolytic cleavage [29, 59].

Oxidatively modified calmodulin binds to the PMCA, but does not induce the formation of the α-helical structure within the CaM-binding sequence normally associated with enzyme activation [47]. Previous measurements have demonstrated that this is the result of altered binding interactions between CaM and the CaM binding sequence on the PMCA that results from global structural changes involving the oxidative modification of a carboxyl terminal methionine [60].

Sarco(endo)plasmic reticulum

Unlike other membrane systems, there is no potential difference across the SR(ER) membrane. The concentration of Ca^{2+} in the extracellular space is between 1 mM and 10 mM, whereas the Ca^{2+} in the cytosol $(Ca^{2+})_{cyt}$ is in the order of 0.1 μM thus creating a large inwardly directed electrochemical gradient forcing Ca^{2+} entry across the plasma membrane. Cells have several mechanisms for maintaining a low $(Ca^{2+})_{cyt}$, which at the same time also ensues that the appropriate transient peak levels of Ca^{2+} are reached during activation. Notable among these are accumulation of the ion into the SR by the SERCA and Na^+ dependent Ca^{2+} uptake. Although these two Ca^{2+} transporting mechanisms are essentially accomplished the same effect of rapidly reducing $(Ca^{2+})_{cyt}$ levels, they have different physicochemical properties and regulatory mechanisms [61].

Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA)

The maintenance of Ca^{2+} stores is the result of equilibrium between Ca^{2+} release and Ca^{2+} refill, which involves opening of cation channels as well as activity of Ca^{2+} pumps. The sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) is a critical molecule which pumps Ca^{2+} from the cytosol into the lumen in order to maintain low intracellular Ca^{2+} [62].

The SERCA family of Ca^{2+}-ATPase includes 3 major isoforms (SERCA1-3) are coded by distinct genes. SERCAs belong to a family of cation transporters, the P-type ATPases, so called because a phosphorylated intermediate involving an Asp residue is formed during the catalytic cycle. The cycle of events by which Ca^{2+} is taken up from the cytosol and pumped against a concentration gradient into the sarco(endo)plasmic reticulum (SR/ER) is shown in Fig. 2. The key features of the model are the presence of at least two conformational forms of the protein. The E1 form binds two Ca^{2+} ions with high affinity from the cytosol. After binding ATP, the pump is phosphorylated and the Ca^{2+} ions are subsequently released to the lumenal side of the membrane following the conversion of the pump to the low affinity E2 conformation. Dephosphorylation follows and the pump returns to the E1 form [62] (Fig. 2).

Modulation of SERCA activity

Given the key role played by calcium pumps in controlling the spatiotemporal patterns of cytosolic Ca^{2+} ions, modulation of calcium pump activity will have profound effects on Ca^{2+} signaling.

TFP, a Ca^{2+}/calmodulin dependent protein kinase inhibitor, acts as a competitive inhibitor of phosphorylation of SERCA [63], and induces conformational change, which enable Ca^{2+} release from the SR through

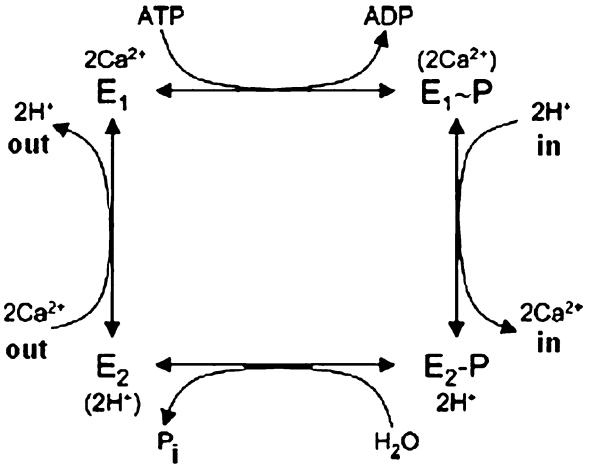

Fig. 2 SERCA pumping cycle. (Taken from reference no 61 of the text with permission)

the ATPase. Addition of TFP during oxidative stress caused by ischemia-reperfusion injury completely blocked the inhibition of ATPase activity. This suggests that TFP may induce conformational changes in the ATPase, which protect it from oxidative damage [63].

In addition to peroxidation of SR membranes and oxidation to ATPase thiols, Moreau et al. [64] demonstrated that oxidative stress caused inhibition of SERCA was involved in the fragmentation of the ATPase polypeptide chain. Reactive oxygen species produced under pathological conditions of iron overload caused reduced ATP hydrolysis. Functional impairment of the Ca^{2+} pump has been suggested to be related to oxidative protein fragmentation [64], which may involve hydrogen abstraction by OH^- from amino acid α carbons, followed by reaction with O_2^- to produce peroxy-species that causes protein fragmentation [65, 66]. Oxidation of proline residues to 2-pyrrolidone [67] may provide an alternative mechanism for peptide bond cleavage [63, 67, 68].

SERCA2a and PLN

Phospholamban (PLN) has 52 amino acid residues. It inhibits the activity of SERCA2, and has the ability to inhibit SERCA1, although phospholamban is not expressed in fast-twitch skeletal muscle. SERCA3 is only weakly inhibited by phospholamban. The inhibitory influence of phospholamban is removed when phospholamban is phosphorylated by protein kinase A and calmodulin kinase at residues ser^{16} or thr^{17}. Transgenic studies indicate that phosphorylation of ser^{16} is a prerequisite for the phosphorylation of thr^{17} [62].

Many studies have demonstrated a reduced expression of SERCA2a protein in failing hearts [69]. Consistently, it has been observed that SR Ca^{2+} uptake (or SERCA2a activity) is reduced in the failing myocardium [24, 62, 70]. The ratio i.e., the protein expression of SERCA2a relative to PLN indicates the extent of Ca^{2+} pump inhibition, and hence the basal level of SERCA2a activity is at a lower level in failing hearts than in normal hearts [71–73]. Regarding the phosphorylation of PLN, the level of ser^{16} phosphorylation has variously been reported to be reduced [74–76] or unaltered [77, 78] in HF, whereas the level of thr^{17} phosphorylation has consistently been reported to be decreased [32, 78]. Thr^{17} phosphorylation is affected by the decreased calmodulin-dependent kinase II (CaMKII) activity in HF, whereas ser^{16} phosphorylation is mainly affected by PKA activity. The level of CaMKII activity also affects the ser^{38} residue, which is the Ca^{2+} binding domain of SERCA2a thereby regulating Ca^{2+} uptake [79]. The altered phosphorylation state of PLN may be responsible for the reduced SR Ca^{2+} uptake activity seen in HF. Type-1 PP1, which makes up a major protein of the serine threonine protein phosphatases present in cardiac myocyte, may also play an important role in regulating PLN phosphorylation. This is apparent from the fact that PP1 may be hyperactivated concurrently with a reduced level of ser^{16} phosphorylation in PLN in several models of HF [80–82]. Overexpression of PP1 catalytic subunit (in the mouse heart was shown to lead to marked left ventricular dilation and premature death due to severe HF [83]. Decreased thr^{35} phosphorylation in inhibitor-1 (I-1), an endogenous inhibitor of PP1, is also associated with increased PP1 activity in the failing heart [80, 83].

PKC regulation of PP1 activity has been demonstrated to be important in HF. Not only that the β-adrenergic system is stimulated, but also the receptor operated signaling triggered by agonists such as angiotensin II, endothelin, and the β-adrenergic system are chronically activated and that was shown to contribute to depressed contractility and to a progression of both remodeling and apoptosis [81–83]. The common key enzyme in the downstream events in these receptor-operated systems is PKC. PKCα, a PKC isoenzyme expressed in the heart, plays a key role in regulating cardiac contractility and Ca^{2+} handling in myocytes. PKCα directly phosphorylates ser^{67} in I-1, thereby augmenting the activity of PP1 and causing hypophosphorylation of PLN. This may, in part, explain why SERCA2a activity decreases in HF, apart from the more obvious possibility of a reduced abundance of SERCA2a protein [80].

Sarcolipin

Another proteolipid, sarcolipin is known to play a role in regulating SERCA1 activity, which has been cloned and sequenced [62]. The sequence of sarcolipin shows some similarities with phospholamban. Coexpression of sarcolipin with SERCA1 reduces the apparent affinity of SERCA1 for Ca^{2+}, while at the same time increasing V_{max} for Ca^{2+} transport [62]. There is no evidence to indicate that sarcolipin can be phosphorylated to modulate its influence on SERCA1, and the most likely mechanism by which sarcolipin exerts control over SERCA1 activity is by alteration of the levels of sarcolipin in SR. Site directed mutagenesis studies of sarcolipin indicate that the transmembrane domain and the C-terminus play essential roles in the interaction with SERCA1. However, despite the similarity of the transmembranous domain of sarcolipin with phospholamban, mutagenesis and coexpression studies suggest that the mechanism of modulation and binding to SERCA1 appear to be different since their combined effects are additive [62].

Nitric oxide

Nitric oxide (NO) has been identified as a potential modulator, stimulating SERCA function in platelets and coronary artery smooth muscle cells. Covalent modifications of SERCAs have also been demonstrated. NO reacts with superoxide anion (O_2^-) to produce peroxynitrite radicals ($ONOO^-$), which can oxidize Cys and Arg residues in SERCA1 [62]. In addition, peroxynitrite can nitrate tyrosine residues. The elevated nitrotyrosine and oxidized Arg and Cys levels in SERCA are associated with a variety of pathophysiological conditions, for example, cardiovascular diseases [84]. Also, the transitions from fast to slow twitch muscle, which accompanies chronic stimulation, is associated with elevated levels of nitrotyrosine in SERCAs [85]. These modifications significantly inhibit the activity of SERCAs [62].

Sarco(endo)plasmic reticular Na^+/Ca^{2+} exchanger

Cardiac sarcolemal vesicles have been used to measure many basic properties of Na^+/Ca^{2+} exchange, such as stoichiometry, electrogenicity, and ion interactions [86]. The Na^+/Ca^{2+} exchanger can be modulated by several interventions. These include protease treatment, high pH, redox modification and an altered lipid environment [86].

The sarcolemmal Na^+/Ca^{2+} exchanger (NCX) is one of the main Ca^{2+} transport mechanisms in cardiac myocytes and is thus essential for the regulation of Ca^{2+} homeostasis and contractile function. Iwamoto et al. [87] demonstrated that the Na^+/Ca^{2+} exchanger of cardiac and vascular smooth muscle cells are activated by protein kinase C-dependent phosphorylation in response to growth factors. However, the effects of phosphorylation on Na^+/Ca^{2+} exchangers in other tissues have not been demonstrated as convincingly as that of the smooth muscle cells. Also, multiple exchanger gene products and splice variants are described and some of these exchangers have been proved to be substrates for different kinases [51].

Oxidants generated during ischemia-reperfusion cause changes of the Na^+/Ca^{2+} exchanger activity in intact cells [88–91]. Diamide, a sulfhydryl oxidizing agent has been shown to inhibit the Na^+/Ca^{2+} exchange current, which suggests that thiol group modification may be involved in the effects of oxidant stress on the Na^+/Ca^{2+} exchanger. Sulfhydryl modification has been suggested to lower the affinity of the Na^+/Ca^{2+} exchange for Ca^{2+}, which indicates the possibility that oxidative stress had a direct sulfhydryl group-mediated effect on the Na^+/Ca^{2+} exchanger; and subsequently inhibition of Na^+/Ca^{2+} exchange by oxidative stress [92, 93].

Role of SERCA in myocardial pathologies

In an early study, Chemnitius et al. [94] have investigated the properties of sarcolemmal membranes purified from isolated perfused canine hearts following intermittent clamping of the inflow and outflow until a stable decline in the rate of force development had been reached. This treatment resulted in irreversible damage of ATP-dependent sarcolemmal Ca^{2+} transport, with depression of both the initial rate of Ca^{2+} uptake and maximal Ca^{2+} uptake capacity. Dhalla and coworkers [31] have observed similar effects and demonstrated that reoxygenation induces a further decline of ATP-dependent Ca^{2+} transport of the sarcolemma. Ca^{2+} pumping activity declined with the duration of ischemia and was accompanied by structural changes of the sarcolemma [95]. In accordance with this Samouilidou et al. [96] have observed a depression of the Ca^{2+} stimulated ATPase activity of sarcolemmal membranes of ischemic hearts in situ. The ATP dependent Ca^{2+} transport of purified membranes from control hearts was inhibited by ischemia/reperfusion, and that this effect was reversed by antioxidant enzymes. Similar effects have also been observed in coronary artery smooth muscle cells [25].

Cardiac hypertrophy and myopathy are other conditions associated with abnormalities in Ca^{2+} handling,

including modifications of the ATP dependent Ca^{2+} transport in sarcolemma. Sarcolemmal membranes isolated from both the hypertension and the cardiac hypertrophy in rats showed depressed activity of the sarcolemmal Ca^{2+} pump and voltage-dependent Ca^{2+} channels. Alterations in the sarcolemmal Ca^{2+} transport activities may serve as an adaptive mechanism facilitating the removal of Ca^{2+} from the myocardial cells during the development of cardiac hypertrophy [25].

The role of oxidative stress may not be restricted to ischemia/reperfusion-related dysfunction. Oxidative damage may also be an important factor in cardiomyopathy caused by excessive amounts of circulating catecholamines. Experimental treatment of rats with a high dose of the synthetic catecholamine inducer, isoproterenol resulted, in addition to morphological and functional changes, in an increased level of oxidative stress products such as malonaldehydes, conjugated dienes and low reduced glutathione. In parallel, a depression of cardiac sarcolemmal ATP dependent Ca^{2+} uptake, Ca^{2+}-ATPase activity, and Na^{+} dependent Ca^{2+} accumulation were also observed. Similar results have demonstrated in isolated hearts perfused with adrenochrome (an oxidized product of catecholamines). These effects were ameliorated by antioxidants, for example, vitamin E. Thus, intracellular Ca^{2+} overload partially caused by defects in sarcolemmal transport may play an important role in catecholamine-induced cardiomyopathy [25].

Inhibitors of the SERCA pump

The study of the function of the SERCA pump has been greatly helped by the availability of four inhibitors. The most specific are thapsigargin and thapsigarcin [51]. Cyclopiazonic acid and 2,5-di(tert-butyl)hydroquinone have relatively lower affinity for the SERCA pump [97].

The inhibition of thapsigargin was demonstrated on all SERCA pump isoforms. Its affinity for the pump is very high. The inhibitor could be removed from the pump only after extensive digestion by trypsin, followed by denaturation. Thapsigargin has been shown to act by locking SERCA in its Ca^{2+}-free E2 conformations by forming a dead-end complex with them [97] (Fig. 3).

Inositol (1,4,5)trisphosphate receptor (IP_3R)

IP_3Rs are abundantly expressed in various myocardial tissues. Of significance was the finding that the IP_3Rs

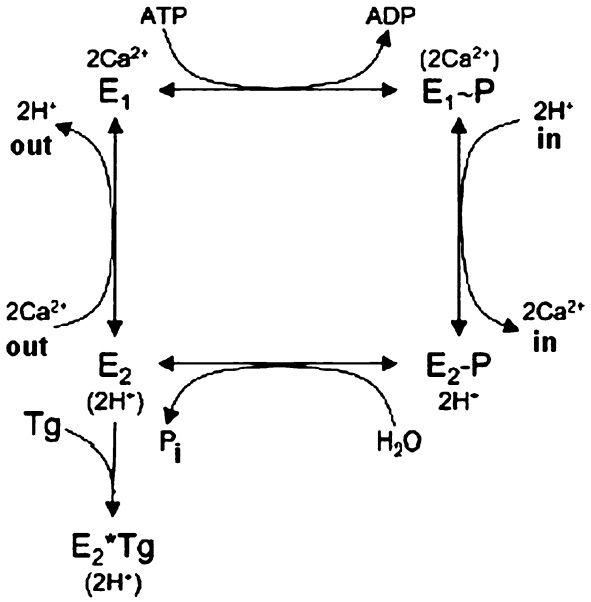

Fig. 3 Model of SERCA inhibition by thapsigargin. (Taken from reference no 61 of the text with permission)

are expressed in both atrial and ventricular myocytes. The release of Ca^{2+} from intracellular stores in response to IP_3 has two effects: first, the rise in the free intracellular Ca^{2+} concentration possibly amplified by Ca^{2+} induced Ca^{2+} release from ryanodine-sensitive stores activates Ca^{2+} dependent chloride channels, producing a membrane depolarization and Ca^{2+} entry through VOCC. Second, depletion of the store acts as the primary stimulus for the activation of capacitative Ca^{2+} entry via SOCCs, responsible for sustaining the contraction and refilling the stores on removal of the agonist [98].

Ryanodine receptor (RyR)

Three types of ryanodine receptors (RyRs) have been isolated, sequenced and cloned to date. The physiological activation of cardiac RyR2 channels in each heartbeat represents the classical CICR response; RyR2 opens in response to Ca^{2+} influx mediated by cardiac L-type voltage-dependent Ca^{2+} channels. As RyR1, RyR2 also possess a few highly reactive SH residues susceptible to redox modification at physiological pH. In the endoplasmic reticulum, ryanodine receptors (RyRs) are a prominent channel type, for which there are also three known gene families. RyR1 is expressed at high levels in skeletal muscle and also in restricted regions of the brain [4, 5]. RyR2 is the predominant isoform expressed in both the heart and the brain [6, 99], and RyR3 is expressed in diverse cell

types [100, 101]. The redox status of cardiac RyR2 channels is a critical determinant of their activity. RyR2 redox modifications affect in vitro single channel activity and Ca^{2+} release from SR vesicles [102].

The role of IP_3Rs in modulating cardiac Ca^{2+} signaling may become particularly significant in the development of heart failure. As end-stage heart failure develops, the ratio of IP_3Rs to RyRs dramatically alters in favour of the former, and cardiomyocytes may therefore become more prone to the proarrhythmogenic effects of IP_3 [103].

Redox modification of RyR

During ischemia there are also important changes in the redox potential of myocytes due to changes in the relative ratios of the redox couples such as NADH/NAD^+. There is evidence that some of the mechanisms participating in E-C coupling can be affected by redox modulation. For example, RyRs contain highly reactive sulfhydryl moieties that may play a redox-sensing function [104]. Oxidation of these thiol groups activate RyRs whereas their reduction inhibits the channel. Redox agents are also known to modulate the SR Ca^{2+}-ATPase [105].

A growing body of evidence documents the importance of reactive oxygen species (ROS) formed during I/R injury in many disorders of cardiovascular system, for example, cardiac ischemia-reperfusion injury. ROS interact with different target molecules including ion transport system that participate in E-C coupling like the RyR and SR Ca^{2+}-ATPase [106].

Cysteine residues on the RyR are likely to be the targets for the reactive oxygen species because oxidation of sulfhydryl (SH) groups induces Ca^{2+} release from SR vesicles and increases single RyR channel activity [107]. There are reports describing either positive or negative effects of NO on cardiac RyR2 function. In isolated cardiomyocytes, NO donors can either enhance or inhibit Ca^{2+} release depending on the concentration of NO donor used [108]. Cardiac RyR2 channels are endogenously S-nitrosylated [109] and it has been proposed that nNOS is directly involved in RyR2 regulation since the neuronal isoform of NOS localizes to the SR [110]. Studies in isolated cardiomyocytes from nNOS knockout mice ($nNOS^{-/-}$) have shown that this isoform is an important determinant of cardiac contractility [111, 112]. Myocytes from neuronal $NOS^{-/-}$ mouse exhibit an increase in resting contractile state and an enhanced inotropic response to β-adrenergic stimulation [112–114]. The endogenous level of RyR2 nitrosylation in these $NOS^{-/-}$ animals, however, is currently unclear.

Role of altered RyR phosphorylation in heart failure

RyR is a Ca^{2+} release channel existing as a homotetramer transversing the SR membrane [115]. Each monomer contains approximately 5000 amino acids and has a molecular mass of 565 kDa. RyR associates with FK506 binding protein (FKBP), calmodulin, protein kinase A (PKA), protein phosphatase-1 (PP1), and protein phosphatase 2A (PP2A). The RyR2 is closely associated with LTCC, and this spatial association of the 2 channels forms a functional unit in cardiac E-C coupling [116].

One of the accessory protein, FKBP12.6 plays an important role in stabilizing the RyR channel; more precisely, in the maintenance of its closed state. FKBP12.6 binds to RyR2 with a stoichiometric ratio of 1FKBP12.6 to 1 RyR2 monomer, or 4FKBP12.6 to 1 tetramer [117]. Marx et al. [118] reported that in human HF, and in an experimental model of HF, PKA mediated hyperphosphorylation of RyR2 occurs due to an enhanced level of circulating catecholamines, and this in turn dissociates FKBP12.6 from RyR2, leading to diastolic Ca^{2+} leak through RyR2. This diastolic leak depresses the SR Ca^{2+} load and serves as a substrate for delayed after depolarization (DAD), which can trigger cardiac arrhythmia and lead to sudden death [119–121]. The dissociation of FKBP12.6 from RyR2 also functionally uncouples multiple RyR2s and disturbs both the simultaneous opening of RyR2s during systole and closing during diastole [118, 122, 123]. In a canine model of pacing induced HF, PKA hyperphosphorylation of RyR2 occurs in association with a conformational change in RyR2 and a subsequent prominent Ca^{2+} leak through RyR2 [124].

PKA dependent phosphorylation of the L-type channels increases the Ca^{2+} current (I_{Ca}), increasing both the Ca^{2+} trigger for SR Ca^{2+} release and the SR Ca^{2+} content [125]. Phosphorylation of phospholamban (PLB) relieves the tonic inhibition that dephosphorylated PLB exerts on the SR Ca^{2+}-ATPase (SERCA) resulting in enhanced SR Ca^{2+} accumulation and enlarged Ca^{2+} release [126].

It is somewhat surprising that hyperphosphorylation of RyR2 is seen in heart failure, in which there is down regulation of β-ARs. Some studies have shown that phosphorylation of phospholamban, another substrate for PKA-mediated phosphorylation, is actually reduced in myocardium from patients with heart failure [127]. However, there are discrete microdomains within cardiac myocytes in which effects of PKA may be regulated by the binding in its anchoring proteins and by colocalization of phosphodiesterases and

phosphatases that can regulate regional concentrations of cAMP and of phosphorylated substrate, respectively [128]. Variable alterations in these factors may explain why one target of PKA might be hyperphosphorylated in the failing myocytes. Reiken et al. [127] suggest that the acute increase in RyR2 phosphorylation induced by PKA may be a component of the ''fight or flight'' response by increasing RyR2 sensitivity to Ca^{2+} and thus enhancing SR Ca^{2+} release and the $(Ca^{2+})_i$ transient [129, 130]. This would cause an acute increase in contractility, along with PKA induced increases in the SR Ca^{2+} uptake induced by phospholamban, and the increase in the L-type Ca^{2+} current induced by phosphorylation of the Ca^{2+} channel. This is plausible, but one might question why a positive inotropic effect, rather than a negative effect resulting from depletion of SR Ca^{2+} due to Ca^{2+} release channel leakiness, would predominate in the presence of hyperphosphorylation induced dissociation of FKBP12.6 from RyR2. The reason may, conceivably, be related to the degree to which SR Ca^{2+} stores can be maintained in the face of an increased SR Ca^{2+} leak. For example, FK506 induces depletion of SR Ca^{2+} in rabbit myocytes and decreases the $(Ca^{2+})_i$ transient [131]. Thus, in normal myocardium, during sympathetic stimulation with activation of β-adrenergic receptors, stimulation of SR Ca^{2+} uptake by phosphorylation of phospholamban might be sufficient to maintain SR Ca^{2+} stores despite a SR Ca^{2+} release channel leak with a resulting positive inotropic effect due to increased sensitivity of RyR2 to Ca^{2+}. In failing myocardium, because of down regulation of SERCA2a and possibly reduced phosphorylation of phospholamban, this might be inadequate to maintain SR Ca^{2+} stores [131].

Spincophilin, junctin, triadin, and calsequestrin in cardiac Ca^{2+} signaling

Ryanodine receptor (RyR) is composed of four subunits that form the channel, which is associated with various proteins that function to modulate it's opening (Fig. 4). The endoplasmic/sarcoplasmic reticulum (ER/SR) luminal Ca^{2+} binding protein calsequestrin modulates the sensitivity of RyR (Fig. 4). The interaction between calsequestrin and RyR2 (the prominent isoform in the heart) is facilitated by the transmembrane proteins triadin and junctin. The reversible phosphorylation of RyR2 by cAMP is controlled by protein kinase A (PKA), which is composed of regulatory (R) and catalytic (C) subunits that are attached through an A kinase anchoring protein (AKAP). Dephosphorylation depends on protein phosphatase 2A (PP2A), which is attached through the isoleucine-zipper-binding scaffolding protein PR130, and on protein phosphatase 1 (PP1), which is attached through spincophilin (SP). RyR2 is also modulated by calmodulin (CaM) and by FKBP12.6 [132].

The RyR2 macromolecular complex also includes the phosphatases PP1 and PP2a, which interact with RyR2 through the leucine/isoleucine-zipper binding scaffolding proteins spincophilin and PR130, respectively [132–134] (Fig. 4). The presence of these phosphatases in the same protein complex as the kinase and substrate ensure that there is a tight regulation of the phosphorylation status of the receptor and, therefore, its activity. The membrane and luminal region of the RyR2 is present in a complex with three other proteins; junctin, triadin and, calsequestrin (Fig. 4). Calsequestrin is the principal Ca^{2+} binding protein of muscle cells and is highly concentrated in the junctional region of the SR. In the lumen of the SR, calsequestrin does not bind directly to the RyR, but is anchored adjacent to the Ca^{2+} release site through triadin and junctin [132–134]. Triadin and junctin interact with calsequestrin in a Ca^{2+} dependent manner, and this interaction provides the sensitivity of RyRs to Ca^{2+} in the lumen. Indeed, transgenic studies have shown that there is a significant role for calsequestrin, junctin and triadin in cardiac Ca^{2+} signaling and hypertrophy [132–134].

cADPR and NAADP

These two nucleotides mobilize intracellular Ca^{2+} through different mechanisms, even though they are generated through the same enzymatic pathway [135, 136]. Mammalian cells express CD38, which is a multifactorial ADP ribosyl cyclase with both synthase and hydrolase activity. The synthase component of CD38 can use either NAD to produce cADPR or NADP to generate NAADP. CD38 has been located on the cell surface. Different activation mechanisms have been proposed for the cytosolic enzyme. External agonists might activate it, but a consistent mechanism for the transduction process is still lacking. An alternate possibility is that the formation of cADPR and NAADP is sensitive to cellular metabolism. In other words, cADPR and NAADP might be metabolic messengers that can relay information about the state of cellular metabolism to the Ca^{2+} signaling pathways. Such an idea is supported by the fact that cADPR metabolism by the hydrolase is inhibited by either ATP or NADH [72, 135, 136].

NAADP functions by releasing Ca^{2+} from an internal store as a reserve granule store. This store is distinct from the store that is regulated by IP_3 and RyRs, because the latter stores can be depleted

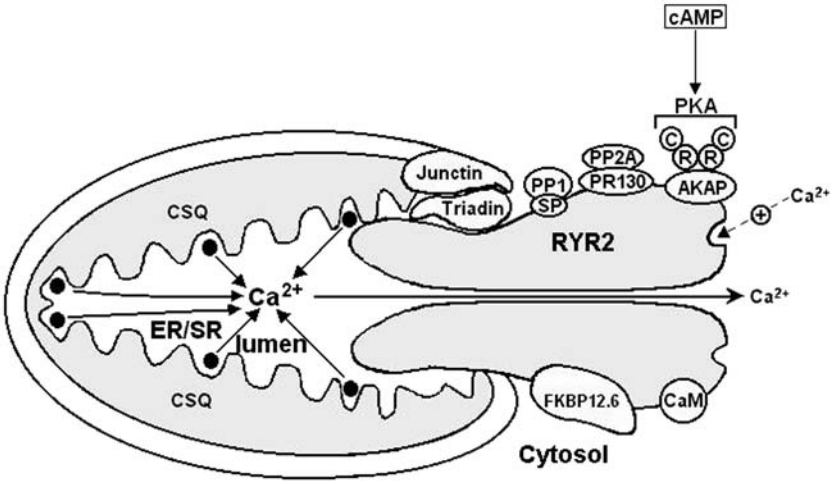

Fig. 4 Schematic representation of the RyR2 Ca^{2+}-release complex in cardiac cells. Ryanodine receptor 2 (RyR2) is composed of four subunits that form the channel, which is associated with various proteins that function to modulate its opening. The endoplasmic/sarcoplasmic reticulam (ER/SR) luminal Ca^{2+} binding protein calsequestrin (CSQ) modulates the sensitivity of RyR2. The interaction between CSQ and RyR2 is facilitated by the transmembrane protein triadin and junctin. The reversible phosphorylation of RyR2 by cyclic AMP (cAMP) is controlled by protein kinase A (PKA), which is composed of regulatory (R) and catalytic (C) subunits that are attached through an A kinase anchoring protein (AKAP). Dephosphorylation depends on protein phosphatase 2A (PP2A), which is attached through the isoleucin-zipper-binding scalffolding protein PR130, and on protein phosphatase1 (PP1), which is attached through spincophilin (SP). RYR2 is also modulated by calmodulin (CaM) and by FK506-binding protein 12.6 (FKBP12.6). (Taken from reference no 132 of the text with permission)

without affecting the ability of NAADP to release Ca^{2+} [135]**.** In contrast to the IP_3Rs and RyRs, the NAADP release mechanism is not sensitive to Ca^{2+} and, therefore, does not support the process of Ca^{2+} induced Ca^{2+} release (CICR). NAADP might sensitize the IP_3Rs and RyRs directly by providing trigger Ca^{2+}, or indirectly by releasing a bolus of Ca^{2+}, which in turn taken up by the other stores in which it can sensitize the release channels.

The function of cADPR resembles that of a modulator rather than a messenger when cADPR is introduced into cells those usually have no immediate effect. In those cases in which it elicits a Ca^{2+} response, there usually is a long latency [137], which indicates that it might be functioning indirectly the Ca^{2+} sensitivity of RyRs, as has been shown in the heart. In the heart, voltage operated Ca^{2+} channels (VOCCs) respond to membrane depolarization by admitting a small pulse of Ca^{2+}, which then stimulates the RyRs to release further Ca^{2+} through CICR. The degree to which the initial entry signal is amplified by CICR, which is referred to as the ''gain'' of signaling system, can be regulated by cADPR. This might have pathological consequences as cardiac arrhythmias can develop if cADPR sets the gain too high [138].

cADPR modulates the sensitivity of the RyRs. cADPR appears to functions as a messenger to stimulate Ca^{2+} release by the RyRs. However, cADPR does not seem to bind directly to the RyR, instead it seems to function through some intermediary component(s), for example, FKBP12.6, a subunit that is associated with the RyR [132].

How are the SR Ca^{2+} stores refilled?

Capacitative Ca^{2+} entry

In most type of cells, unloading of the ER or SR opens ''store-operated'' Ca^{2+} permeable channels (SOCs) that help to refill the Ca^{2+} stores [98]. The refilling phenomenon is known as capacitative Ca^{2+} entry (CCE). CCE explains the relationship between Ca^{2+} entry and internal release [139], according to which Ca^{2+} flows into the cytosol of cells through processes of constant refilling and discharging of the internal pools. Apart from the involvement of SOCC with the participation of TRP (transient receptor potential), CRAC (Ca^{2+}-release-activated Ca^{2+} channel) [140] and STIM1 (stromal interaction molecule1) [141] during IP_3-mediated ER store depletion, VDCC also contribute to CCE under certain conditions, for example, during emptying of ER Ca^{2+} by RyR activation [142] (Fig. 5).

ER when emptied of its Ca^{2+}, begins to release a factor which then diffuses to the membrane to open

I_{CRAC}. This factor has been named CIF (Ca^{2+} influx factor) and is postulated to be a low molecular mass phosphorylated compound that is stored in the ER awaiting the onset of store depletion for its release. There also are indications that protein phosphatases may regulate the responsiveness of the entry channel to CIF, implying that the later may act by promoting channel phosphorylation. Phosphatase inhibitors such as okadaic acid, cyclosporin and calyculin were able to enhance the responsiveness of cells to threshold levels of either CIF or the Ca^{2+} mobilizing agents thapsigargin and carbachol. Inactivation of entry by Ca^{2+} can be explained on the basis of a Ca^{2+} dependent activation of the enzymic cascade that degrades CIF [143].

In order for CIF mediating capacitative Ca^{2+} entry, mechanisms must exist regulating its release to the cytoplasm and its action at the plasma membrane. There are evidences suggesting that at least one guanine nucleotide dependent step exists in the capacitative Ca^{2+} entry pathway. Thus, nonhydrolyzable derivatives of GTP inhibited Ca^{2+} entry due to intracellular Ca^{2+} store depletion, and that this inhibition could be reversed by excess GTP. Thus, this process is somehow involved the hydrolysis of GTP rather than simply the binding of GTP, which occurs with the heterotrimeric G proteins and many small G protein (SMG), such as ras. Thus, the GTP dependent step reflects the involvement of a small G protein, which is known to require hydrolysis of GTP for function. Such a small G protein could be the diffusible signal. Alternatively, if CIF does turn out to be the diffusible signal, then either its release or action at the plasma membrane could involve GTP dependent mechanisms [144].

Stromal interaction molecule1 (STIM1) that has been suggested to play a role in CCE is unlikely to be a store-operated channel. It has no channel like sequence, and over expression of the protein only modestly enhances Ca^{2+} entry. Apparently, the protein is located both on the plasma and intracellular membranes, presumably the ER. The protein sequence suggests that it spans the membrane once, with its NH_2 terminus oriented toward the lumen of the ER or the extracellular space (Fig. 5). STIM1 can oligomerize and the protein in the ER and plasma membrane could interact bridging the two, according to which ER stores communicate with the plasma membrane by means of protein–protein interactions. Thus, STIM1 could play a

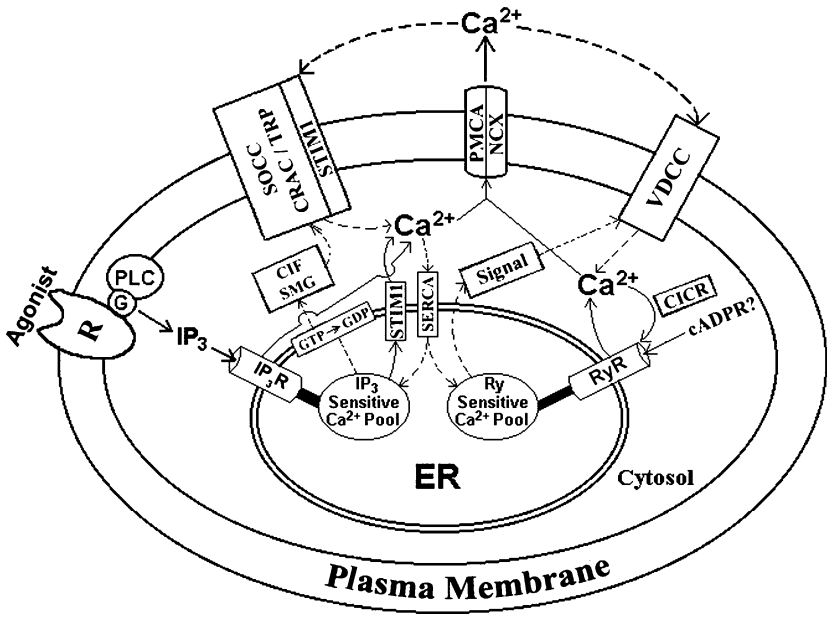

Fig. 5 Activation of surface membrane receptor (R) by an agonist causes activation of PLC leading to the formation of IP_3, which then activates IP_3 receptor (IP_3R) channel of a critical compartment of ER causing release of Ca^{2+} to the cytosol. Another critical ER stored Ca^{2+} may also be activated by a CICR mechanism associated with the ryanodine receptor (RyR) calcium channel. The RyR calcium channel may, in some instances, be regulated by cyclic adenosine diphosphate ribose (cADPR). The increased cytosolic Ca^{2+} then effluxed from the cell predominantly by PMCA and NCX. The fall in ER calcium then signals (via CIF and/or SMG) plasma membrane store operated channels (SOC) through mechanisms involving TRP gene product and CRAC, which interacts with STIM1 leading to CCE (for IP_3-mediated Ca^{2+} regulation); and with the involvement of voltage dependent Ca^{2+} channels (VDCC) (signals transmitted during RyR receptor mediated ER Ca^{2+} store depletion) leading to CCE. Details are in the text

role in activating CCE that occurs especially during IP_3-mediated ER Ca^{2+} store depletion [141, 145, 146].

Thus, in the sustained presence of an agonist, when the Ca^{2+} releasings are being maintained continuously at an elevated level, the Ca^{2+} content of this pool would presumably remain low, maintaining an open pathway from the extracellular space. This favours Ca^{2+} to enter the pool and subsequently releases to the cytosol through the IP_3 and RyR activated channels; thereby further aggravating a situation that favours $(Ca^{2+})_i$ under ischemic injury to heart where cell membrane Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger activities are depressed [147].

Capacitative Ca^{2+} entry has been demonstrated in excitation/contraction coupling and cell proliferation [148, 149]. Consequently, malfunction of capacitative Ca^{2+} entry could have widespread pathophysiological consequences. Certainly, it is known that the Ca^{2+} handling capacity of the SR is defective in disease states such as hypertension. Blood vessels from hypertensive rats show an enhanced contractile response to Ca^{2+}-ATPase inhibition and although a large fraction of this over reaction involves VOCCs, it is likely that changes in capacitative Ca^{2+} entry might contribute to the altered contractile function [61, 98]. Thus, the development of selective inhibitors of SOCCs would not only be of great benefit for the scientific investigation of CCE in smooth muscle and other cells, but could also provide agents with important therapeutic application. The most commonly used inhibitor is SKF96365 [98].

TRP channels, SOCs, and CCE

Transient receptor potential (TRP) channels are known as candidate molecular correlates of receptor activated or store operated Ca^{2+} entry. The regulation and control of plasma membrane Ca^{2+} fluxes is critical for the initiation and maintenance of a variety of signal transduction cascades. Recently studies on the transient receptor potential channels (TRPs) have suggested that these proteins have an important role to play in mediating CCE (Fig. 5). TRP channels mediate the trans-membrane flux of cations down their electrochemical gradients thereby raising intracellular Ca^{2+} and Na^+ concentrations. TRPs regulate $(Na^+)_i$, $(Ca^{2+})_i$ and transmembrane voltage (V_m) in the heart [150].

The SOCCs responsible for CCE are probably composed of mammalian homologs of transient receptor potential (TRP) and transient receptor potential-like (TRPL) proteins. To date seven of these homologous TRPC proteins (TRPC1-7) have been cloned and sequenced [151].

TRPC3, TRPC6, and TRPC7 are regulated by a store dependent mechanism by directly interacting with the IP_3 receptor under low expression conditions. Evidence is accumulating that demonstrates a store-dependent receptor-mediated activation of this TRPC sub-family. The membrane confined lipid second messenger diacylglycerol has been shown to link PLC (phospholipase C) activity to the activation of TRPC3, TRPC6, and TRPC7 [152]. TRP-3, -6, and -7 are gated by products of G protein coupled receptor (GPCR) signaling but cannot be activated by depletion of Ca^{2+} stores with thapsigargin [152]. TRP3 is considered to play a role in maintaining depolarization after Ca^{2+} signal transduction or variables such as an arrhythmogenic transient inward current (I_{T1}) in the heart [153].

It has been suggested that TRPC1 and -6 are components of CCE channels that contribute to the regulation of growth in PASMCs. Thus, CCE through sarcolemmal SOCCs composed of TRPC proteins could play an important role in pulmonary vascular reactivity [154, 155].

NO/cGMP and CCE

The NO/cGMP system interacts with CCE in smooth muscle cells [156]. NO, acting via cGMP, has been shown to strongly inhibit CCE in smooth muscle cells, which is considered to be an important mechanisms underlying the smooth muscle relaxant effects of nitrovasodilator drugs [157]. Furthermore, both nitrovasodilators and cGMP have been found recently to inhibit a Ca^{2+} permeable, nonselective cation current activated by endothelin in rat aortic smooth muscle cells [158]. There are two main mechanisms by which NO/cGMP might inhibit CCE, either directly by blocking the SOCC or indirectly by refilling the SR and therefore removing the primary stimulus for SOCC activation. In support of the latter mechanism, cGMP has been shown to increase Ca^{2+} sequestration by the SR in the smooth muscle cells [159]. Thus, the interactions between CCE and intracellular signaling pathways, for example, that mediated by cGMP appears to be important for furthering our understanding the role played by this process in vascular endothelial and smooth muscle cells [160].

Sphingolipid derived messengers

Sphingolipids are ubiquitous constituents of eukaryotic cells [161]. Sphingolipid metabolites include second messenger sphingosine (SPH), sphingosine-1-phos-

phate (S1P), ceramide (Cer), and ceramide-1-phosphate (C1P) all of which are involved in common signaling pathways controlling cell function (Fig. 6).

Sphingolipids plays important role in regulating vascular tone in health and disease [21, 22, 162]. Two of the major intermediates in the sphingolipid signaling pathway, ceramide and sphingosine, are suspected to mediate some of the vascular effects, such as angiogenesis, atherogenesis, coronary vasoconstriction and arrhythmias [163].

Na^+/Ca^{2+} exchange activity was inhibited by ceramide and sphingosine. They interfere with the activation of exchange activity of Ca^{2+} and suggest that this interaction provides a means for monitoring and regulating diastolic Ca^{2+} levels in beating cardiac myocytes. The regulation of exchange activity in intact cells involve multiple interactions with various lipid species, cytosolic Ca^{2+}, organelle Ca^{2+} stores, and the cytoskeleton [164, 165].

Sphingosine-1-phosphate

Sphingosine-1-phosphate (S1P) is suggested to function together with IP_3 and generates the Ca^{2+} signals that underlie the synthesis and release of inflammatory mediators. The dual activation of these pathways leads to a Ca^{2+} signal with a rapid peak (S1P dependent) and a sustained plateau (IP_3 dependent) [166]. Exactly, how S1P stimulates Ca^{2+} release from intracellular stores is unclear. Until recently, the best candidate for the S1P receptor was a widely expressed protein known as sphingolipid Ca^{2+} release mediating protein of endoplasmic reticulum (SCaMPER). However, it has no similarity to any known intracellular Ca^{2+} channels and is a small (~20 kDa) protein with only one transmembrane domain. Expression of SCaMPER was shown to confer sensitivity to sphingolipids or to affect Ca^{2+} sensitivity to sphingolipids or to affect Ca^{2+} homeostasis [167].

S1P induces tyrosine phosphorylation of $K_v1.2$, one of the K^+ delayed rectifier channel isoforms predominantly expressed in vascular smooth muscle [168]. In addition, this phosphorylation and the subsequent inhibition of the voltage gated K^+ current are dependent on PKC activation of Ca^{2+}-CaM dependent protein kinase. This whole episode was determined to be dependent on an increase in Ca^{2+}, which occurs via two mechanisms: (i) intracellular Ca^{2+} release via an inositol 1,4,5 trisphosphate (IP_3)-dependent pathway and (ii) Ca^{2+} entry through voltage operated Ca^{2+} channels (VOCC). Activation of VOCC occurred through S1P-induced inhibition (~50%) of the voltage gated potassium (K_v) channels and more specifically $K_v1.2$. S1P activates the transcription factor CREB through different Ca^{2+} dependent pathways including intracellular Ca^{2+} release and inhibition of voltage-gated K^+ channels leading to Ca^{2+} influx, which suggests a potential role for S1P in regulation of gene expression in vascular smooth muscle [169, 170] (Fig. 7).

S1P is a potentially important mitogenic factor and is found throughout the cardiovascular system [171]. It is an sphingolipid stored in high concentrations in platelets [172]. Activation of pro-thrombotic stimuli results in the release of S1P from platelets achieving

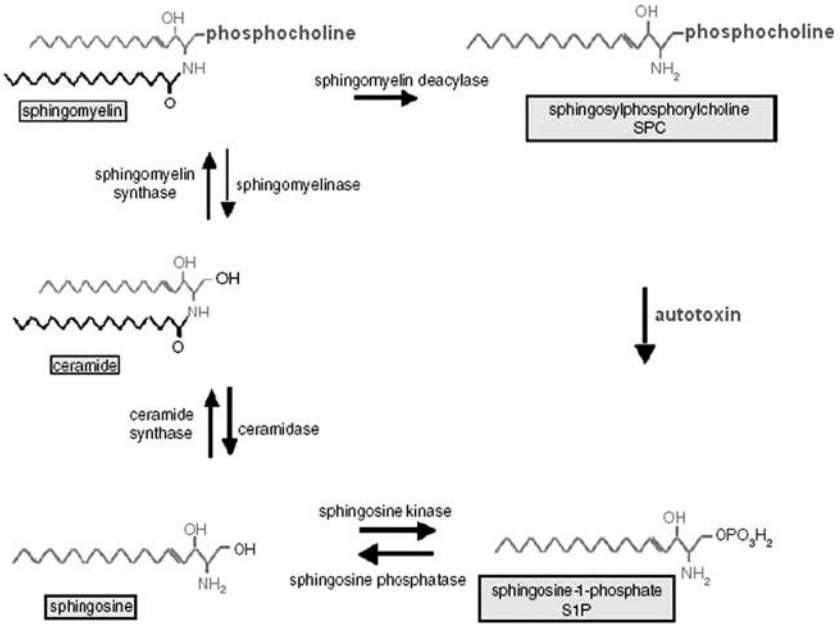

Fig. 6 Sphingomyelin metabolism. (Taken from reference no 161 of the text with permission)

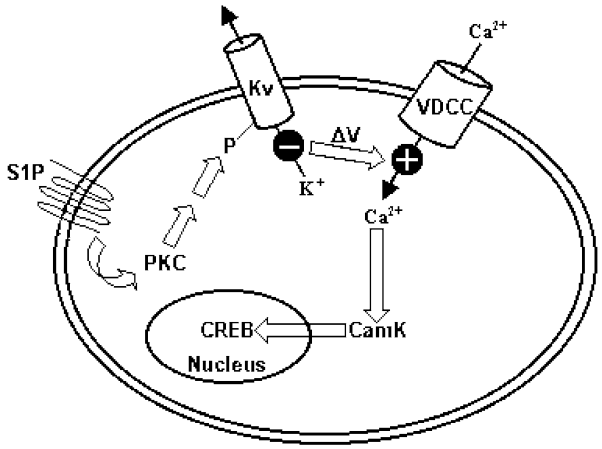

Fig. 7 Schematic diagram of S1P-induced CREB activation via an inhibition of the voltage-gated K^+ current. S1P activates one or more PKC isoforms, leading to tyrosine phosphorylation and inhibition of the K_v channels. This inhibition results in a depolarization, activating VDCC and influx of Ca^{2+}. This influx activates CamK and subsequently results in the phosphorylation of CREB. (Taken from reference no 170 of the text with permission)

high concentrations in serum (nmol/L to μmol/L range) [173]. Due to lipophilic nature of S1P and the levels reached in vivo, vascular smooth muscle cells are exposed to relatively high concentrations in pathophysiological conditions. S1P receptors are G protein coupled receptors and it is now known that these receptors could play important roles in regulating proliferation in vascular smooth muscle. In the pathogenesis of vascular disease, the switch from a contractile to a proliferating phenotype may involve activation of S1P receptors [173]. Studies using smooth muscle cells have implicated that several transcription factors, including cAMP response element-binding protein (CREB) drive the proliferation process in vascular smooth muscle cells [174], which suggests a potential role of S1P in regulating gene expression in vascular smooth muscle cells (Fig. 7).

Sphingosylphosphocholine

Sphingosylphosphocholine (SPC) modulates Ca^{2+} release from isolated cardiac sarcoplasmic reticulum. SPC has been reported to activate G protein-coupled receptors (GPCRs) to induce phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), although the molecular identity of specific receptors to which SPC binds with high affinity is still unclear. In intact cardiac myocytes, even in the absence of extracellular Ca^{2+}, SPC causes a rise in diastolic Ca^{2+}, which is greatly reduced when the sarcoplasmic reticulum is depleted of Ca^{2+} by prior thapsigargin treatment. SPC action on the ryanodine receptor is Ca^{2+} dependent. SPC seems to play as a physiological regulator of $(Ca^{2+})_i$ in cardiac muscle sarcoplasmic reticulum when sphingomyelinase is activated. SPC has been known to induce proliferation, migration, and morphogenesis of endothelial cells. It has been suggested that SPC-induced activation of PLD generates intracellular ROS through NADPH oxidase dependent pathway, and also ROS play important roles in SPC-induced physiological responses in endothelial cells [175, 176].

Ceramide

Ceramide has been shown to mediate some biological effects of IL-1β and TNF-α, two primary proinflammatory cytokines [177] and prostaglandin E2 [12, 17]. Ceramide functions as a lipid second messenger and has been demonstrated to cause an increase in $(Ca^{2+})_i$. B-type Ca^{2+} channels are good candidates for the Ni^{2+}-insensitive Ca^{2+} entry because their activity was increased by chronic and acute applications of ceramide, which was blocked by eosin. Moreover, pharmacological modulations of B-type Ca^{2+} channels regulate the apoptosis of myocytes induced by ceramide [178].

Ceramide reduces L-type Ca^{2+} channel current (I_{CaL}) in adult rat ventricular myocytes. In patch-clamped myocytes, ceramide increased contraction concomitant with reductions in I_{CaL}. In intact myocytes, ceramide increased cell shortening and relaxation and the duration of contraction. In myocytes, ceramide increased systolic Ca^{2+} and the magnitude and maximum rates of the rising and declining phases of Ca^{2+} transients. It appears that regardless of decreasing I_{CaL}, ceramide elicits distinct positive inotropic and lucitropic effects, resulting probably from enhanced SR Ca^{2+} cycling, and increased Ca^{2+} sensitivity of ventricular myocytes [165]. Ceramide elicited effects can be beneficial (as a Ca^{2+} sensitizing and positive inotropic agent) or deleterious (as a potential arrhythmogenic agent). The cardiac effects of ceramide could play an important role in cytokine-related pathophysiological conditions such as ischemia-reperfusion and sudden cardiac death [165].

The mechanisms involved in ceramide-induced alterations in Ca^{2+} sensitivity and SR Ca^{2+} cycling are currently unknown. The dissociation of its inotropic effects from its electrophysiological effect could result from its diversity of cell signaling mechanisms and alterations in the lipid microenvironment of ion channels. For example, ceramide has been reported to

activate protein kinases (including PKC and MAPK) as well as protein phosphatases. Activation of protein phosphatases could reduce I_{CaL}, whereas activation of protein kinases could enhance contractile protein activities. Similarly, the target proteins involved in the ceramide-induced positive inotropic effect in ventricular myocytes also remains largely unknown [165].

SR cholesterol content and Ca^{2+} permeability

Cholesterol enrichment and depletion in model membranes were found to change the permeability to numerous electrolytes and nonelectrolytes. Removal of cholesterol from liposomes resulted in a 40- to 280-fold increase in permeability of H^+ in cholesterol fed rabbits. An increase in Na^+ content of the myocardium has also been observed in rabbits upon cholesterol feeding. Increase of the cholesterol phospholipid ratio, which occurs under pathological conditions changes the fluidity of the ER membrane and inhibits SERCA. This effect can be avoided by pharmacologic manipulations that block cholesterol trafficking to the ER [54]. Pfeiffer et al. [179] have also demonstrated that myocardial Ca^{2+} level was elevated in cholesterol-fed rabbits. In this regard, it is interesting to point out that enrichment of the sarcoplasmic reticulum with cholesterol inhibited the sarcoplasmic reticulum Ca^{2+} pump ATPase activity.

The effects of cholesterol loading on allosteric properties of the Ca^{2+} pump ATPase have been studied in different systems [54]. The Ca^{2+}-ATPase activity is inversely related to cardiac sarcolemmal cholesterol content; high cholesterol content inhibited Ca^{2+}-ATPase activity whereas withdrawal of a small amount of cholesterol caused a stimulatory effect on its activity [54].

Moffat and Dhalla [180] observed a generalized depression in cardiac sarcolemmal ATPase activity in rats 24 weeks after being fed a high cholesterol diet. Kutryk and Pierce [181] also observed a marked depression of Ca^{2+}-ATPase activity by cholesterol enrichment in isolated cardiac sarcolemmal vesicles. Cardiac sarcolemmal Ca^{2+}-ATPase as well as arterial smooth muscle Ca^{2+} pump was also shown to be depressed by an increase in cholesterol and 25-hydroxycholesterol content in rat [180].

Interrelationship between different transporters

Active Na^+ transport across the cardiac sarcolemma, driven by Na^+/K^+ ATPase is an important regulator of cardiac function. The intracellular Na^+ concentration affects a number of physiological processes in cardiac myocytes, including intracellular Ca^{2+} handling, contraction-relaxation processes, pH regulation, energy metabolism, and cell growth. Alterations in the maintenance of normal intracellular Ca^{2+} homeostasis results in heart failure [182].

Na^+/K^+ ATPase is a heterodimer composed of α and β subunits. The α subunit is a catalytic subunit, and it binds translocating cations and ATP. The α subunit is also the pharmacological receptor for cardiac glycosides. These compounds inhibit Na^+/K^+ ATPase activity and are used in the treatment of congestive heart failure. Depending on the species, different combinations of these α-isoforms are present in heart. The α_1 and α_2 isoforms are expressed in rodent heart, whereas three α isoforms: α_1, α_2 and α_3 are expressed in human heart. As multiple isoforms are expressed in heart, it is possible that they play different physiological roles [182, 183].

In human heart expressing three α isoforms: α_1, α_2, and α_3 all are sensitive to ouabain. α_1 isoform along with α_2 isoform plays important role in the increased heart contraction of patients who died eventually with congestive heart failure [184].

Both α_1 and α_2 isoforms have been demonstrated to complex with the Na^+/Ca^{2+} exchanger in the plasma membrane of heart, and regulates cardiac contractility through the reverse mode of the Na^+/Ca^{2+} exchanger [182].

During ischemic injury to heart, intracellular Na^+ concentration rises because of its increased influx and decreased efflux. Na^+ influx increases because H^+ generation by glycolysis activates the Na^+/H^+ exchanger and also possibly because of activation of voltage dependent Na^+ channels. Inhibition of Na^+/K^+ ATPase activity by oxidants generated in ischemic heart also contributes to the increase in intracellular Na^+. Additionally, reduced ATP generation slows Na^+ efflux that occurs via the Na^+/K^+ ATPase. In addition, the cell membrane potential depolarizes during ischemia because of a combination of direct effects on K^+ channels and run down of the transmembrane K^+ gradient. These forces combine to reverse the driving force on the Na^+/Ca^{2+} exchanger inducing ''reverse'' mode transport that brings Ca^{2+} into the cell. The mechanisms by which a cell can get rid of the intracellular increase in Ca^{2+} level would be through Ca^{2+}-ATPase and forward mode of Na^+/Ca^{2+} exchanger. Oxidants, generated during ischemia-reperfusion, depress these transporters resulting in an increase in intracellular Ca^{2+} mobilization and subsequently to heart failure [182, 185, 186].

Some investigators [187] have shown an increase in the Na^+/Ca^{2+} current but normal Ca^{2+} transport in cardiomyocytes from infarcted animals; whereas others

[188] have reported an increase in the concentration of intracellular Na^+ but no change in Na^+/K^+ pump function in hearts failing due to aortic insufficiency. Such conflicting results are consistent with the view that alterations in SL Na^+/K^+ ATPase and Na^+/Ca^{2+} exchange activities are dependent on the stage and type of heart failure. A depression in SL Na^+/Ca^{2+} exchange activity could arise due to depressed protein content and mRNA levels for Na^+/Ca^{2+} exchanger in the infarcted heart. Gene expression for Na^+/Ca^{2+} exchanger in infarcted hearts has been found to be decreased at early stages and increased at later stages of heart failure; however, the Na^+/Ca^{2+} exchange activity at both early and later stages was depressed in the MI-induced model of heart failure in rats [189]. A decrease in mRNA level for Na^+/Ca^{2+} exchanger was reported in the hypertensive heart in the guinea pig [177]. On the otherhand, an increase or no change in mRNA levels has been observed at the later stages of human heart failure [190]. Both enhanced protein expression of Na^+/Ca^{2+} exchanger and its activity have been reported in the end stage human heart failure and the increased protein expression of Na^+/Ca^{2+} exchange was suggested to preserve diastolic function [191]. Depression in Na^+/K^+ ATPase activity in human heart failure was reported by several investigators. In fact, the reduced Na^+/K^+ ATPase activity in human heart failure was shown to be due to varying degrees of changes in Na^+/K^+ ATPase isoforms [192]. A recent study demonstrated a decrease in protein content and mRNA levels for α_1, α_2 and β_1 isoforms, and increased protein content for α_3-isoform in the infarcted heart, which suggest that changes in protein and gene expression may account for a depression in the SL Na^+/K^+ ATPase activity [193]. Semb et al. [193] also observed an association of decreased Na^+ pump capacity with a decrease and an increase in protein content of α_2-isoform and α_3-isoform, respectively; however, these investigators did not detect any change in the α_1 or β_1 isoforms in heart failure due to MI. Although such differences in the expression of α_1 and β_1 isoforms in these two studies may be due to differences in the degree of heart failure, these data when taken together reflect remodeling of SL membrane with respect to the molecular structure for both Na^+/K^+ ATPase and Na^+/Ca^{2+} exchange in the failing heart [194].

Cardiac hypertrophy

Two components provide a conceptual framework to understand how cardiac hypertrophy might develop and how it switches into CHF. The first response is driven mainly by extrinsic factors that act through a variety of signaling mechanisms to induce the transcription events that modification of the Ca^{2+} transients, by an increase in its frequency, amplitude or width, is the primary signal for hypertrophy. It is the upregulation of the Ca^{2+} signaling pathway that may provide the connection to the second component, which is irreversible phenotypic remodeling that results in CHF [195].

In broad terms, there are three types of cardiac hypertrophy: normal growth, growth induced by physical conditioning (i.e., physiologic hypertrophy), and growth induced by pathologic stimuli. Recent evidences suggest that normal and exercise induced cardiac growth are regulated in large part by the growth hormone/IGF axis via signaling through the P13 K/Akt pathway. In contrast, pathological or reactive cardiac growth is triggered by autocrine and paracrine neurohormonal factors released during biomechanical stress that signal through the Gq/PLC/PKC pathway, which involve via an increase in cytosolic Ca^{2+} and activation of PKC [196].

PKC and cardiac hypertrophy

In cardiac tissue, PKC enzyme activity is increased after ischemia and acute or chronic pressure overload where it is postulated to mediate ischemic preconditioning and to transduce hypertrophy signaling [69]. However, the heterogeneity of PKC isoform expression and differences in PKC isoform regulation and activation in the heart has complicated attempts to precisely define the role of PKC in adaptive cardiac responses and related maladaptive sequelae [196].

Although it is the most highly expressed of the myocardial PKC isoforms, PKCα, unlike PKCδ and ε, it is not regulated in acute myocardial ischemia; and in contrast to PKCβ, PKCα is not regulated in diabetes [196]. In vivo analysis of PKCα effects in the mouse heart utilizing gene ablation and transgenic overexpression revealed no effect of PKCα overexpression on cardiac growth and no effect of PKCα inhibition on the hypertrophic response to pressure overload. Instead, ablation of PKCα improved contractility, while overexpression diminished it [83].

PKCα is more important as a regulator of myocardial contractility than cardiac hypertrophy. Proposed mechanisms for PKCα-mediated contractile dysfunction include regulation of sarcoplasmic reticular ATPase-mediated Ca^{2+} cycling through the phospholamban/protein phosphatase inhibitor-1 axis [83] and phosphorylation-mediated uncoupling of β-adrenergic receptors from adenylate cyclase [196]. The relative

contribution of myocardial contractility of these two equally plausible mechanisms is yet to be established. PKCδ can regulate normal cardiomyocyte growth, but suggestions have been made that PKCδ plays a role in cardiac ischemia than myocardial growth. It regulates critical stress-response gene that performs varied tasks as per physiological requirements [196]. PKCε has been shown to be selectively translocated to the particulate ventricular fractions during acute or chronic pressure overload [196] and after AngII stimulation [197]. An interesting report also noted an association between PKCε activation by chronic ethanol consumption in guinea pigs and ethanol-induced cardioprotection from ischemic reperfusion injury [198].

Conventional and inducible cardiac specific transgenesis has been used to explore the direct effects of PKCβ signaling in vivo. Although there is controversy over whether PKCβ is expressed in the adult mouse heart [196], Wakasaki et al. [199] expressed PKCβII under control of a truncated α-myosin heavy-chain promoter and observed a phenotype of hypertrophy, fibrosis, and systolic dysfunction. In a follow up study, PKCβ-mediated phosphorylation of troponin I was suggested as a mechanism for contractile dysfunction in these mice [200].

Studies in the recent past have focused on the more abundant adult cardiac PKC isoforms, such as PKCε. Mochly-Rosen and Dorn [196, 201] have used transgenic techniques to express PKCε activating and inhibiting peptides in mouse heart. The initial description of this model demonstrated that increasing basal translocation of PKCε by approximately 20% was sufficient to exert a powerful protective effect on cardiac contractile function and myocyte integrity in isolated hearts subjected to global ischemia with reperfusion [201]. Subsequent studies using the complementary approaches of PKC isoform inhibition and activation demonstrated that PKCε activation causes a physiologic form of hypertrophy, whereas inhibition of PKCε translocation with a RACK-binding peptide (εVI) caused the opposite response, that is, thinning of the ventricular walls and lethal heart failure leading to a dilated cardiomyopathy. These studies suggested that PKCε activation is a necessary component of normal trophic growth of cardiomyocytes during postnatal development [10].

MAPKs and associated kinases

p38MAPK and cardiac hypertrophy

An increase in free radicals generated and an increase in PKC expression due to I-R injury to heart has been shown to increase $(Ca^{2+})_i$, and decrease expression of sarcoplasmic reticular Ca^{2+}-ATPase with increased phosphorylation of extracellular signal regulated protein kinase and p38MAPK [202]. In cardiac myocytes, mechanical deformation, GPCR ligands (angiotensin II, ET-1, and PE) and mitogens are potent activators of p38MAPK [203]. Pharmacologic inhibition by SB203580 and SB202190 blocked agonist stimulated cardiomyocyte hypertrophy in culture [203]. In addition, adenovirus mediated gene transfer of a dominent negative p38MAPK blunted the hypertrophic response of neonatal cardiomyocytes [203].

Ang II can modulate cardiac contractility. AngII produces negative inotropic effect (NIE) in cardiac myocytes. The NIE of AngII was not associated with a parallel decrease in the intracellular Ca^{2+} transients, indicating that a decrease in myofilament responsiveness to Ca^{2+} underlies the reduction in contractility. Ang II induced NIE was abrogated by the inhibitors of PKC (calphostin), tyrosine kinase (genistein), and p38MAPK (SB202190). The NIE was significantly exacerbated by p38MAPK overexpression. This indicates p38MAPK as the putative effector of the reduction in myofilament responsiveness to Ca^{2+} and the decrease in contractility induced by the peptide [203].

p38MAPK, MKP-1, and calcineurin

Calcineurin, a Ca^{2+} regulated protein phosphatase, promotes down regulation of p38MAPK activity and enhances expression of the dual specificity phosphatase, MAPK phosphatase-1 (MKP-1). Transgenic mice expressing activated calcineurin in the heart were characterized by inactivation of p38MAPK and increased MKP-1 expression during early postnatal development, before the onset of cardiac hypertrophy. In vitro cultured neonatal cardiomyocytes infected with a calcineurin-expressing adenovirus and stimulated with phenylephrine demonstrated reduced p38MAPK phosphorylation and increased MKP-1 protein levels. Activation of endogenous calcineurin with the calcium ionophore decreased p38MAPK phosphorylation and increased MKP-1 protein levels. Inhibition of endogenous calcineurin with cyclosporin A decreased MKP-1 protein levels and increased p38MAPK activation in response to agonist stimulation. Investigation for the potential cross talk between calciuneurin and p38MAPK through alteration in MKP-1 expression revealed that the MKP-1 promoter appeared to be calcineurin-responsive indicating that calcineurin enhances MKP-1 expression in cardiac myocytes, which is associated with p38MAPK inactivation [204].

MEK1 and ERKs in cardiac hypertrophy

Two separate extracellular signal regulated kinase (ERK) isoforms have been described ERK1 and ERK2, which are coordinately phosphorylated and activated by a wide array of mitogenic stimuli. MEK1 and MEK2 are the major upstream activators of ERKs and MAPKs [205].

In response to agonist stimulation or cell stretching, ERK1 and 2 become activated both in cultured cardiac myocytes and in isolated perfused hearts [10, 206]. These observations have implicated ERK-1 and -2 signaling factors as regulators of the hypertrophic response. In support of this notion, transfection of a constitutively active MEK-1 encoding construct (immediate upstream activator of ERK1 and 2) augmented ANF promoter activity in cultured cardiomyocytes, whereas a dominant negative MEK-1 encoding construct attenuated activity [207]. Using antisense oligonucleotides, Glennon et al. [208] demonstrated that ERK signaling is necessary for the α-1 agonist, PE-induced cardiomyocyte hypertrophy in culture. Using the MEK1 inhibitor PD98059, Clerk et al. [209] reported that ERKs were required for sarcomeric organization induced by hypertrophic agonists, suggesting that ERKs play a more specialized role in cardiomyocyte hypertrophy.

C-jun NH2 terminal kinase and cardiac hypertrophy

Three distinct JNK or SAPK (stress activated protein kinase) gene have been identified in mammalian cells. Each is activated by the upstream MAPK kinase and MKK7, which in turn are activated by MEKK1 or MEKK2 [205]. The MEKKKs upstream of MEKK1 and 2 include members of the low molecular weight G proteins (Ras) [205]. JNK are directly phosphorylated by MKK4 or MKK7. JNK1 and JNK2 were shown to be expressed in the heart, whereas JNK3 expression is mostly restricted to the brain [210].

In cardiomyocytes, JNK isoforms become phosphorylated in response to stress stimuli (stretching) or GPCR activation [211, 212]. JNK activation has also been associated with load induced cardiac hypertrophy in rat myocardial infarction, and human heart failure [10, 211].

A number of studies have shown that JNK1 and 2 are critical regulators of cardiac hypertrophy in vitro and in vivo. In transfection experiments, an activated MEKK1-MKK4 induced promoter expression of certain hypertrophy-associated gene and transfection of a dominent negative MEKK1 encoding expression vector attenuated ANF promoter activity [212]. Nemoto and others [213] demonstrated that MEKK1 and JNK activation blocked ANF expression in cultured cardiomyocytes. JNK activation appeared to be a necessary molecular event in the cardiac hypertrophic response, both in vitro and in vivo [10, 214].

C-Jun, an immediate early gene is a key transcription factor for apoptosis. Ca^{2+} influx was shown to be involved in C-Jun N-terminal kinase (JNK) signaling pathway-mediated by IL-1β induced apoptosis. Pharmacological blockers of the Ca^{2+} channel, for example, dihydropyridine suppressed IL-1β induced C-Jun phosphorylation. Treatment with Ca^{2+} mobilizing compounds such as A23187 and ionomycin were shown to cause an amplification of IL-1β induced JNK activation that subsequently leads to apoptosis [215].

Mechanical stress induced cardiac hypertrophy

Mechanical stress is considered to be the trigger inducing a growth response in the overloaded myocardium. Furthermore, mechanical stress induces the release of growth promoting factors, such as angiotensin II, endothelin I and transforming growth factor β; which provide a second line of growth induction [216]. Mechanical stress may be coupled to intracellular signals that are responsible for the hypertrophic response via integrins and the cytoskeleton or via sarcolemmal proteins, such as ion channels and ion exchangers. The signal transduction pathways that may be involved belong to two groups: (i) the mitogen activated protein kinases (MAPK) pathway; and (ii) the janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway. The MAPK pathway can be subdivided into the extracellular-regulated kinase (ERK), the c-jun N-terminal kinase (JNK), and p38MAPK pathway. Alternatively, the stress signal may be directly submitted to the nucleus via the cytoskeleton without the involvement of signal transduction pathways. Finally, by promoting an increase in intracellular Ca^{2+} concentration stretch may stimulate the calcium/calmodulin dependent phosphatase calcineurin, a novel hypertrophic signaling pathway [217].

SERCA2a regulation by MAPKs

The nonreceptor protein tyrosine kinase (PTK) proline rich tyrosine kinase 2 (PyK2) has been implicated in cell signaling pathways involved in left ventricular hypertrophy and heart failure. PyK2 overexpression significantly decreased SERCA2 mRNA; while PTK inhibitor PP2 (which blocks PyK2 phosphorylation by

src family PTKs) significantly increased SERCA2 mRNA levels. PyK2 overexpression was found to be ineffective on ERK1/2, but markedly increased JNK1/2 and p38MAPK phosphorylation. Activation of both ''stress activated'' protein kinase cascades appeared necessary to reduce SERCA2 mRNA levels [218].

NHE-1 regulation by ERK during I-R injury

Generation of reactive oxygen species (ROS) and intracellular Ca^{2+} overload are involved during ischemia/reperfusion (I/R)-induced myocardial injury. Oxidants have been shown to cause an increase in plasma membrane NHE-1 activity in an ERK dependent manner. Oxidants have been shown to increase diastolic intracellular Ca^{2+} concentration that was blocked by inhibition of ERK-1/2 activation with U-0216 or inhibition of NHE-1 with HOE-642. Increased NHE-1 activity was associated with phosphorylation of the NHE-1 carboxyl tail that was blocked by U-0216, which indicated that oxidants induced Ca^{2+} overload is partially mediated by NHE-1 activation secondary to phosphorylation of NHE-1 by the ERK1/2 MAP kinase pathway [219].

Regulation of NCX by mitogen activated protein kinase

An increase in the reverse mode of plasma membrane Na^+/Ca^{2+} exchanger (NCX1) is regulated at the transcriptional level in cardiac hypertrophy, ischemia, and heart failure. Following pressure overload, activation of MAPKs coincides with the kinetics of NCX1 gene upregulation in adult cardiomyocytes. Inhibition of ERK with the MEK inhibitor U0126, the ERK protein phosphatase MKP-3, inhibited ERK activation, but only inhibited α-adrenergic induced NCX1 upregulation by 30%. Overexpression of JNK lowered basal NCX1 expression. Overexpression of activated MKK3 was sufficient for α-adrenergic stimulated upregulation of the reporter gene. These studies suggested that (i) JNK mediates basal cardiac expression of the NCX1 gene; (ii) ERK and p38MAPK play a role in α-adrenergic stimulated NCX1 upregulation, and (iii) p38MAPK activation alone is sufficient for NCX1 upregulation [220].

Na^+/K^+ ATPase and mitogen activated protein kinases

In neonatal rat cardiac myocytes partial inhibition of Na^+/K^+ ATPase causes hypertrophic growth and transcriptional regulations of genes that are markers of cardiac hypertrophy. Inhibition of Na^+/K^+ ATPase regulates the protein tyrosine phosphorylation and p42/44 MAPKs, and increases both systolic and diastolic Ca^{2+}. Pretreatment of myocytes with several Src kinase inhibitors, or overexpression of a dominant negative Ras, antagonized ouabain induced activation of MAPKs and increase $(Ca^{2+})_i$. Treatment with PD-98059 (a MAPK kinase inhibitor) or overexpression of a dominant negative MAPK kinase I also ablated the effect of ouabain, a Na^+/K^+ ATPase inhibitor on MAPKs and $(Ca^{2+})_i$. Thus, Ras/MAPK cascade regulates $(Ca^{2+})_i$ in rat cardiac myocytes by modulating Na^+/K^+ ATPase activity [221, 222].

Cross-talk between CNP and ET-1 in cardiac hypertrophy

C-type natriuretic peptide (CNP) is known to play a role in the local regulation of vascular tone. Cardiac ventricular cells produce CNP. However, its local effect on myocyte hypertrophy remains to be elucidated. CNP has been shown to attenuate basal and ET-1 augmented protein synthesis, atrial natriuretic peptide secretion, hypertrophy related gene expression, calcium-calmodulin dependent kinase II activity, and ERK phosphorylation [223]. CNP also inhibited ET-1 induced increase in intracellular Ca^{2+} concentration. These effects of CNP were mimicked by a cGMP analog, 8-bromo cGMP. However, the inhibitory effects of CNP on the hypertrophic response of myocytes were significantly diminished at high concentration of ET-1. Although CNP increased cGMP levels in myocytes, ET-1 suppressed CNP-induced cellular cGMP accumulation. Protein kinase C activator and Ca^{2+} ionophore mimicked suppressive effect of ET-1. CNP and 8-bromo cGMP significantly inhibited ET-1 secretion [223]. Combined treatment with CNP and 8-br-cGMP significantly attenuated ET-1 induced protein synthesis in cardiac myocytes. Thus, CNP inhibits ET-1 induced cardiac myocyte hypertrophy via a cGMP-dependent mechanism; whereas, ET-1 inhibits CNP signaling by a protein kinase C and Ca^{2+} dependent mechanism, suggesting mutual interference between CNP and ET-1 signaling pathways [223].

Role of angiotensin II

The physiology of angiotensinII (AngII) continues to be a major field of investigation. In rat cardiac myocytes, Ang II produces a negative inotropic effect

(NIE). NIE of Ang II was not associated with a parallel decrease in the intracellular Ca^{2+} transients, indicating that a decrease in myofilament responsiveness to Ca^{2+} underlies the reduction in contractility. Ang II induced NIE was abrogated by the inhibitor of PKC (calphostin C), tyrosine kinase (genistein), and p38MAPK (SB202190). The NIE was significantly exacerbated by p38MAPK overexpression [203].

Activation of PKC in vitro leads to phosphorylation of several myofibrillar proteins such as troponin I (TnI), Troponin T and actomyosin ATPase, any of which could potentially lead to a reduction in myofilament responsiveness to Ca^{2+} [224]. The intermediate signaling events caused by Ang II was shown to be mediated by p38MAPK. However, phosphorylation, for example, of TnI did not occur by p38MAPK, indicating that TnI is not a direct downstream target of p38MAPK [225]. Previous studies indicated that heat shock protein HSP27 is activated by p38MAPK, causing its translocation to the Z-line of the sarcomere. p38MAPK-mediated activation of HSP27 leads to a decrease in actomyosin ATPase activity and contractile depression, possibly through modifications in sarcomere scaffolding proteins,for example, α-actin [226]. Another possibility could arise from the proapoptotic activity of p38MAPK. Caspase-3 has been shown to directly target the contractile proteins and decrease myofibrillar responsiveness to Ca^{2+} [227]. Identifying the molecular mechanisms that are responsible for the AngII/p38MAPK-mediated decrease in myofilament responsiveness needs further investigation.

Angiotensin type I (AT1) receptor activation such as receptor transactivation of tyrosine kinase receptors and stimulation of reactive oxygen species (ROS) production suggest that AngII has growth factor and cytokine-like properties in addition to its vasoconstrictor actions. AngII induced activation of NF-κB has been causally implicated in the inflammatory vasculopathy in rats. Treatment with an AT1 receptor antagonist or an antioxidant inhibited these effects [228]. In rat vascular smooth muscle cells, AngII treatment stimulated degradation of cytosolic IκB binding protein, which was paralleled by translocation of the activated heterotrimeric protein form of NF-κB, p50/p65 to the nucleus. These effects are attenuated by AT1 receptor and phosphotyrosine kinase inhibition. Furthermore, AT1 receptor inhibition abrogated NF-κB induced gene transcription [228].

Many of the actions of angiotensin type II (AT2) receptor activation are diametrically opposite to those of the AT1 receptor. For example, AT1 receptor-induced rat cardiomyocyte hypertrophy is augmented by AT2 receptor inhibition, suggesting that this receptor may exert a tonic inhibitory effect on cardiac hypertrophy.

MAPKs play important roles in the development of cardiac hypertrophy. It has been reported that AngII tightly regulates the activity of MAPKs in cardiac myocytes. Hiroi et al. [229] demonstrated that AngII increased MAPK phosphatase 1 (MKP-1) gene expression. ATI specific antagonists, CV11974, completely suppressed the AngII induced increase in MKP-1 gene expression, while AT2 specific antagonist, PD-123319, had no significant effects. Induction of MKP-1 gene expression by AngII was inhibited by pretreatment with an intracellular Ca^{2+} chelator, BAPTA-AM, or with the PKC inhibitor, H-7 and calphostin C. PMA and Ca^{2+} ionophore both significantly increased MKP-1 mRNA levels and showed synergistic action. Overexpression of MKP-1 cDNA blocked the AngII-induced increase in expression of immediate early response genes. In addition, AngII induced MAPK activation was significantly inhibited by pretreatment with CV-11974, but significantly enhanced by pretreatment with PD-123319. Addition of the AT2 agonist, CGP42112A reduced basal MAPK activity and pretreatment with PD-123319 abolished MAPK inactivation that AngII negatively regulates MAPKs through ATI receptors by increasing MKP-1 mRNA levels and through AT2 receptors by unknown mechanisms [229].

Thus, cross talk between signaling mechanisms of the angiotensin receptors, therefore, appear to modulate the physiological effects of AngII [228].

Ca^{2+} sensing receptor (CaR)

Both intra- and extra- cellular Ca^{2+} play multiple roles in the physiology and pathophysiology of cardiomyocytes, especially in stimulus-contraction coupling. The intracellular Ca^{2+} level is closely controlled through the concerted actions of calcium channels, exchangers and pumps; however, the expression and function(s) of the so-called Ca^{2+} sensing receptor (CaR) in the heart remain less well characterized. The CaR is a seven transmembrane receptor, which, in response to non-covalent binding of extracellular Ca^{2+}, activates intracellular effectors, including G proteins and extracellular signal regulated kinases (ERK1/2). CaR is functionally expressed in cardiac myocytes and plays a role in regulating cardiac development, function and homeostasis [230].

Antiapoptotic effect of extracellular Ca^{2+} was also observed in c-myc overexpressing serum deprived cells where all the cells were shown to express CaR [231].

PI3 K and p38MAPK were suggested to the downstream pathways that mediate the mitogenic response to extracellular Ca^{2+} activated CaR [215, 232]. However, the downstream mechanism by which CaR prevents apoptosis has not yet been clearly elucidated.

β-AR agonists and ER in apoptosis

A long-term and increased plasma catecholamine level is a prognostic indicator of a worse cardiovascular outcome. Catecholamines activate β-adrenergic receptor (β-AR) to increase the force of contraction and heart rate [233]. In mice, an increase in β-adrenergic receptor activation and expression and caspase12 activation cause endoplasmic reticulum driven apoptosis. Although chronic in vivo infusion of β-AR agonists partially activates the apoptosis program, but for its full activation requires the caspase activation too [234].

Apoptosis in heart, in common with other tissues is the result of changes in the ratio of antiapoptotic and proapoptotic proteins, activation of initiator caspases and, eventually executioner caspases [234]. Despite the association of high plasma catecholamine levels with a worse outcome and catecholamine induced apoptosis in vitro [235], few studies have examined the effect of chronic in vivo infusion of catecholamines on cardiomyocyte apoptosis. Chronic norepinephrine infusion into ferrets for 4 weeks led to decreased Bcl-2 and increased Bax expression. The changes in the Bcl-2/Bax ratio along with activation of the initiator or executioner caspase cascade were suggestive of the full activation of apoptosis [234, 236].

Alterations in Ca^{2+} homeostasis and oxidative stress have been shown to cause unfolded proteins to accumulate in the ER and caused initiation of the unfolded protein response and subsequently produced activation of caspase 12 [237]. Altered Ca^{2+} handling and increased oxidative stress might cause catecholamine stimulation. The molecular chaperones that are increased in ER stress may contribute to the refolding of the damaged proteins, thus preserving the function of the potentially critical proteins. Clusterine is a molecular chaperone induced when ER is stressed [238]. Clusterin overexpression in hearts exposed to chronic catecholamine infusion suggests that sufficient damage had occurred to activate the unfolded protein response. Clusterin expression may be a more sensitive marker of ER stress in heart than GRP78, but full activation of clusterin in the ER apoptosis pathway was not observed. GRP78 is an ER chaperone whose expression is induced and translocated to the cytosol in response to ER stress, where it function to block caspase activity [239]. However, GRP78 expression was not increased in catecholamine infused hearts, indicating that the chronic catecholamine infusion is an insufficient stimulus for full induction of ER stress. It, therefore, appears conceivable that catecholamines alone set in motion the initial steps of apoptosis, but that other insults are required to continue apoptosis activation. The pathway to heart failure from hypertrophy involves the factors that could push these catecholamine-challenged hearts into failure that might include changes in the rennin-angiotensin-aldosterone system or concomitant inflammation due to cytokine activation [240].

SERCA2 and apoptosis

When ER stress persists, apoptosis is induced. This allows elimination of the damaged cells without causing inflammation or tissue damage [241]. The contribution of ER induced apoptosis might differ among different cells. This may be related to the fact that some cells have more active capacitative Ca^{2+} entry pathways than others. Also, differences in the cytotoxic apoptotic stimuli used in these studies (unnatural ones like Ca^{2+} ionophores, thapsigargin, or the natural ones like glucocorticoids, ceramide etc) may also have contributed to the controversy. According to an early view, it is the decrease in lumenal Ca^{2+} in the ER that is more directly linked to apoptosis [242]. Depletion by luminal ER Ca^{2+} by harsh interventions like the application of ionophores or thapsigargin is likely to cause a deadly blow to the cell. The more generally accepted view on apoptosis, however, holds that it is the rise in $(Ca^{2+})_i$ that causes cell death. Procaspase-12 bound to the cytosolic side of the ER appeared to be cleaved and activated after Ca^{2+} release from the ER and Ca^{2+} dependent translocation of cytosolic caspase-7 to the ER surface. Caspase-12 was found to be involved in ER-stress induced apoptosis but not in plasma membrane or mitochondrial induced apoptosis. The latter forms make use of other caspases [243]. The expression of SERCA1 truncated proteins was unable to pump Ca^{2+}, but instead apparently causing a Ca^{2+} leak through the ER membranes of several nonmuscle human tissues [244]. Apoptosis caused by overexpression of these SERCA1 mutants was confirmed from results of the antiapoptotic effect of Bcl-2 [241, 242].

Hypoxia inducing factor (HIF) and heart diseases

Ischemia preconditioning elicits myocardial protection. A critical component of myocardial ischemia is

hypoxia. Role of hypoxia on $(Ca^{2+})_i$ dysregulation in the cardiovascular system is now fairly well established [57, 58].

Acute hypoxia causes a marked increase in pulmonary arterial pressure and is attributable to hypoxic pulmonary vasoconstriction (HPV). The primary mechanisms of HPV are contained entirely within pulmonary vascular tissue. The main locus of response is small distal pulmonary arteries. The smooth muscle effector pathway depends on an increase in $(Ca^{2+})_i$ caused by influx of Ca^{2+} from extracellular fluid. VDCCs provide a major influx pathway; however, release of Ca^{2+} from sarcoplasmic reticulum (SR) seems to be essential, and influx also occurs through other pathways, such as channels dependent on internal Ca^{2+} stores [245, 246]. One hypothesis suggests that hypoxia first causes SR Ca^{2+} release, which in turn leads to SOC influx, altered activity of sarcolemmal ion channels, membrane depolarization, and Ca^{2+} influx through VDCC. The resulting increase in $(Ca^{2+})_i$ causes calmodulin-mediated activation of myosin light chain kinase, actin-myosin interaction, and contraction [245].

Hypoxia activates a number of genes, for example, vascular endothelial growth factors (VEGF) that are important in cellular and tissue adaptation to low oxygen conditions. The hypoxic expression of these different genes is controlled at the transcriptional level by the ubiquitously expressed transcription factor, hypoxia inducing factor-1 (HIF-1). Under hypoxic conditions, the hydroxylation of specific proline and asparagine residues in HIF-1α is inhibited due to substrate (O_2) limitation, resulting in HIF-1α protein stabilization and transcriptional activation. HIF-1 mediates transcriptional activation of several mediators and other cardioprotective genes including iNOS, HSP70 and VEGF, which are also known as hypoxia responsive or HIF-1 target genes [247, 248].

Ischemic preconditioning has been shown to up regulate VEGF expression and neovascularization, which in return reduces infarct size on subsequent lethal ischemia. It is also known that transcription of iNOS, an important mediator/effector of myocardial preconditioning is directly activated by HIF-1 in response to hypoxia. Thus, HIF-1 is involved in the development of late phase preconditioning in cardiomyocytes by inducing multiple protective genes [247, 248].

HIF induces VEGF expression in pulmonary vascular endothelial cells. VEGF has been shown to play a crucial role in the life and death of pulmonary vascular endothelial cells. Treatment of neonatal or adult rats with a VEGF receptor blocker destroys lung capillaries by inducing endothelial cell apoptosis and causes emphysema. Human lung tissue samples from patients with end-stage emphysema have decreased levels of VEGF messenger RNA and protein and impairs endothelial cell survival in emphysematous lungs. Combination of VEGF receptor blockade with chronic hypoxia (3-week exposure) results in obliteration of small precapillary pulmonary arteries by proliferating endothelial cells, severe pulmonary hypertension, and death caused by right ventricular hypertrophy and failure. Thus, HIF-1α is a useful marker of a jeopardized myocardium [249].

Phosphatidylinositol 3 kinase (PI3K)-dependent Akt phosphorylation was found to be activated by mechanical stress and completely blocked by wortmannin (a PI3 K inhibitor). Moreover, the stress-mediated induction of HIF-α and VEGF was suppressed by gadolinium (a stretch activated channel inhibitor) and wortmannin. HIF-1α also plays an important role in the induction of VEGF in nonischemic and mechanically stressed myocardium, which is regulated by stretch-activated channels and the PI3 K/Akt pathway. Moreover, this signaling pathway, which induces HIF-α seems to play an important role in the adaptation of the myocardium to stresses [250].

Myocardial stunning and heat shock proteins

Reperfusion of ischemic myocardium during the early phase of myocardial infarction has become the treatment of choice in the management of acute myocardial infarction. Myocardial stunning is defined as mechanical dysfunction that persists after reperfusion of previously ischemic tissue in the absence of irreversible damage including myocardial necrosis. Unstable angina or acute myocardial infarction with early reperfusion, cardiac operation with cardioplegic arrest, and cardiac transplantation all subject the myocardium to transient ischemia, and therefore, may be associated with myocardial stunning. The physiological implication of myocardial stunning is an increase in $(Ca^{2+})_i$ [244]. The underlying mechanism for depressed SR(ER) Ca^{2+}-ATPase and plasma membrane Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger, which regulates $(Ca^{2+})_i$ could be due to their misfolding caused by oxidants generated under ischemia-reperfusion injury to the myocardium [251, 252].

Misfolded proteins do not remain soluble and are become denatured proteins. Induction of stress or heat shock proteins (HSPs) protects the cells against harmful consequences of ''protein denaturation'' [253, 254]. According to the mol wt., the major HSPs are classified. These are: small HSPs (26–28 kD), HSP60

family (which are located in the mitochondria), HSP70, HSP90, and HSP100 gene families. HSP70 was identified in neonatal and adult heart tissue for several species, including dog, rat, and rabbit [255]. The HSP70 serves an important role by associating with nascently formed proteins that have not reached their permanent folding state and preventing their denaturation [256]. In addition, they serve as unfoldases by associating with proteins and pairing them into a translocation competent configuration [257]. These effects of HSP70 have been termed their chaperone function [257]. Marber et al. [258] have shown that HSP70 transgene in the mouse heart protected against ischemia/reperfusion injury. Qian et al. [259] and Kukreja et al. [260] have shown that quercetin, the inhibitor of heat shock transcription factor, blocked ischemic tolerance, and synthesis of HSP70 in heat stressed rat hearts, which further lend evidence in favour of the role of HSPs in myocardial protection. Thus, it appears that HSP may have a cause-and-effect relationship with myocardial protection, which may be exploited to develop therapy for attenuation of stunning or reducing I-R related damage in the myocardium.

Cross-talk between endothelin and catecholamines

During the course of cardiovascular disorders, such as congestive heart failure and ischemic heart disease, plasma levels of both endothelin-1 (ET-1) and norepinephrine (NE) tend to increase [261]. The signal transduction processes that are triggered by the activation of receptors for these endogenous agonists are different, and thus it seems likely that cross-talk between ET-1 and NE might play a critical role in the regulation of cardiac function determining hemodynamic responses to antagonists of β-adrenoceptor or endothelin receptor under various pathophysiological conditions. The available evidences suggest that these endogenous regulators are engaged in cross-talk at different levels of their respective signaling pathways. For example, the positive feedback mechanism seems to exist at the level of the synthesis of NE by which ET-1 increases the plasma concentration of NE, whereas NE facilitates the expression of mRNA that encodes the prepro-ET-1 and the production of ET-1 [262].

ET-1 has a positive inotropic effect (PIE) in ventricular myocardium of most mammals [262, 263]. In contrast, ET-1 has a negative inotropic effect (NIE) in the presence of catecholamines and antagonizes β-adrenoceptor mediated regulation of contractile function in several mammalian species [264]. Acceleration of the hydrolysis of phosphoinositide and the subsequent generation of inositol 1,4,5-trisphosphate and 1,2-diacylglycerol has been suggested to be responsible for the PIE of ET-1 [263], indicating that the PIE of ET-1 is associated definitively with an increase in the myofilament sensitivity to Ca^{2+} [265].

In contrast to the PIE, the NIE of ET-1 was accompanied by a pronounced decrease in Ca^{2+} transients. In dog ventricular myocytes, ET-1 inhibited significantly the increase in L-type Ca^{2+} current (I_{CaL}) induced by isoproterenol [266]. Because the inhibitory action of ET-1 on the isoproterenol induced increase in I_{CaL} was suppressed by the treatment with pertussis toxin (PTX) in rabbit myocytes, the inhibition of the cAMP-mediated increase in the I_{Ca} via the PTX sensitive inhibitory pathway activated by ET-1 might contribute, to some extent, to the ET-1 induced decrease in Ca^{2+} transients. However, involvement of effects on other processes, such as PKA on SR Ca^{2+} release cannot be excluded [261].

The cross talk between ET-1 and NE might play a crucial role in the regulation of myocardial contractility under pathophysiological conditions that are associated with elevated plasma levels of the endogenous regulators [261].

Protective role of Mg^{2+}

A discernible increase in extracellular Mg^{2+} concentration [$(Mg^{2+})_o$] reduces Ca^{2+} accumulation during reoxygenation of hypoxic cardiomyocytes and exerts protective effects [267]. Intracellular free [$(Mg^{2+})_i$] has been shown to inhibit L-type Ca^{2+} currents through $Ca_V1.2$ channels in cardiac myocytes. [$(Mg^{2+})_i$] acts through the COOH-terminal E-F hand of $Ca_V1.2$, which is important in regulating Ca^{2+} influx in physiological and pathophysiological states [268].

Oral Mg^{2+} supplementation lowers systolic as well as diastolic blood pressure and thus prevents the development of hypertension. The influence of Mg^{2+} in the regulation of blood pressure seems complex. Alterations in Mg^{2+} metabolism have been demonstrated in genetic and experimentally induced hypertension as well as patient with essential and malignant hypertension [269, 270]. Mg^{2+} supplementation produces a small but significant decrease in platelet aggregation with concomitant inhibition in the synthesis of thromboxanes. Intracellular Ca^{2+} concentration plays a critical role in platelet activation and aggregation. Antiaggregatory effect of Mg^{2+} may also be related to its influence on cytosolic Ca^{2+}, which were decreased by Mg^{2+} supplementation [269, 270].

Myocardial Mg^{2+} and Ca^{2+} interactions have already been reported [271]. The opposing effects of Mg^{2+} and Ca^{2+} on myocardial contractility could be due to the competition between Mg^{2+} and Ca^{2+} for the same binding site(s) on key myocardial contractile proteins such as troponin C, myosin and actin [271]. In myocyte from patients with heart failure, resting $(Ca^{2+})_i$ level was found to be increased compared with normal subjects. Mg^{2+} reduces the intracellular Ca^{2+} availability through several mechanisms. These are (i) Mg^{2+} inhibits the sarco(endo)plasmic Na^+/Ca^{2+} exchange; (ii) Mg^{2+} displaces Ca^{2+} from sarco(endo)plasmic stores; and (iii) Mg^{2+} influences Ca^{2+} pumps on sarco(endo)plasmic reticular surface [272].

An increase in extracellular Ca^{2+} has been shown to cause extrusion of $(Mg^{2+})_i$ in response to α-adrenergic receptor activation, for example, by the α1-adrenergic activator, phenylephrine (PE). Pretreatment with Ca^{2+} channel blockers such as nifedepine and verapamil prevented the Mg^{2+} response caused by PE. This indicates that Ca^{2+}/Mg^{2+} exchanger may play a role in this scenario. Pretreatment with PD98059 (a MAP kinase inhibitor) has been shown to prevent the response caused by PE. An increase in extracellular Ca^{2+} or addition of PE was found to cause activation of ERK and that is inhibited by PD98059 in cardiac myocytes. Thus, stimulating the cardiac α-1 adrenergic receptors by PE causes the extrusion of Mg^{2+} via the Ca^{2+} activated Na^+ dependent transport pathway, and the ERKs assist in Mg^{2+} transport in the heart [273].

It has been established that RyR Ca^{2+} channels, which play a key role for Ca^{2+} induced Ca^{2+} release (CICR) are activated by Ca^{2+}, positively modulated by ATP, and inhibited by Mg^{2+} [274]. There is compelling evidence from studies on both multicellular and isolated myocyte preparations to suggest that Ca^{2+} entry via L-type Ca^{2+} (I_{CaL}) channel is an important trigger for CICR. Thus, Mg^{2+} modulates I_{CaL} and also influences both Ca^{2+} uptake and Ca^{2+} release in the SR. The ATP bound form of Mg^{2+} (Mg^{2+}-ATP) is essential for force development by the myofilaments and the level of free Mg^{2+} also influences the Ca^{2+} sensitivity of the myofilaments [274–276].

Mg^{2+} plays a key role in several important cellular functions related to Ca^{2+} channels in the heart. cAMP-mediated phosphorylation reduces the sensitivity to Mg^{2+} of regulatory gates present in the Ca^{2+} channel leading to dissociation of Mg^{2+} from these blocking sites, and consequently to an increase in current through the Ca^{2+} channels [277] (Fig. 8).

GTP suppresses Ca^{2+} channel activities by reducing the level of Mg^{2+}. The suppression is not mediated by G protein activation but by direct binding of GTP to Ca^{2+} channels. The inhibitory action of both GTP and Mg^{2+} are exerted directly, but independently, on the channel [277]. Regulatory gates having binding sites for Mg^{2+} and GTP control Ca^{2+} channels. Most Ca^{2+} channels bind Mg^{2+} and/or GTP and remain in a nonavailable state under basal conditions. When Ca^{2+} channels are phosphorylated, their sensitivity to Mg^{2+} and GTP decreases allosterically. Subsequently, Mg^{2+} and GTP are related at their binding sites and the channel becomes available. Depletion of intracellular Mg^{2+} relieves the Mg^{2+} block, because blocking device, which is assumed to reside in the C-terminal region of α-subunit of L-type Ca^{2+} channels can no longer block the channel when free of Mg^{2+}. However, in the presence of GTP, depletion of Mg^{2+} does not make the

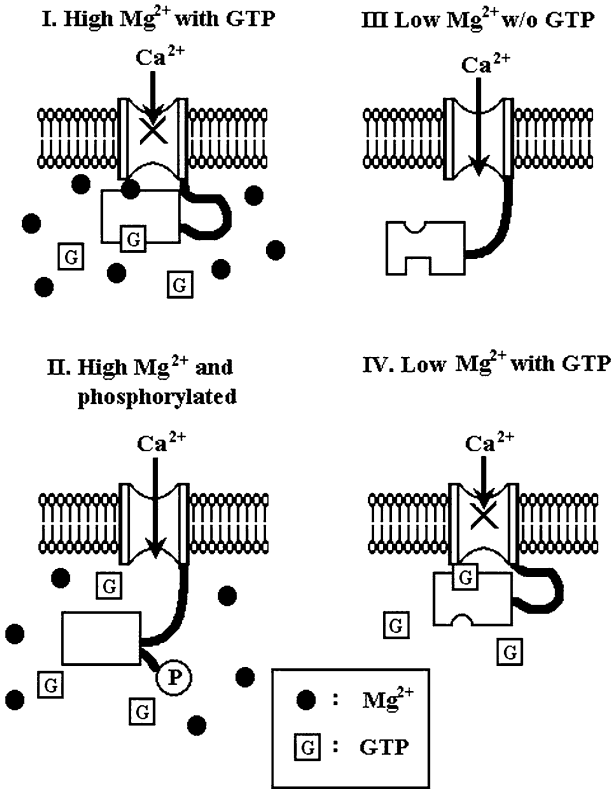

Fig. 8 A schematic representation of Mg^{2+} and GTP-mediated block of L-type Ca^{2+} channels under phosphorylated and dephosphorylated conditions. Mg^{2+} and GTP binding sites are assumed to reside in the intracellular C-terminal side of the α_1 subunit of the channel. Basal conditions (i.e. the channel dephosphorylated and Mg^{2+} and GTP abundant on the intracellular side) are depicted in I, where Mg^{2+} and GTP binding to C-terminal inhibit the current conduction. A decrease in $(Mg^{2+})_i$ without intracellular GTP produces a current conducting state (III) but addition of GTP blocks the channel (IV). Phosphorylation results both Mg^{2+} and GTP blocks by unbinding these blocking substances through conformational change of the channel protein (II). (Taken from reference no 277 of the text with permission)

channel available because GTP also can block the channel in a manner similar to Mg^{2+} [277]. The possible physiological relevance of such substantial inhibition by Mg^{2+} and GTP is that Ca^{2+} channels can be controlled over a wide dynamic range. Because Ca^{2+} channels are inherently able to allow the passage of ten times more Ca^{2+} than is physiologically necessary under normal conditions, Ca^{2+} channels in which the gates are ''partially closed'' by both Mg^{2+} and GTP are able to limit the amount of Ca^{2+} flowing through the channel, which otherwise may harm the cells [278] (Fig. 8).

Heart failure

SERCA2a is responsible for the removal of about 70% of the contraction activating Ca^{2+} in human ventricular muscle (as well as rabbit, dog or cat; over 90% in rat or mouse), with most of the balance removed by the Na^{+}/Ca^{2+} exchanger. The speed and extent of diastolic relaxation (lusitropy), as well as the systolic contractile force (inotropy) are crucially affected by SERCA2a performance. Heart failure is a serious and prevalent clinical condition with high morbidity and mortality [24]. Most studies measuring expression of SERCA2a in failing myocardium from animal models or in strips of human hearts obtained at the time of transplantation have shown a decrease in amounts of SERCA2a mRNA and/or protein, accompanied by deficits in SERCA2a activity [69]. Ca^{2+} dynamics in isolated cardiomyocytes from human myopathic hearts showed prolonged cytosolic Ca^{2+} transients, elevated diastolic Ca^{2+} levels and decreased systolic Ca^{2+} concentrations. These abnormalities were hypothesized to reflect largely a diminished SERCA2a functionally.

Genetic causes of human heart failure

Human mutations in the genes encoding protein components of the sarcomere cause either hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM). While progression to heart failure occurs with both patterns of remodeling, the histopathology, hemodynamic profiles, and biophysical consequences of HCM or DCM mutations suggest that distinct molecular processes are involved [279].

Sarcomere gene mutations that cause HCM produce a shared histopathology with enlarged myocytes that are disorganized and die prematurely, which results in increased cardiac fibrosis. The severity and pattern of ventricular hypertrophy, age at onset of clinical manifestations, and progression to heart failure is in part dependent on the precise sarcomere protein gene mutation. For example, TNNT2 mutations are generally associated with a high incidence of sudden death despite only mild left ventricular hypertrophy [279].

DCM sarcomere protein gene mutations affect distinct amino acids from HCM-causing mutations, although the proximity of altered residues is remarkable. The histopathology of sarcomere DCM mutations is quite different from those causing HCM, and is remarkably nonspecific. There are two mechanisms by which sarcomere mutations may cause DCM and heart failure: deficits of force production and deficits of force transmission. Diminished force may occur in myosin mutations (e.g., MYH7Ser532Pro) that alter actin-binding residues involved in initiating the power stroke of contraction. Impaired contractile force may also occur in DCM troponin mutations, for example, TNN13AlaVal that alter residues implicated in tight binary troponin interactions. Because troponin molecules modulate calcium-stimulated actomyosin ATPase activity, these defects may cause inefficient ATP hydrolysis and decreases contractile power [279].

To date, more than 30 mutations have been found in the analogous RyR2 regions in patients with arrhythmogenic right ventricular cardiomyopathy type-2 (ARVD/C2) or catecholaminergic polymorphic ventricular tachycardia (CPVT) [280]**.** This suggests that those regions represent domains that are critical for the regulation of both RyR1 and RyR2 and that these domains are also involved in the pathogenesis of RyR-linked skeletal and cardiac muscle diseases [280].

Marks et al. [281] demonstrated the pathogenic role of RyR2 mutations by evaluating channel activity in recombinant RyR2 containing the same single-point mutation as that seen in CPVT patients**.** Wehrens et al. [282] found that FKBP12.6 deficient mice and CPVT-associated RyR2 mutants exhibited a significantly increased open probability of the channel only during exercise or in the PKA phosphorylated state, that these RyR2 mutants displayed a reduced affinity of FKBP12.6 for RyR2, and that a constitutively active recombinant FKBP12.6 mutant (FKBP12.6-D373), a mutant form of FKBP12.6 was serine residue 37 substituted for aspartic acid that can bind to PKA phosphorylated RyR2 reversed the hyper activity of channel gating seen in PKA phosphorylated mutated RyR2 [282]. These findings are comparable within the clinical findings that CPVT patients do not exhibit arrhythmia at rest but may suffer lethal arrhythmia during exercise [281]. However, Jiang et al. [283] reported that the mutant RyR2 linked to CPVT and sudden death increased the sensitivity of single RyR2

channels to activation by luminal Ca^{2+} and enhanced the basal level of [^{3}H] ryanodine binding, even without PKA phosphorylation. The discrepancies between these reports might be explained by FKBP12.6 being absent from the RyR2 mutant studied by Jiang et al. [283] but present in the RyR2 mutant studied by Wehrens et al. [282]. It remains to be determined whether the resting channel gating property of the FKBP12.6 depleted, mutant RyR2 linked to CPVT and sudden cardiac death can be altered even without PKA phosphorylation and activity in the FKBP12.6-depleted mutant RyR2 [279].

Rare human PLN mutations cause familial DCM and heart failure. The pathogenic mechanism of one mutation (PLN Arg9Cys) was elucidated through biochemical studies, which indicated unusual PKA interactions that inhibited phosphorylation of mutant and wild type PLN. The functional consequences of the mutation were predicted to be constitutive inhibition of SERCA2a, a result confirmed in transgenic mice expressing mutant, but not wild type, PLN protein. In mutant transgenic mice, Ca^{2+} transients markedly prolong, myocyte relaxation was delayed, and these abnormalities were unresponsive to β-adrenergic stimulation. Profound biventricular cardiac dilation and heart failure developed in mutant mice, proves clear evidence of both detrimental effects of protracted SERCA2A inhibition due to altered PLN activity [279].

New treaments for heart diseases

In the cardiovascular system, two types of voltage gated Ca^{2+} channels are present: the L-type and the T-type. Under normal conditions, T-type Ca^{2+} channels are involved in the maintenance of vascular tone and cardiac automaticity but, since they are not present in contractile myocardial cells, they do not contribute significantly to myocardial contraction. In experimental models of cardiac hypertrophy, myocardial T-type Ca^{2+} channels are upregulated, which could contribute to the increased incidence of ventricular arrhythmia. In addition, T-type Ca^{2+} channels might participate in myocardial remodeling. Mybefradil, a Ca^{2+} antagonist that is 10–50 times more potent at blocking T-type than L-type Ca^{2+} channels, has been found to be effective in ameliorating the pathological role of T-type Ca^{2+} channels in heart failure [284].

The gating of large conductance Ca^{2+} activated K^+ [BK(Ca)] channel is primarily controlled by intracellular Ca^{2+} and/or membrane depolarization. These channels play a role in the coupling of excitation–contraction and stimulus secretion. A variety of structurally distinct compounds may influence the activity of these channels. Squamocin, an annonaceous acetogenin, could interact with the BK(Ca) channel to increase the amplitude of Ca^{2+} activated K^+ current in coronary smooth muscle cells. Its stimulatory effect is related to intracellular Ca^{2+} concentration. ICI-182.780, an estrogen rceptor antagonist, was found to modulate BK(Ca)-channel activity in cultured endothelial cells and smooth muscle cells in a mechanism unlinked to the inhibition of estrogen receptors [285].

Oral dipyridamole induces accumulation of endogenous adenosine, which in a hypoxic milieu exerts experimentally an angiogenic effect on coronary collateral circulation. Dipyridamole showed benefit in the treament of angina pectoris, especially with longer duration treatment [286].

In the recent past, a number of newer anti-anginal agents, including nicorandil, trimelazidine, and ivabradine have been synthesized, but ronalazine, a piperazine derivative that partially inhibits fatty acid oxidation and the late I_{Na} current in animal models gained prime interest as an anti-anginal drug [287].

The RyRs has been shown to be hyperphosphorylated by PKA in both human and experimental HF [118, 124, 127, 288]. Many large clinical trials have shown that treatment with a β blocker restores cardiac function and reduces the rate of mortality in patients with HF. β-blockers reversed PKA mediated hyperphosphorylation of RyR2, restored the stoichiometry of the RyR2 macromolecular complex, restored normal single channel function and inhibited the Ca^{2+} leak [127]. These findings provide a molecular basis for the common clinical observation that the use of β receptor blockers improves the prognosis of patients with HF. In a canine model of HF, the angiotensin II receptor blocker valsartan, which has been used in the treatment of HF in the clinical setting, also normalize the Ca^{2+} regulatory processes through a β blocker-like action [288]. By acting on the presynaptic angiotensin II receptor, valsartan inhibited norepinephrine release and stimulated norepinephrine uptake back into the synaptic pool, with the result that adrenergic signals were not overtransmitted into the cell. This would lead to a reduction in the PKA hyperphosphorylation of RyR2 and to an inhibition of the Ca^{2+} leak in the failing heart [288].

Since a conformational change in RyR2 precedes the Ca^{2+} leak [127], an amelioration of this conformational change could be a new therapeutic strategy against HF (Fig. 9). Using a canine model of HF, it was found that chronic administration of a new compound, the 1,4 benzothiazepine derivative JTV519 improved

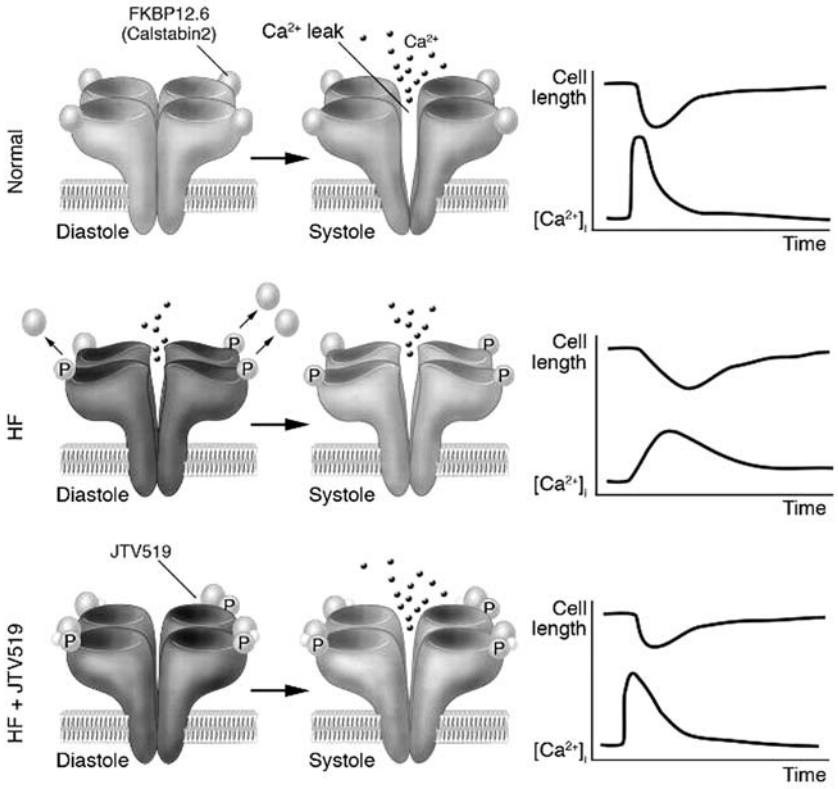

Fig. 9 Therapeutic strategy involving FKBP12.6-mediated stabilization of RyR. A small influx of Ca^{2+} through the LTCC leads to the release of a large amount of Ca^{2+} from the SR through RyR in the normal heart. In HF, however, PKA-mediated hyperphosphorylation of RyR2 occurs, and this in turn dissociates FKBP12.6 from RyR2, leading to a diastolic Ca^{2+} leak through RyR2. This results in the Ca^{2+} transient being diminished (due to the reduced SR Ca^{2+} content and dyssynchronous Ca^{2+} release). Administration of a new compound, the 1,4-benzothiazepine derivative JTV519, normalizes this abnormal channel gating by restoring the conformational state of RyR and by rebinding FKBP12.6 to the channel complex. Thereby, JTV519 normalizes Ca^{2+} cycling and contractile function in failing cardiac myocytes and hence provides chronic suppression of progressive left ventricular dysfunction in HF. P, PKA phosphorylation at serine 2809; $[Ca^{2+}]_i$, intracellular Ca^{2+}. (Taken from reference no 80 of the text with permission)

contractility and prevented the development of LV remodeling and HF, presumably by stabilization of RyR2 [76]. In JTV519-untreated hearts, RyR2 was PKA-hyperphosphorylated with a dissociation of FKBP12.6 whereas the reverse of these states was true of JTV519-treated hearts toward the levels seen in the normal heart [76, 118, 289].

The NO donor, S-nitroso-N-acetyl-D-penicillamine (SNAP) has been shown to induce HIF-1α protein in vascular endothelial and smooth muscle cells, and activated expression of HIF-1 target gene, Heme oxygenase-1 (HO-1). Epigallocatechin-3-gallate (EGCG), a green tea catechn, produces modest increase in HIF-1 activity by increasing the expression of HIF-1 target genes that include VEGF. EGCG appears to activate HIF-1 by chelating the iron ions and ascorbate suggests that EGCG may activate the expression of HIF-1 target genes including VEGF. Interestingly, desferrexochalin, an activator of HIF has been shown to prevent cardiac reperfusion injury in cultured rat adult cardiac myocytes and in isolated rabbit hearts. It also inhibits human vascular smooth muscle cell proliferation in vitro and prevented restenosis in a porcine coronary artery restenosis model [290].

Extracellular matrix turnover is important in many cardiovascular pathologies such as arterial remodeling, plaque rupture, restenosis, aneurysm formation and heart failure. In this aspect MMP inhibitors are likely to be useful in the development of pharmacological approaches to reduce cardiovascular death; considering the positive outcome after usage of MMP inhiitors in restenosis and arterial remodeling [291].

Conclusions and future directions

Contractile force in cardiomyocytes is generated via an increase in cytosolic Ca^{2+} concentrations during systole due mainly to SR Ca^{2+} release via RyR2, which can be measured as Ca^{2+} transients [80, 292]. Depolarization

of the plasma membrane during the cardiac action potential activates voltage-gated L-type Ca^{2+} channels in the sarcolemmal membrane encompassing the T-tubules. Additional Ca^{2+} may enter via mechanisms such as the T-type Ca^{2+} channels (TTCC) [293] or the Na^{+}/Ca^{2+} exchanger (NCX) operating in its reverse mode. The ensuing Ca^{2+} influx then triggers a much greater Ca^{2+} release from the SR via RyR2 through a process called Ca^{2+} induced Ca^{2+} release (CICR). The increase in cytosolic Ca^{2+} concentrations results in actin-myosin cross-bridge formation that is activated by Ca^{2+} binding to troponin C, which causes displacement of tropomyosin, translocation of the myosin heads along the actin filaments, and contraction of the myocyte [292].

Myocardial relaxation during diastole is initiated by the removal of Ca^{2+} from the cytosol. Cytosolic Ca^{2+} is pumped back into the SR by SR Ca^{2+}-ATPase (SERCA2a) [294, 295]. Activity of this enzyme is inhibited by binding of phospholamban (PLB) [296]. In its nonphosphorylated form, PLB inhibits SERCA2a activity whereas phosphorylation of PLB reverses the inhibition [296]. Cytosolic Ca^{2+} can also be extruded from the cardiomyocytes via the sarcolemmal NCX [297].

In the failing heart, maladaptive changes result in depressed intracellular Ca^{2+} cycling and decreased SR Ca^{2+} concentrations, which produces less force during EC coupling [298–300]. While the role of TRPC, CRAC and STIM1 in CCE is evident, an important question involves the nature of the signal(s) for activation of the plasma membrane channels. Two fundamentally different ideas have been suggested. Depletion of ER Ca^{2+} stores may trigger release or formation of a signaling substance that diffuses to the plasma membrane to activate the channels [301]. Alternatively, proteins in the endoplasmic reticulum, especially the IP_3R and the RyR may directly interact with calcium channels in the plasma membrane via protein–protein interactions. This idea was based on an analogy with the known interaction between RyRs and VDCC in skeletal muscle [302].

An important unexplained question is how does CaMKII distinguish between somewhat 10-fold elevations of Ca^{2+} required for heart muscle contraction and the pathologic signals elicited by the chronic hyperadrenergic state? It is possible that CaMKII requires perturbations of Ca^{2+} signaling, which could explain how these signals are initiated. Alternatively, CaMKII-dependent signals are mediated via β1-adrenergic receptors but they do not involve Ca^{2+} [303]. For example, increased CaMKII-mediated effects could reflect a downregulation of the protein phosphatase that is paired with CaMKII, or an increase in CaMKII protein levels due to transcriptional effects. If one accepts that it would be incompatible with life to have a sustained elevation of Ca^{2+} in the heart at levels sufficient to activate CaMKII, then another possibility is that localized signaling complexes could be exposed to chronic elevations of Ca^{2+} presumably through a ''leaky'' channel that would not be reflected in global elevations of Ca^{2+}. Another important task is to identify the β1-adrenergic receptor-mediated activation of CaMKII in normal physiology. Importantly, it will indeed be an important contribution for understanding the pathogenesis of heart failure if prolonged β1-adrenergic receptor stimulation appears to be responsible for cardiomyocyte cell death via a CaMKII mediated pathway [304].

Several studies have demonstrated that the activity of Na^{+}/H^{+} exchanger isoform 1 (NHE-1) is increased in circulating cells of a subset of patients with essential hypertension [305]. NHE-1 is phosphorylated to a greater extent in cells from patients with essential hypertension and animals with genetic hyperension [306]. However, it is not clear at present whether increased NHE-1 phosphorylation contributes to its enhanced activity in essential hypertension. In additon, recent studies have shown that protein kinase C does not directly phosphorylate the NHE-1. Rather, Ca^{2+} induced activation of the NHE-1 appears to occur through a Ca^{2+}-calmodulin site on the cytosolic domain of the exchanger, which modulates its affinity to cytosolic protons [307]. This finding firmly establishes a direct link between the cytosolic Ca^{2+} and the NHE-1.

Glycosphingolipids, for example, lactosyl ceramide (lac-cer) present in vascular cells such as endothelial and smooth muscle cells contribute to atherosclerosis. Indeed lac-cer accumulate in fatty streaks, intimal plaque and calcified intimal plaque, along with oxidized LDL and proinflammmatory cytokines [162]. The role of lac-cer in Ca^{2+} signaling phenomena in the cardiovascular system is not clearly known and is an important aspect of future research.

Two likely targets for the apoptotic effects of Ca^{2+} signals have been identified over the past two years. One is mitochondrion and the other is ER. In ER emptying of its Ca^{2+} content induces a form of cell stress that ultimately leads to apoptosis. Further work is required to interpret the signals that interconnect ER stress, caspase activation, and apoptosis. The etiologies of some forms of heart diseases were demonstrated to be linked to an improper regulation of apoptosis. There is much hope that a better understanding of the molecular events in apoptosis which eventually will help developing better preventive and

therapeutic strategies to control apoptosis and hence the development of heart diseases.

Recent progress in molecular cardiology makes it possible to envision a new therapeutic approach to HF, targeting key molecules involved in intracellular Ca^{2+} handling (such as RyR, SERCA2a, PLN and others). Controlling these molecular functions by different agents have been found to be beneficial in some experimental conditions [80]. Therefore, it is important to notice whether the animal models accurately reflect the human disorders or the underlying human biology. Because of the hetergeneous nature of human HF examined in various studies involving different stages and etiologies caution should be exercised when trying to decide whether this approach might be generally applicable to the treatment of HF.

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