Targeting calcium transport in ischaemic heart disease

M.A. Hassan Talukder1, Jay L. Zweier1,2, and Muthu Periasamy1,2*

1 Davis Heart and Lung Institute and The Division of Cardiovascular Medicine, Department of Internal Medicine, The Ohio State University College of Medicine, Columbus, OH 43210, USA; and 2 Department of Physiology and Cell Biology, The Ohio State University College of Medicine, 304 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210, USA

Received 22 April 2009; revised 14 July 2009; accepted 20 July 2009; online publish-ahead-of-print 29 July 2009

Time for primary review: 22 days

KEYWORDS
Ca2+ overload;
Ischaemic myocardium;
Ischaemia-reperfusion;
Heart failure

Ischaemic heart disease (IHD) is the leading cause of morbidity and mortality worldwide. While timely reperfusion of acutely ischaemic myocardium is essential for myocardial salvage, it leads to a unique type of injury known as ‘myocardial ischaemia/reperfusion (I/R) injury’. Growing evidence suggests that a defect in myocardial Ca2+ transport system with cytosolic Ca2+ overload is a major contributor to myocardial I/R injury. Progress in molecular genetics and medicine in past years has clearly demonstrated that modulation of Ca2+ handling pathways in IHD could be cardioprotective. The potential benefits of these strategies in limiting I/R injury are vast, and the time is right for challenging in vivo systemic work both at pre-clinical and clinical levels.

1. Introduction

Ischaemic heart disease (IHD) or heart attack is the single largest cause of death and disability worldwide,1,2 and acute myocardial infarction (AMI) is the fundamental clinical manifestation of heart attack following coronary thrombosis. AMI treated with early revascularization leads to myocardial reperfusion. The primary manifestations of myocardial I/R injury are myocyte death, arrhythmias, and contractile dysfunction.3–5 Patients surviving AMI are susceptible to recurrent angina, reinfarction, arrhythmias, heart failure (HF), and sudden cardiac death.6

Over the centuries, tremendous progress has been made in terms of diagnosis of MI from the post-mortem era to real-time clinical scenario of modern cardiac imaging.7,8 Management of AMI evolved in the 1960–80s from passive bed rest and symptomatic treatment to active revascularization with thrombolytic agents, angioplasty, or coronary artery bypass grafting (CABG).9–11 With these advancements and lifestyle changes, mortality from IHD has also fallen during the last three decades.12–14 While reperfusion-associated myocardial injury limits myocardial salvage, reperfusion therapies and interventions that are cardioprotective in animal models have not yet been successfully translated to achieve improved clinical outcome in patients.15 In view of this, one of the major ongoing research efforts in cardiovascular medicine has been development of approaches to prevent reperfusion injury and maximize myocardial salvage in patients with IHD.

Ca2+ is a ubiquitous signal for regulating cellular function, including survival and death.16 While a small amount of Ca2+ is necessary for the optimal physiological function of the heart, growing evidence suggests that an increased cytosolic free Ca2+ overload is one of the major contributors of myocardial I/R-induced injury.17–21 Therefore, Ca2+ handling in the post-ischaemic myocardium has become a prime target to treat patients with AMI. Progress in molecular genetics has led to the cloning and characterization of cardiac Ca2+ handling proteins.22 Ca2+ handling in the cardiac muscle is orchestrated by a set of proteins that include: (i) L-type Ca2+ channel (LTCC), (ii) sarco(endo)plasmic reticulum (SR) Ca2+ release channel--ryanodine receptor (RyR), (iii) sarco(endoplasmic reticulum Ca2+-ATPase (SERCA), (iv) Na+-Ca2+ exchanger (NCX), and (v) phospholamban (PLB) (Figure 1).

In this review, we will discuss the therapeutic potential and impact of targeting Ca2+ handling machinery to improve post-ischaemic myocardial function and injury.

2. Historical perspective of IHD

The history of IHD—myocardial ischaemia and angina pectoris, AMI, and sudden cardiac death belongs to atypical and interlacing presentations dated back to the 17th century.22–23 The first classic description of angina pectoris that is still valid until today was made by William Heberden in 1772 as Pectoris Dolor.24 John Hunter made the first self-diagnosis of angina in 1794 and it was confirmed in his own post-mortem by Edward Jenner.25–27 In 1910, William Osler summarized his views on angina pectoris for the Lumleian lecture based on his observation with 250 patients.28 The pathology and clinical features of MI were finally summarized his views on angina pectoris for the Lumleian lecture based on his observation with 250 patients.28

* Corresponding author. Tel: +1 614 292 2310; fax: +1 614 292 4888.
E-mail address: periasamy.1@osu.edu
recognized in 1912 when John B. Herrick published his classic paper on the clinical features of coronary artery occlusion and MI.29 The distinction between angina and MI was clarified by Parkinson and Bedford in 1928. 30 In 1933, Thomas Lewis clearly described relative ischaemia in contrast to the absolute ischaemia that follows total coronary obstruction.31 However, the worldwide morbidity and mortality with MI have led the experts to redefine MI in 2007, 1 and it is now phrased that ‘the term myocardial infarction should be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia’.

3. Ca²⁺ handling in cardiac muscle

The fundamental role of Ca²⁺ within cardiomyocyte is to enable excitation–contraction (E–C) coupling. The essential steps in this process involve membrane-potential-dependent small amount of Ca²⁺ entry through LTCC, subsequent large Ca²⁺-induced Ca²⁺ release (CICR) from the SR via RyR, and finally, initiation of contraction by binding of cytosolic Ca²⁺ to myofilaments (Figure 1). The concentration of free cytosolic Ca²⁺ determines the extent of muscle contraction and therefore the force development. Subsequent rapid removal of the cytosolic Ca²⁺ into SR by SERCA and extrusion of Ca²⁺ from the cell by sarcolemmal NCX and Ca²⁺-ATPase result in muscle relaxation. Additionally, dynamic interaction between SERCA and phospholamban plays a critical role in Ca²⁺ handling. Thus, E-C coupling is a finely tuned and coordinated mechanism where electrical activation is translated into cardiac contraction.32

4. Ca²⁺ overload hypothesis

A number of mechanisms have been proposed to mediate myocardial I/R injury. These include: intracellular Ca²⁺ overload; the occurrence of a no reflow phenomenon due to cell swelling, impaired vascular relaxation or the formation of white cell plugs; and the formation of oxygen radicals. The oxyradical hypothesis of myocardial I/R has been reviewed in detail.33 The Ca²⁺ overload hypothesis was first demonstrated by Zimmerman et al.34 in 1967 as ‘calcium paradox’ where the sudden readmission of Ca²⁺ to the perfusate after a brief period in which hearts are perfused with Ca²⁺-free media causes massive tissue disruption, enzyme release, development of contracture, and marked reductions of high-energy phosphate stores. In 1981, Nayler showed that ‘calcium paradox’ does not mimic the sequence of events that occurs when heart muscle is made ischaemic and then reperfused.35 However, it was shown that in both instances, the end result was the same: mitochondrial Ca²⁺ overload and depletion of tissue ATP stores. Nayler’s group in 1984 showed that the gain in Ca²⁺ that occurs in rat hearts during Ca²⁺ repletion after a Ca²⁺-free perfusion is rapid, complex, and comprises both an early and a late phase of entry. The early phase was shown to be a Ca²⁺-antagonist-sensitive as well as a Ca²⁺-antagonist-insensitive component, and the latter was shown for NCX activity.36 Pool-Wilson highlighted the abnormalities of Ca²⁺ regulation and their role in possible reperfusion injury entitled ‘calcium out of control’.37 Marban’s group in 1987 proposed that Ca²⁺ entry upon reperfusion plays a major role in the pathogenesis of myocardial stunning.38 In 1991, Opie proposed the two-stage

![Figure 1 Strategies to modulate Ca²⁺ transport in the post-ischaemic heart. The primary targets to modulate Ca²⁺ overload and improve post-ischaemic myocardial performance are (i) Sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA), (ii) Na⁺/Ca²⁺ exchanger (NCX), (iii) SR Ca²⁺ release channel RyR, (iv) L-type Ca²⁺ channel (LTCC), (v) Na⁺-H⁺ exchanger (NHE), and (vi) Ca²⁺ and calmodulin-dependent protein kinase II (CaMKII).](https://academic.oup.com/cardiovascres/article-abstract/84/3/345/457243)
model of Ca\(^{2+}\)-mediated reperfusion damage, the first phase due to excess Ca\(^{2+}\) and the second stage the consequences of that excess Ca\(^{2+}\). The pathophysiology of myocardial stunning has been extensively reviewed where elevated Ca\(^{2+}\) has been shown to cause activation of protein kinase and alter Ca\(^{2+}\) sensitivity or maximal Ca\(^{2+}\)-activated force through phosphorylation of contractile proteins.\(^{3,40,41}\) Compared with stunning, cellular mechanisms of lethal or irreversible myocardial I/R injury are complex and incremental consisting of several independent aetiologies. The pathophysiology and pharmacology of myocardial I/R injury have been elegantly reviewed by several investigators with primary emphasis on cytosolic Ca\(^{2+}\) control.\(^{3,4,21,42}\) The long-standing popular hypothesis of ‘calcium paradox’ has now been revisited with distinction that Ca\(^{2+}\) overload in cellular I/R is always preceded by Na\(^{+}\) overload, but, under the classical calcium paradox protocol Na\(^{+}\) overload does not occur prior to massive Ca\(^{2+}\) overload.\(^{43}\)

4.1 Role of LTCC in ischaemic Ca\(^{2+}\) overload

Sarcoplasmatic LTCCs are integral component of the E-C coupling mechanism in cardiomyocytes. Membrane depolarization opens these channels, allowing rapid influx of Ca\(^{2+}\) into the cell. Increased Ca\(^{2+}\) accumulation could be augmented by ischemia-induced depolarization of the membrane potential, which allows opening of the LTCC and further Ca\(^{2+}\) entry into the cell. About two decades ago, the protective effect of LTCC blockers were demonstrated in vivo canine models of I/R.\(^{44-47}\) and later, in a wide variety of experimental models.\(^{48-50}\) The expression of LTCCs was reported to be decreased\(^{51}\) or unchanged\(^{52}\) following in vitro I/R. Recently, in a rat model of chronic MI, expression of LTCC mRNA not the protein was reported to decrease 1 day after MI followed by recovery towards control values in a 4-week follow-up period.\(^{53}\) Although Ca\(^{2+}\) channel blockers initially suggested a role for LTCCs and subsequent Ca\(^{2+}\) overload in I/R injury, a reduction in myocardial oxygen consumption due to negative inotropic and chronotrophic effects of Ca\(^{2+}\) channels blockers were later referred to the anti-ischaemic effects of these compounds.\(^{3}\) In humans, LTCC blockers have yielded conflicting results on myocardial I/R either with no beneficial effects on patient survival or with reduced cellular damage after AMI.\(^{54-56}\) Overall, the clinical use of LTCC blockers in MI is still in dispute because of their marked haemodynamic effects.

4.2 SERCA and Ca\(^{2+}\) overload

SERCA2a is primarily responsible for SR Ca\(^{2+}\) uptake and replenishing the SR Ca\(^{2+}\) load during the contraction-relaxation cycle of the heart.\(^{57-59}\) The SR has been shown to suffer substantial damage with impaired Ca\(^{2+}\) uptake function during I/R. Its nearly five decades since Stuckey and associates in 1967 demonstrated a decrease of Ca\(^{2+}\) uptake in isolated SR following coronary artery occlusion in a canine model of cardiopulmonary bypass.\(^{60}\) In 1978, a study in a canine model of MI reported that there is simultaneous decrease in the SR Ca\(^{2+}\) uptake rate, Ca\(^{2+}\)-Mg\(^{2+}\)-stimulated ATPase activity and ATPase protein expression, but, all of these changes returned to normal levels by 4 weeks.\(^{61}\) Later, several investigators using various ischaemic durations reported a decrease in SR Ca\(^{2+}\) reuptake and/or SR Ca\(^{2+}\)-ATPase activity in the ischaemic myocardium of different mammals.\(^{62-64}\) In humans, a significant decrease in the SR Ca\(^{2+}\) uptake of post-ischaemic atrial myocardium was reported after reversible I/R.\(^{65}\) Recently, the critical role of SR Ca\(^{2+}\) transport system in myocardial I/R has been consistently demonstrated both in transgenic animals and by gene therapy to improve Ca\(^{2+}\) handling. Hajjar and associates showed that adenoviral overexpression of SERCA2a in the heart reduces arrhythmias and improves ventricular thickening in a rat model myocardial I/R.\(^{66}\) Subsequently, several studies have demonstrated that increasing SR Ca\(^{2+}\) uptake function by SERCA overexpression or gene transfer strongly protects the heart against I/R injury and/or HF.\(^{67-69}\) Using both gain-of-function (SERCA overexpression) and loss-of-function (SERCA deficiency) models,\(^{70-72}\) we have clearly demonstrated that SERCA2a function is critical for post-ischaemic injury because sub-lethal ischemia caused significant in vivo myocardial infarction in SERCA-deficient mice,\(^{71}\) whereas lethal ischemia in SERCA overexpression mice exhibited markedly small myocardial infarction.\(^{72}\) Taken together, these data suggest that the reduced functional level of SERCA is one of the factors that determines intracellular Ca\(^{2+}\) overload and contractile dysfunction following I/R. While SERCA2a-mediated gene therapy has gained considerable attention to treat MI, studies also suggested that excessive SERCA expression might cause impaired post-ischaemic contractile function.\(^{73-75}\)

4.3 SR Ca\(^{2+}\) release channel and Ca\(^{2+}\) overload

The cardiac SR Ca\(^{2+}\) release channel RyR is a key component in cardiac E-C coupling, and it is responsible for the release of Ca\(^{2+}\) from the SR during cardiac muscle contraction (Figure 1). The involvement of RyR in myocardial I/R injury stems from two different sets of experiments. First, several groups demonstrated that inhibition of RyR by pharmacological inhibitors, such as ryanodine, caffeine, or dantrolene, could be beneficial during I/R because it would attenuate cytosolic Ca\(^{2+}\) overload, improve contractile recovery, decrease myocardial infarction, and reduce the incidence of arrhythmias.\(^{76-79}\) Second, in contrast to the first, other investigators have reported that a decrease in the amount of RyR protein, RyR mRNA, and/or the RyR binding sites following I/R might be responsible for contractile dysfunction in the ischaemic and reperfused post-ischaemic myocardium.\(^{80,81}\) Studies also reported that mRNA levels of RyR were differentially expressed in the scar and viable myocardium of post-MI rat hearts.\(^{82}\) Recently, in a rat in vivo model of MI, the expression of RyR mRNA not the RyR proteins was found to be reduced 1 day after MI, but the expression levels recovered by 4 weeks.\(^{53}\) Oxidative modifications of Ca\(^{2+}\)-handling proteins have been reported during I/R.\(^{83-84}\) The redox modulation of RyR activity is mediated by the redox modification of sulf-hydryl groups of cysteine residues. Similarly SERCA and LTCC channel are subject to redox modulation. Interestingly, a recent in vitro report suggests that NADH oxidation and acute cytosolic redox status is involved in negative-feedback regulation of the RyR.\(^{85}\) Thus redox-mediated alteration of ion channels and pumps could be important in I/R injury.
4.4 NCX and Ca$^{2+}$ overload

In mammalian heart, NCX plays an important role in cardiac E–C coupling by extruding Ca$^{2+}$ from the cytosol during diastole. NCX is electrogenic and influenced by the membrane potential as well as by Na$^+$ and Ca$^{2+}$ gradients across cell membrane. The exchanger operates in the ‘forward mode’ under normal physiological conditions to extrude Ca$^{2+}$ from the cell, however, it can also operate in the ‘reverse mode’ under certain conditions to transport Ca$^{2+}$ into the cell. It is more than two decades when two independent groups demonstrated that Na$^+$-dependent increase in Ca$^{2+}$ during ischaemia and early reperfusion is due to reduced Ca$^{2+}$ efflux by a reduced Na$^+$ gradient or due to Ca$^{2+}$ entry via reverse-mode NCX. Several investigators have consistently reported that pharmacological inhibition of reverse-mode NCX reduces cytosolic Ca$^{2+}$ overload in various experimental conditions. This observation was subsequently verified using genetic models of mice, and it was established that inhibition or ablation of NCX function provides cardioprotection against I/R injury. Recently, administration of an NCX inhibitor at the time of reperfusion has been shown to reduce contractile dysfunction and myocardial injury in vitro and in vivo swine model of I/R. These findings indicate that inhibition of reverse-mode NCX at the time of reperfusion could be an attractive strategy to maximize clinical benefit of reperfusion therapy in patients with AMI.

4.5 Na$^+$/H$^+$ exchanger activity during I/R

Myocardial NHE plays an important role in the maintenance of intracellular pH, Na$^+$, and Ca$^{2+}$ homeostasis. During myocardial ischaemia, intracellular acidosis develops quickly, activating the exchanger to extrude H$^+$ into the extracellular space and bring Na$^+$ into the cell. However, with continued ischaemia, the cell becomes unable to handle the overload of Na$^+$, and in turn, stimulates Ca$^{2+}$ influx through NCX and causes intracellular Ca$^{2+}$ overload. This can lead to detrimental cardiac injury, such as contracture and necrosis. During myocardial reperfusion, these events are magnified because the blood flow lowers the extracellular Na$^+$ concentration, stimulating NHE to extrude more intracellular H$^+$. This leads to intracellular Na$^+$ overload and eventually, Ca$^{2+}$ overload that mediates the unfavourable sequels of myocardial I/R such as expansion of MI, myocardial stunning, and arrhythmias. Karmazyn was the first to show that the NHE inhibitor could improve the post-ischaemic contractile recovery in isolated hearts. Later, several investigators have repeatedly shown that various NHE inhibitors can prevent myocardial I/R injury in animal models.

4.6 PLB activity during I/R

PLB is an SR membrane protein and the endogenous regulator of SERCA2a. In the dephosphorylated state, PLB inhibits the activity of SERCA2a and SR Ca$^{2+}$ transport. Phosphorylation of PLB at Ser16 by protein kinase A (PKA) or at Thr17 by Ca$^{2+}$-calmodulin protein kinase II (CaMKII) relieves its inhibitory effect on SERCA2a and SR Ca$^{2+}$ transport. Thus, PLB is a critical regulator of SR function, myocardial relaxation, and contractility. A decrease in the activity of SERCA2a and/or the rate of SR Ca$^{2+}$ uptake have been reported in most studies of myocardial I/R. PLB phosphorylation was found to be decreased after ≥30 min ischaemia in a porcine model of coronary ligation; however other reports showed increased, decreased, or unchanged phosphorylation during myocardial I/R with variable post-ischaemic recovery in different mammalian species. Interestingly, studies with PLB knockout mice demonstrated that ablation of PLB exacerbates post-ischaemic myocardial injury. While published data clearly indicates that phosphorylation status of PLB is altered following I/R, future long-term in vivo studies will be required to correlate the time course of phosphorylation status with post-ischaemic myocardial recovery.

4.7 CaMKII and Ca$^{2+}$ overload

The multifunctional Ca$^{2+}$/calmodulin-dependent protein kinase (CaMK) is an intracellular protein that is dynamically activated by intracellular Ca$^{2+}$ and serves as a major downstream effector for Ca$^{2+}$ signalling in the heart. When intracellular Ca$^{2+}$ is low, CaMKII is auto-inhibited in the resting state. Activation occurs with elevation of Ca$^{2+}$ and calmodulin binding to an autoinhibitory domain, and upon activation it can phosphorylate many different proteins. CaMKIIδ is the predominant isoform in the heart and it modulates an array of proteins involved in the cardiac E–C coupling and Ca$^{2+}$ handling, including PLB, RyR, and L-type Ca$^{2+}$ channels. Because of its modulatory role on different Ca$^{2+}$ handling apparatus, CaMKII is postulated to play a key role in myocardial physiology and disease. An increased activity of myocardial CaMKII has been reported in different species following I/R. Interestingly, CaMKII activation has been shown to be beneficial on reversible (stunning) and detrimental on irreversible myocardial I/R injury. Importantly, whether these effects of CaMKII on myocardial I/R are present or relevant in human heart remains to be determined.

5. Therapeutic approaches to target Ca$^{2+}$ overload

The clinical manifestations of IHD such as myocardial ischaemia and angina pectoris, AMI, and sudden cardiac death are the leading causes of death worldwide. During the last decades, several novel pharmacological and mechanical therapies have been developed to rapidly restore coronary artery patency. Currently, the single established strategy to limit infarct size in impending AMI is early reperfusion with thrombolysis or percutaneous coronary intervention. Since an acute ischaemic attack is unpredictable, the size established platform for target AMI.
5.1 LTCC blockers

Ca\(^{2+}\) channel blockers, although, proved to prevent Ca\(^{2+}\) overload during prolonged ischaemia and reperfusion, and limit irreversible myocardial damage in experimental models of coronary occlusions (Section 3.1), the clinical benefit of Ca\(^{2+}\) channel blockers in the setting of acute MI remains inconsistent because of limited clinical trials.\(^{105}\) One unjustifiable limitation is that, even in experimental models of I/R, Ca\(^{2+}\) channel blockers are cardioprotective only when administered prior to ischaemia.\(^{107}\) While LTCC blockers have been available for decades, there is no consistent evidence that they are protective in man and, therefore, they are not routinely recommended in the setting of AMI, unless, there is a clear indication such as hypertension or arrhythmias.\(^{108}\) Interestingly, two recent meta-analysis have, however, indicated that LTCC blockers may be safer with less cardiovascular complications after cardiac surgery,\(^{109}\) or may not increase the risk of cardiovascular death and myocardial infarction.\(^{110}\) Therefore, a definitive conclusion on the use of LTCC blockers awaits further evaluations.

5.2 Enhancing SERCA expression and activity

There is now convincing evidence that impaired SR calcium handling pathways are directly involved in the cardiomyocyte cell death during I/R.\(^{73,111,112}\) Adenoviral- and lentiviral-mediated gene transfer to increase SERCA2a expression or the use of transgenic animals with SERCA overexpression have clearly established the potent beneficial effects during I/R.\(^{67,68}\) and thus promoted the field of potential SERCA gene therapy. Recently, one novel drug with SERCA2a stimulating properties has emerged with great promise.\(^{113,114}\) Istaroxime that concomitantly inhibits Na\(^+\)/K\(^-\) ATPase and stimulates SERCA2a is currently in phase Ib clinical trials.\(^{113}\) Istaroxime has been shown to improve cardiac performance and haemodynamics without affecting blood pressure, heart rate, and renal function, and without inducing the pro-arrhythmic consequences of intracellular Ca\(^{2+}\) overload in patients with chronic HF.\(^{114}\) The impact of myocardial SERCA upregulation requires a more highly integrated, systematic, and focused research for in vivo models.

5.3 Inhibiting SR Ca\(^{2+}\) release channel

Although much needs to be understood regarding the role of RyR in experimental I/R, dantrolene that selectively inhibit SR Ca\(^{2+}\) release channel has been shown to protect the heart from post-ischaemic injury regardless of the timing of administration.\(^{115}\) The advantage with dantrolene is that it is already used in humans as a therapeutic agent for malignant hyperthermia,\(^{116}\) and it has been used in cardiomyopathic patients without relevant side effects.\(^{117}\) At present, there is not enough pre-clinical and clinical data to demonstrate the potential beneficial effects of systemic inhibition of Ca\(^{2+}\) release channels in post-MI subjects. Therefore, future studies will justify the usefulness of dantrolene in the setting of AMI.

5.4 NCX inhibitors

Increased intracellular Ca\(^{2+}\) overload during myocardial I/R is due to Ca\(^{2+}\) influx via the reverse mode of sarcolemmal NCX and reduced SR Ca\(^{2+}\) uptake activity. In animal models, inhibition of the NCX or heterozygous knockout of NCX has been shown to reduce the susceptibility to I/R injury by preventing Ca\(^{2+}\) influx and/or diminishing the cell injury.\(^{86,89,91}\) NCX inhibitors are effective when they are administered at the time of reperfusion,\(^{97}\) and thus, it appeared to be more useful to alleviate the tissue damage in a clinical setting with AMI patients who receive reperfusion therapy. Currently, three NCX inhibitors, KB-R7943, SEA0400, and SN-6, are available with major therapeutic potential.\(^{118}\) However, there is no pre-clinical report or clinical trial as to whether long-term systemic inhibition is beneficial or detrimental.

5.5 NHE inhibitors

Despite highly encouraging cardioprotective effects of NHE inhibitors in pre-clinical studies,\(^{119}\) clinical studies in patients with evolving MI and those at risk of MI have provided mixed and largely disappointing data.\(^{120,121}\) In the GUARDIAN trial,\(^{120}\) the cardioprotective efficacy of cariporide was limited to the subset of high-risk patients who underwent CABG. In contrast, no cardioprotective benefit was observed in the ESCAMI trial in patients with acute MI.\(^{120}\) The failure in clinical trials could be attributed to many reasons, but the majority of pre-clinical data suggest that NHE inhibitors show most benefit when administered during ischaemia alone or during ischaemia and reperfusion.\(^{121}\) Interestingly, the recent EXPEDITION trial in CABG patients had revealed compelling evidence that NHE inhibitor could significantly reduce myocardial injury in patients at risk of I/R injury.\(^{122}\) Thus, it appears that the timing of administration plays a critical role in the positive outcome of NHE inhibitors, and it is expected that future clinical trials will extend the role of NHE inhibition in therapeutic application.

5.6 Ca\(^{2+}\) handling modulators

Recently, caldaret, (5-methyl-2-(1-piperazinyl) benzene sulfonic acid monohydrate; MCC-135) a novel drug having the ability to modulate intracellular Ca\(^{2+}\) handling has been reported. In pre-clinical studies, caldaret inhibits I/R-induced Ca\(^{2+}\) overload, enhances Ca\(^{2+}\) uptake into and inhibits Ca\(^{2+}\) leakage from the SR, reduces myocardial infarction, decreases cardiac markers of I/R injury, and improves LV function.\(^{123}\) Caldaret has been found safe and well tolerated in early clinical studies involving healthy subjects, and it has demonstrated a good safety profile as an adjunctive in patients undergoing primary percutaneous coronary intervention (PCI) for ST-elevation MI (STEMI).\(^{123}\) While ‘EVOLVE’ trial showed no significant benefit of caldaret on preservation of LV ejection fraction and reduction of infarct size in patients with STEMI undergoing primary PCI, ‘CASTEMI’ trial showed a significant decrease in the incidence of LV dysfunction in patients with STEMI undergoing primary PCI.\(^{124}\) Outcomes of current and future clinical trials will establish the therapeutic benefits of caldaret for the treatment of AMI.

6. Conclusions and perspective

In this review, we have made an attempt to identify the key players in Ca\(^{2+}\) homeostasis, whose function is modified...
during I/R. We have also attempted to highlight the ongoing efforts to develop therapeutics to treat several of these known targets to improve calcium transport. Despite significant advances in defining many of the targets that are modified during I/R injury, there are many challenges to determine which of these targets are of primary importance for developing rationale therapy in a clinical setting. In this review, we have not been able to address every aspect of calcium signalling. Apart from E–C coupling, Ca\textsuperscript{2+} plays important roles in signal transduction pathways that contribute to cardiac growth/hypertrophy, apoptosis, and HF. We should also point out that there is increasing evidence that redox modulation of ion channels, pumps, and transporters play important roles in post-ischaemic myocardial dysfunction. Therefore, the potential roles of redox modification on Ca\textsuperscript{2+} handling and mitochondrial Ca\textsuperscript{2+} overload induced apoptosis should be considered in the crucial outcome of novel therapeutic strategies. Thus, despite mounting evidence and better understanding of the Ca\textsuperscript{2+}–IHD link and the benefits from potential novel therapeutic strategies (Figure 1), challenging work lies ahead both at pre-clinical and clinical levels to determine the definite importance of these interventions in limiting myocardial damage and improving patient’s survival.

Conflict of interest: none declared.

Funding
This work was supported by NIH grants HL64140-08, HL080551-01 to M.P.; and HL63744, HL65608, and HL38324 to J.L.Z.

References
46. Pryzylkien K, Ghabafar GB, Eitzman DT, Klener RA. Nedifepine adminis-
tered after reperfusion ablates systolic contractile dysfunction of post-
47. Gross GJ, Farber NE, Pieper NE. Effects of amiodopine on myocardial
48. duToit EF, Opie LH. Modulation of severity of reperfusion stunning in the
ischemically injured hearts by the agents altering calcium flux at the onset of
49. Hoff PT, Tamura Y, Lucchesi BR. Cardioprotective effects of amiodopine
Galina N. Diltiazem and protection of myocardial ischemic injury during coronary
51. Bersohn MM, Moresy AK, Weiss RS. Sarcolemmal calcium transporters in
myocardial I/R injury to infarction and affects postischemic functional recovery.
52. Zucchi R, Ronca-Testoni S, Yu G, Galbani P, Ronca G, Mariani M. Are dihy-
dropyridine receptors downregulated in the ischemic myocardium? J Mol Cell Cardiol 1997;29:2525–2532.
53. Periasamy M, Huke S. SERCA pump level is a critical determinant of
calcium-induced calcium release.
54. The HINT Research Group. Early treatment of unstable angina in the cor-
55. Gross GJ, Farber NE, Pieper NE. Effects of amlodipine on myocardial
56. Sheiban I, Tonni S, Chizzoni A, Marini A, Trevi G. Recovery of left ventri-
57. Misquitta CM, Mack DP, Grover AK. Sarco/endoplasmic reticulum Ca(2+
58. Periasamy M, Huke S. SERCA pump level is a critical determinant of
calcium-induced calcium release.
59. The HINT Research Group. Early treatment of unstable angina in the cor-
60. Lee KS, Ladinsky H, Stuckey JH. Decreased Ca2+
þ-Ca2+
þ exchange.
61. Del Monte F, Lebeche D, Guerrero JL, Tsuji T, Doyle AA, Gwathmey JK,
et al. Abrogation of ventricular arrhythmias in a model of ischemia
62. Lee KS, Ladinsky H, Stuckey JH. Decreased Ca2+
þ-Ca2+
þ exchange.
64. Mubagwa K. Sarcoplasmic reticulum function during myocardial ischae-
dropyridine receptors downregulated in the ischemic myocardium? J Mol Cell Cardiol 1997;29:2525–2532.
66. del Monte F, Lebeche D, Guerrero JL, Tsuji T, Doyle AA, Gwathmey JK,
et al. Abrogation of ventricular arrhythmias in a model of ischemia
Lentiviral vector-mediated SERCA2 gene transfer protects against
69. Byrnes MJ, Powers JM, Prevooulos A, Mariani JA, Hajar JR, Kaye DM. Recir-
culating cardiac delivery of AAV2/1SERCA2a improves myocardial func-
70. Talukder MA, Kalyanasundaram A, Zuo L, Velayutham M, Nishijima Y, Periasamy M et al. Is reduced SERCA2a expression detrimental or ben-
eficial to postischemic cardiac function and injury? Evidence from het-
þ transport properties improves postischemic cardiac function, Ca2+
73. Piper HA, Kasseckert S, Abdallah Y. The sarcoplasmic reticulum as the primary target of reperfusion protection. Cardiovasc Res 2006;70:
170–173.
74. Teunier N, Prestle J, Seidler T, Currie S, Elliott BE, Reynolds DF et al. Excessive sarcoplasmic/endoplasmic reticulum Ca2+
þ-ATPase expression causes increased sarcoplasmic reticulum Ca2+
þ uptake but decreases cellular viability: implications for cardiac
1978;83:97–103.
75. Chen Y, Escobert B, Prunier F, Amour J, Simonides WS, Vivien B et al. Constitutive cardiac overexpression of sarcoplasmic/endoplasmic reti-
culum Ca2+
þ-ATPase delays myocardial failure after myocardial infarc-
77. Du Toit EF, Opie LH. Modulation of severity of reperfusion stunning in iso-
79. Zucchi R, Ronca-Testoni S, Flameng W. The sarcoplasmic reticulum Ca2+
80. Zucchi R, Ronca-Testoni S, Yu G, Galbani P, Ronca G, Mariani M. Effect of ischemia and reperfusion on cardiac ryanodine receptors-sarcoplasmic reticulum Ca2+
82. Xu Y-J, Chapman D, Dixon IMC, Sethi R, Guo X, Dhalla NS. Differential
genotype expression in infarct scar and viable myocardium from rat heart
83. Dixon IM, Kaneko M, Hata T, Panagia V, Dhalla NS. Alterations in cardiac
membrane Ca2+
84. Zima AV, Blatter LA. Redox regulation of cardiac calcium channels and trans-
86. Blaustein M, Lederer J. Sodium/calcium exchange: its physiological
significance and the role of the Na+/Ca2+
87. Tani M, Neely JR. Role of intracellular Na+
þ on myocardium.
88. Periasamy M, Huke S. SERCA pump level is a critical determinant of
calcium-induced calcium release.
89. The HINT Research Group. Early treatment of unstable angina in the cor-
90. Periasamy M, Huke S. SERCA pump level is a critical determinant of
calcium-induced calcium release.
þ exchanger at the time of
networksdoi.org/10.1093/acadspec/asc88/13645624/195061 by guest on 10 February 2019


