Cardiovascular Research (2009) **84**, 345–352
doi:10.1093/cvr/cvp264

**Review**

# Targeting calcium transport in ischaemic heart disease

**M.A. Hassan Talukder[1], Jay L. Zweier[1,2], and Muthu Periasamy[1,2]***

[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 209

**Time for primary review: 22 days**

**KEYWORDS**
$Ca^{2+}$ 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 $Ca^{2+}$ transport system with cytosolic $Ca^{2+}$ 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 $Ca^{2+}$ 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.

$Ca^{2+}$ is a ubiquitous signal for regulating cellular function, including survival and death.[16] While a small amount of $Ca^{2+}$ is necessary for the optimal physiological function of the heart, growing evidence suggests that an increased cytosolic free $Ca^{2+}$ overload is one of the major contributors of myocardial I/R-induced injury.[17–21] Therefore, $Ca^{2+}$ 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 $Ca^{2+}$ handling proteins. $Ca^{2+}$ handling in the cardiac muscle is orchestrated by a set of proteins that include: (i) L-type $Ca^{2+}$ channel (LTCC), (ii) sarcoplasmic reticulum (SR) $Ca^{2+}$ release channel—ryanodine receptor (RyR), (iii) sarco(endo)-plasmic reticulum $Ca^{2+}$-ATPase (SERCA), (iv) $Na^{+}$–$Ca^{2+}$ exchanger (NCX), and (v) phospholamban (PLB) (*Figure 1*).

In this review, we will discuss the therapeutic potential and impact of targeting $Ca^{2+}$ 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

* Corresponding author. Tel: +1 614 292 2310; fax: +1 614 292 4888.
*E-mail address*: periasamy.1@osu.edu

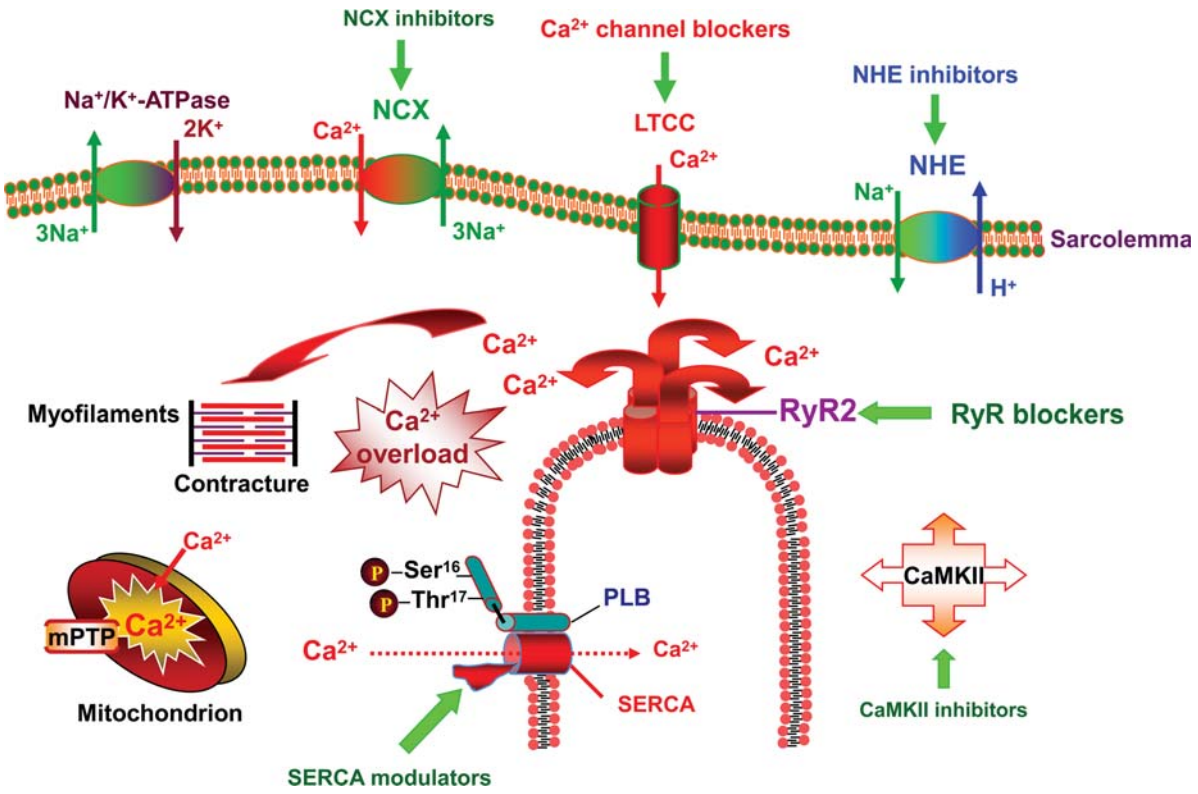


**Figure 1** Strategies to modulate $Ca^{2+}$ transport in the post-ischaemic heart. The primary targets to modulate $Ca^{2+}$ overload and improve post-ischaemic myocardial performance are (i) Sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase (SERCA), (ii) $Na^+/Ca^{2+}$ exchanger (NCX), (iii) SR $Ca^{2+}$ release channel RyR, (iv) L-type $Ca^{2+}$ channel (LTCC), (v) $Na^+$–$H^+$ exchanger (NHE), and (vi) $Ca^{2+}$ and calmodulin-dependent protein kinase II (CaMKII).

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. $Ca^{2+}$ handling in cardiac muscle

The fundamental role of $Ca^{2+}$ within cardiomyocyte is to enable excitation–contraction (E–C) coupling. The essential steps in this process involve membrane-potential-dependent small amount of $Ca^{2+}$ entry through LTCC, subsequent large $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) from the SR via RyR, and finally, initiation of contraction by binding of cytosolic $Ca^{2+}$ to myofilaments (*Figure 1*). The concentration of free cytosolic $Ca^{2+}$ determines the extent of muscle contraction and therefore the force development. Subsequent rapid removal of the cytosolic $Ca^{2+}$ into SR by SERCA and extrusion of $Ca^{2+}$ from the cell by sarcolemmal NCX and $Ca^{2+}$-ATPase result in muscle relaxation. Additionally, dynamic interaction between SERCA and phospholamban plays a critical role in $Ca^{2+}$ handling. Thus, E–C coupling is a finely tuned and coordinated mechanism where electrical activation is translated into cardiac contraction.[32]

## 4. $Ca^{2+}$ overload hypothesis

A number of mechanisms have been proposed to mediate myocardial I/R injury. These include: intracellular $Ca^{2+}$ 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 $Ca^{2+}$ overload hypothesis was first demonstrated by Zimmerman *et al.*[34] in 1967 as 'calcium paradox' where the sudden readmission of $Ca^{2+}$ to the perfusate after a brief period in which hearts are perfused with $Ca^{2+}$-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 $Ca^{2+}$ overload and depletion of tissue ATP stores. Nayler's group in 1984 showed that the gain in $Ca^{2+}$ that occurs in rat hearts during $Ca^{2+}$ repletion after a $Ca^{2+}$-free perfusion is rapid, complex, and comprises both an early and a late phase of entry. The early phase was shown to be a $Ca^{2+}$ antagonist-sensitive as well as a $Ca^{2+}$ antagonist-insensitive component, and the latter was shown for NCX activity.[36] Pool-Wilson highlighted the abnormalities of $Ca^{2+}$ regulation and their role in possible reperfusion injury entitled 'calcium out of control'.[37] Marban's group in 1987 proposed that $Ca^{2+}$ entry upon reperfusion plays a major role in the pathogenesis of myocardial stunning.[38] In 1991, Opie proposed the two-stage

model of $Ca^{2+}$-mediated reperfusion damage, the first phase due to excess $Ca^{2+}$ and the second stage the consequences of that excess $Ca^{2+}$.[39] 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

Sarcolemmal 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 ischaemia-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 chronotopic 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, $Ca^{2+}$–$Mg^{+}$-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 ischaemia caused significant *in vivo* myocardial infarction in SERCA-deficient mice,[71] whereas lethal ischaemia 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 sulfhydryl 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.[86] 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.[87,88] Several investigators have consistently reported that pharmacological inhibition of reverse-mode NCX reduces cytosolic $Ca^{2+}$ overload in various experimental conditions.[86] This observation was subsequently verified using genetic models of mice,[89,90] 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 *in vitro* rat and *in vivo* swine model of I/R.[91] 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.[92] 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.[92] Karmazyn[93] 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.[94]

### 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.[95] 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.[60–65] PLB phosphorylation was found to be decreased after $\geq$30 min ischaemia in a porcine model of coronary ligation;[96] however other reports showed increased, decreased, or unchanged phosphorylation during myocardial I/R with variable post-ischaemic recovery in different mammalian species.[97,98] Interestingly, studies with PLB knockout mice demonstrated that ablation of PLB exacerbates post-ischaemic myocardial injury.[99] 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.[100] 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. CaMKII$\delta$ 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.[101] Because of its modulatory role on different $Ca^{2+}$ handling apparatus, CaMKII is postulated to play a key role in myocardial physiology and disease.[102] An increased activity of myocardial CaMKII has been reported in different species following I/R.[103,104] Interestingly, CaMKII activation has been shown to be beneficial on reversible (stunning) and detrimental on irreversible myocardial I/R injury.[105] 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.[42,105–108] 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, majority of the patients end up with infarcts involving a large fraction of the myocardial area at risk. With this view, development of strategies aimed at limiting cell death secondary to transient coronary occlusion by directly interfering with the mechanisms of I/R injury appears to be an attractive strategy. From the preceding sections of this review, it is obvious that modulation of $Ca^{2+}$ handling pathways (*Figure 1*) might salvage the myocardium at risk. Here, we will briefly discuss the potential therapeutic candidates for 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 IIb 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, $Ca^{2+}$ 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 $Ca^{2+}$ handling and mitochondrial $Ca^{2+}$ overload induced apoptosis should be considered in the crucial outcome of novel therapeutic strategies. Thus, despite mounting evidence and better understanding of the $Ca^{2+}$–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

1. Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. *J Am Coll Cardiol* 2007;**50**:2173–2195.
2. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N *et al*. Heart disease and stroke statistics-2008 update. A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2008;**117**:e25–e146.
3. Gross GJ, Kersten JR, Warltier DC. Mechanisms of postischemic contractile dysfunction. *Ann Thorac Surg* 1999;**68**:1898–1904.
4. Piper HM, García-Dorado D. Prime causes of rapid cardiomyocyte death during reperfusion. *Ann Thorac Surg* 1999;**68**:1913–1919.
5. Verma S, Fedak PW, Weisel RD, Butany J, Rao V, Maitland A *et al*. Fundamentals of reperfusion injury for the clinical cardiologist. *Circulation* 2002;**105**:2332–2336.
6. Woo KS, White HD. Factors affecting outcome after recovery from myocardial infarction. *Annu Rev Med* 1994;**45**:325–339.
7. Fleming PR. *A Short History of Cardiology.* Amsterdam/Atlanta, GA: Rodopi Publishers; 1997.
8. Saraste A, Nekolla S, Schwaiger M. Contrast-enhanced magnetic resonance imaging in the assessment of myocardial infraction and viability. *J Nucl Cardiol* 2008;**15**:105–117.
9. Van Der Werf JJ, Topol EJ, Sobel BE. The impact of fibrinolytic therapy for ST-segment-elevation acute myocardial infarction. *J Thromb Haemost* 2009;**7**:14–20.
10. Grech ED. ABC of interventional cardiology: percutaneous coronary intervention. I: history and development. *BMJ* 2003;**326**:1080–1082.
11. Sellke F, Swanson S, del Nido PJ. *Sabiston & Spencer Surgery of the Chest*. Saunders, PA: Elsevier Health Sciences; 2004.
12. Heidenreich PA, McClellan M. Trends in treatment and outcomes for acute myocardial infarction: 1975–1995. *Am J Med* 2001;**110**:165–174.
13. Hardoon SL, Whincup PH, Lennon LT, Wannamethee SG, Capewell S, Morris RW. How much of the recent decline in the incidence of myocardial infarction in British men can be explained by changes in cardiovascular risk factors? Evidence from a prospective population-based study. *Circulation* 2008;**117**:598–604.
14. Luepker RV. Decline in incident coronary heart disease. Why are the rates falling? *Circulation* 2008;**117**:592–593.
15. Bolli R, Becker L, Gross G, Mentzer R Jr, Balshaw D, Lathrop DA. Myocardial protection at a crossroads: the need for translation into clinical therapy. *Circ Res* 2004;**95**:125–134.
16. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodeling. *Nat Rev Mol Cell Biol* 2003;**4**:517–529.
17. Elz JS, Nayler WG. Contractile recovery and reperfusion-induced calcium gain after ischemia in the isolated rat heart. *Lab Invest* 1988;**58**:653–659.
18. Steenbergen C, Fralix TA, Murphy E. Role of increased cytosolic free calcium concentration in myocardial ischemic injury. *Basic Res Cardiol* 1993;**88**:456–470.
19. Siegmund B, Schlüter KD, Piper HM. Calcium and the oxygen paradox. *Cardiovasc Res* 1993;**27**:1778–1783.
20. Meissner A, Morgan JP. Contractile dysfunction and abnormal $Ca^{2+}$ modulation during postischemic reperfusion in rat heart. *Am J Physiol Heart Circ Physiol* 1995;**268**:H100–H111.
21. Piper HM, García-Dorado D, Ovize M. A fresh look at reperfusion injury. *Cardiovasc Res* 1998;**38**:291–300.
22. Clarendon EH. *The life of Edward, Earl of Clarendon*. Oxford: Clarendon Press; 1759.
23. McNee JW. The clinical syndrome of thrombosis of the coronary arteries. *Q J Med* 1925;**19**:44–52.
24. Heberden W. Some account of a disorder of the breast. *Med Trans R Coll Physicians (Lond)* 1772;**2**:59–67.
25. Parry CS. *An Inquiry into the Symptoms and Causes of the Syncope Anginosa, Commonly Called Angina Pectoris*. Bath: R Crutwell; 1799.
26. Hunter J. *A Treatise in Blood, Inflammation and Gun-shot Wounds to which is Prefixed a Short Account of the Author's Life by his Brother-in-law Everard Home*. London: G Nichol; 1794.
27. Livesley B. The spasms of John Hunder: a new interpretation. *Med Hist* 1973;**17**:70–75.
28. Osler W. Lumleian lecture on angina pectoris. *Lancet* 1910;I:839–844.
29. Herrick JB. Clinical features of sudden obstruction of the coronary arteries. *J Am Med Assoc* 1912;**59**:2015–2020.
30. Parkinson J, Bedford DE. Cardiac infarction and coronary thrombosis. *Lancet* 1928;**1**:4–11.
31. Lewis T. *Diseases of the Heart*. London: MacMillan; 1933.
32. Bers DM. Cardiac excitation-contraction coupling. *Nature* 2002;**415**:198–205.
33. Zweier JL, Talukder MA. The role of oxidants and free radicals in reperfusion injury. *Cardiovasc Res* 2006;**70**:181–190.
34. Zimmerman ANE, Daems W, Hulsmann WC, Snijder J, Wisse E, Durrier D. Morphological changes of heart muscle caused by successive perfusion with calcium-free and calcium-containing solutions (calcium paradox). *Cardiovasc Res* 1967;**1**:201–209.
35. Nayler WG. The role of calcium in the ischemic myocardium. *Am J Pathol* 1981;**102**:262–270.
36. Nayler WG, Perry SE, Elz JS, Daly MJ. Calcium, sodium and the calcium paradox. *Circ Res* 1984;**55**:227–237.
37. Poole-Wilson PA, Harding DP, Bourdillon PD, Tones MA. Calcium out of control. *J Mol Cell Cardiol* 1984;**16**:175–187.
38. Kusuoka H, Porterfield JK, Weisman HF, Weisfeldt ML, Marban E. Pathophysiology and pathogenesis of stunned myocardium. Depressed $Ca^{2+}$ activation of contraction as a consequence of reperfusion-induced cellular calcium overload in ferret hearts. *J Clin Invest* 1987;**79**:950–961.
39. Opie LH. Role of calcium and other ions in reperfusion injury. *Cardiovasc Drugs Ther* 1991;**5**:237–248.
40. Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 1999;**79**:609–634.
41. Kim S-J, Depre C, Vatner SF. Novel mechanisms mediating stunned myocardium. *Heart Fail Rev* 2003;**8**:143–153.
42. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiol Rev* 2008;**88**:581–609.
43. Piper HM. The calcium paradox revisited: an artifact of great heuristic value. *Cardiovasc Res* 2000;**45**:123–127.
44. Bush LR, Romson JL, Ash JL, Lucchesi BR. Effect of diltiazem on extent of ultimate myocardial injury resulting from temporary coronary artery occlusion in dogs. *J Cardiovasc Pharmacol* 1982;**4**:285–296.
45. Lamping KA, Gross GJ. Improved recovery of myocardial segment function following a short-coronary occlusion in dogs by nicorandil, a potential new antianginal agent, and nifedipine. *J Cardiovasc Pharmacol* 1985;**7**:158–166.

46. Przyklenk K, Ghafari GB, Eitzman DT, Kloner RA. Nifedipine administered after reperfusion ablates systolic contractile dysfunction of postischemic 'stunned' myocardium. *J Am Coll Cardiol* 1989;**13**:1176–1183.
47. Gross GJ, Farber NE, Pieper NE. Effects of amlodipine on myocardial ischemia-reperfusion injury in dogs. *Am J Cardiol* 1989;**64**:94I–100I.
48. duToit EF, Opie LH. Modulation of severity of reperfusion stunning in the isolated rat hearts by the agents altering calcium flux at the onset of reperfusion. *Circ Res* 1992;**70**:960–967.
49. Hoff PT, Tamura Y, Lucchesi BR. Cardioprotective effects of amlodipine in the ischemic-reperfused heart. *Am J Cardiol* 1989;**64**:101I–115I.
50. García-Dorado D, Theroux P, Fernandez-Aviles F, Elizaga J, Solares J, Galinanes M. Diltiazem and progression of myocardial ischemic damage during coronary artery occlusion and reperfusion in porcine hearts. *J Am Coll Cardiol* 1987;**10**:906–911.
51. Bersohn MM, Morey AK, Weiss RS. Sarcolemmal calcium transporters in myocardial ischemia. *J Mol Cell Cardiol* 1997;**29**:2525–2532.
52. Zucchi R, Ronca-Testoni S, Yu G, Galbani P, Ronca G, Mariani M. Are dihydropyridine receptors downregulated in the ischemic myocardium? *Cardiovasc Res* 1995;**30**:769–774.
53. Sallinen P, Mänttäri S, Leskinen H, Ilves M, Ruskoaho H, Saarela S. Time course of changes in the expression of DHPR, RyR2, and SERCA2 after myocardial infarction in the rat left ventricle. *Mol Cell Biochem* 2007;**303**:97–103.
54. The HINT Research Group. Early treatment of unstable angina in the coronary care unit, a randomized, double-blind placebo controlled comparison of recurrent ischemia in patients treated with nifedipine or metoprolol or both. *Br Heart J* 1986;**56**:400–413.
55. Theroux P, Gregoire J, Chin C, Pelletier G, de Guise P, Juneau M. Intravenous diltiazem in acute myocardial infarction. DATA trial. *J Am Coll Cardiol* 1998;**32**:620–628.
56. Sheiban I, Tonni S, Chizzoni A, Marini A, Trevi G. Recovery of left ventricular function following early reperfusion in acute myocardial infarction: a potential role for the calcium antagonist nisoldipine. *Cardiovasc Drugs Ther* 1997;**11**:5–16.
57. Misquitta CM, Mack DP, Grover AK. Sarco/endoplasmic reticulum $Ca^{2+}$ (SERCA)-pumps: link to heart beats and calcium waves. *Cell Calcium* 1999;**25**:277–290.
58. Periasamy M, Huke S. SERCA pump level is a critical determinant of Ca(2+)homeostasis and cardiac contractility. *J Mol Cell Cardiol* 2001;**33**:1053–1063.
59. Frank KF, Bolck B, Erdmann E, Schwinger RH. Sarcoplasmic reticulum $Ca^{2+}$-ATPase modulates cardiac contraction and relaxation. *Cardiovasc Res* 2003;**57**:20–27.
60. Lee KS, Ladinsky H, Stuckey JH. Decreased $Ca^{2+}$ uptake by sarcoplasmic reticulum after coronary artery occlusion for 60 and 90 min. *Circ Res* 1967;**21**:439–444.
61. Toba K, Katagiri T, Takeyama Y. Studies of the sarcoplasmic reticulum in myocardial infarction. *Jpn Circ J* 1978;**42**:447–453.
62. Krause S, Hess ML. Characterization of cardiac sarcoplasmic reticulum dysfunction during short-term, normothermic, global ischemia. *Circ Res* 1984;**55**:176–184.
63. Kaplan P, Hendrikx M, Mattheussen M, Mubagwa K, Flameng W. Effect of ischemia and reperfusion on sarcoplasmic reticulum calcium uptake. *Circ Res* 1992;**71**:1123–1130.
64. Mubagwa K. Sarcoplasmic reticulum function during myocardial ischaemia and reperfusion. *Cardiovasc Res* 1995;**30**:166–175.
65. Zucchi R, Ronca-Testoni S, Di Napoli P, Yu G, Gallina S, Bosco G *et al*. Sarcoplasmic retciculum calcium uptake in human myocardium subjected to ischemia and reperfusion during cardiac surgery. *J Mol Cell Cardiol* 1996;**28**:1693–1701.
66. del Monte F, Lebeche D, Guerrero JL, Tsuji T, Doye AA, Gwathmey JK *et al*. Abrogation of ventricular arrhythmias in a model of ischemia and reperfusion by targeting myocardial calcium cycling. *Proc Natl Acad Sci USA* 2004;**101**:5622–5627.
67. Niwano K, Arai M, Koitabashi N, Watanabe A, Ikeda Y, Miyoshi H *et al*. Lentiviral vector-mediated SERCA2 gene transfer protects against heart failure and left ventricular remodeling after myocardial infarction in rats. *Mol Ther* 2008;**16**:1026–1032.
68. Miyamoto MI, del Monte F, Schmidt U, DiSalvo TS, Kang ZB, Matsui T *et al*. Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic-banded rats in transition to heart failure. *Proc Natl Acad Sci USA* 2000;**97**:793–798.
69. Byrne MJ, Power JM, Preovolos A, Mariani JA, Hajjar RJ, Kaye DM. Recirculating cardiac delivery of AAV2/1SERCA2a improves myocardial function in an experimental model of heart failure in large animals. *Gene Ther* 2008;**15**:1550–1557.
70. Talukder MA, Kalyanasundaram A, Zuo L, Velayutham M, Nishijima Y, Periasamy M *et al*. Is reduced SERCA2a expression detrimental or beneficial to postischemic cardiac function and injury? Evidence from heterozygous SERCA2a knockout mice. *Am J Physiol Heart Circ Physiol* 2008;**294**:H1426–H1434.
71. Talukder MA, Yang F, Nishijima Y, Chen CA, Kalyanasundaram A, Periasamy M *et al*. Reduced SERCA2a converts sub-lethal myocardial injury to infarction and affects postischemic functional recovery. *J Mol Cell Cardiol* 2009;**46**:285–287.
72. Talukder MA, Kalyanasundaram A, Xue Z, Zuo L, Bhupathy P, Babu GJ *et al*. Expression of SERCA isoform with faster $Ca^{2+}$ transport properties improves postischemic cardiac function, $Ca^{2+}$ handling, and decreases myocardial infarction. *Am J Physiol Heart Circ Physiol* 2007;**293**:H1418–H1428.
73. Piper HM, Kasseckert S, Abdallah Y. The sarcoplasmic reticulum as the primary target of reperfusion protection. *Cardiovasc Res* 2006;**70**:170–173.
74. Teucher N, Prestle J, Seidler T, Currie S, Elliott EB, Reynolds DF *et al*. Excessive sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase expression causes increased sarcoplasmic reticulum $Ca^{2+}$ uptake but decreases myocyte shortening. *Circulation* 2004;**110**:3553–3559.
75. Chen Y, Escoubet B, Prunier F, Amour J, Simonides WS, Vivien B *et al*. Constitutive cardiac overexpression of sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase delays myocardial failure after myocardial infarction in rats at a cost of increased acute arrhythmias. *Circulation* 2004;**109**:1898–1903.
76. Northover BJ. Effects of pretreatment with caffeine or ryanodine on the myocardial response to simulated ischaemia. *Br J Pharmacol* 1991;**103**:1225–1229.
77. Du Toit EF, Opie LH. Modulation of severity of reperfusion stunning in isolated rat heart by agents altering calcium flux at onset of reperfusion. *Circ Res* 1992;**70**:960–970.
78. Mubagwa K, Kaplan P, Flameng W. The effects of ryanodine on calcium uptake by the sarcoplasmic reticulum of ischemic and reperfused rat myocardium. *Fundam Clin Pharmacol* 1997;**11**:315–321.
79. Zucchi R, Ronca-Testoni S. The sarcoplasmic reticulum $Ca^{2+}$ channel/ryanodine receptor: modulation by endogenous effectors, drugs and disease states. *Pharmacol Rev* 1997;**49**:1–51.
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 $Ca^{2+}$ channels. *Circ Res* 1994;**74**:271–280.
81. Valdivia C, Hegge JO, Lasley RD, Valdivia HH, Mentzer R. Ryanodine receptor dysfunction in porcine stunned myocardium. *Am J Physiol Heart Circ Physiol* 1997;**273**:H796–H804.
82. Xu Y-J, Chapman D, Dixon IMC, Sethi R, Guo X, Dhalla NS. Differential gene expression in infarct scar and viable myocardium from rat heart following coronary ligation. *J Cell Mol Med* 2004;**8**:85–92.
83. Dixon IM, Kaneko M, Hata T, Panagia V, Dhalla NS. Alterations in cardiac membrane $Ca^{2+}$ transport during oxidative stress. *Mol Cell Biochem* 1990;**99**:125–133.
84. Zima AV, Blatter LA. Redox regulation of cardiac calcium channels and transporters. *Cardiovasc Res* 2006;**71**:310–321.
85. Cherednichenko G, Zima AV, Feng W, Schaefer S, Blatter LA, Pessah IN. NADH oxidase activity of rat cardiac sarcoplasmic reticulum regulates calcium-induced calcium release. *Circ Res* 2004;**94**:478–486.
86. Blaustein M, Lederer J. Sodium/calcium exchange: its physiological implications. *Physiol Rev* 1999;**79**:763–854.
87. Tani M, Neely JR. Role of intracellular $Na^+$ in $Ca^{2+}$ overload and depressed recovery in ventricular function of reperfused ischemic rat hearts. Possible involvement of $H^+$-$Na^+$ and $Na^+$-$Ca^{2+}$ exchange. *Circ Res* 1989;**65**:1045–1056.
88. Cross HR, Lu L, Steenbergen C, Philipson KD, Murphy E. Overexpression of the cardiac $Na^+/Ca^{2+}$ exchanger increases susceptibility to ischemia/reperfusion in male, but not female, transgenic mice. *Circ Res* 1998;**83**:1215–1223.
89. Ohtsuka M, Takano H, Suzuki M, Zou Y, Akazawa H, Tamagawa M *et al*. Role of $Na^+$-$Ca^{2+}$ exchanger in myocardial ischemia/reperfusion injury: evaluation using a heterozygous $Na^+$-$Ca^{2+}$ exchanger knockout mouse model. *Biochem Biophys Res Commun* 2004;**314**:849–853.
90. Imahashi K, Christian P, Goldhaber JI, Steenberger C, Philipson KD, Murphy E. Cardiac-specific ablation of the $Na^+$-$Ca^{2+}$ exchanger confers protection against ischemia/reperfusion injury. *Circ Res* 2005;**97**:916–921.
91. Inserte J, Garcia-Dorado D, Ruiz-Meana M, Padilla F, Barrabés JA, Pina P *et al*. Effect of inhibition of the $Na^+/Ca^{2+}$ exchanger at the time of

myocardial reperfusion on hypercontracture and cell death. *Cardiovasc Res* 2002;**55**:739–748.
92. Karmazyn M, Gan XT, Humphrevs RA, Yoshida H, Kusumoto K. The myocardial Na(+)-H(+) exchange: structure, regulation, and its role in heart disease. *Circ Res* 1999;**85**:777–786.
93. Karmazyn M. Amiloride enhances postischemic ventricular recovery: possible role of $Na^+$-$H^+$ exchange. *Am J Physiol Heart Circ Physiol* 1988;**255**:H608–H615.
94. Levitsky J, Gurell D, Frishman WH. Sodium ion/hydrogen ion inhibition: a new pharmacologic approach to myocardial ischemia and reperfusion injury. *J Clin Pharmacol* 1998;**38**:887–897.
95. MacLennan DH, Kranias EG. Phospholamban: a crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol* 2003;**4**:566–577.
96. Schoutsen B, Blom JJ, Verdouw PD, Lamers JM. Calcium transport and phospholamban in sarcoplasmic reticulum of ischemic myocardium. *J Mol Cell Cardiol* 1989;**21**:719–727.
97. Vittone L, Mundina-Weilenmann C, Mattiazzi A. Phospholamban phosphorylation by CaMKII under pathophysiological conditions. *Front Biosci* 2008;**13**:5988–6005.
98. Weber T, Neumann J, Meißner A, Hartlage MG, Aken HV, Hanske G *et al*. Reduced serine-16 and threonine-17 phospholamban phosphorylation in stunning of conscious dogs. No evidence for any involvement of protein kinase A or protein phosphatases. *Basic Res Cardiol* 2006;**101**:253–260.
99. Cross HR, Kranias EG, Murphy E, Steenbergen C. Ablation of PLB exacerbates ischemic injury to a lesser extent in female than male mice: protective role of NO. *Am J Physiol Heart Circ Physiol* 2003;**284**: H683–H690.
100. Maier LS, Bers DM. Role of $Ca^{2+}$/calmodulin-dependent protein kinase (CaMK) in excitation-contraction coupling in the heart. *Cardiovasc Res* 2007;**73**:631–640.
101. Anderson ME. Calmodulin kinase signaling in heart: an intriguing candidate target for therapy of myocardial dysfunction and arrhythmias. *Pharmacol Ther* 2005;**106**:39–55.
102. Couchonnal LF, Anderson ME. The role of calmodulin kinase II in myocardial physiology and disease. *Physiol* 2008;**23**:151–159.
103. Vila-Petroff M, Salas MA, Said M, Valverde CA, Sapia L, Portiansky E *et al*. CaMKII inhibition protects against necrosis and apoptosis in irreversible ischemia-reperfusion. *Cardiovasc Res* 2007;**73**:689–698.
104. Currie S, Smith GL. Calcium/calmodulin-dependent protein kinase II activity is increased in sarcoplasmic reticulum from coronary artery ligated rabbit hearts. *FEBS Lett* 1999;**459**:244–248.
105. Dirksen MT, Laarman GJ, Simoons ML, Duncker DJGM. Reperfusion injury in humans: a review of clinical trials on reperfusion injury inhibitory strategies. *Cardiovasc Res* 2007;**74**:343–355.
106. Moens AL, Claeys MJ, Timmermans JP, Vrints CJ. Myocardial ischemia/reperfusion-injury, a clinical view on a complex pathophysiological process. *Intl J Cardiol* 2005;**100**:179–190.
107. Wang Q-D, Pernow J, Sjöquist P-O, Rydén L. Pharmacological possibilities for protection against myocardial reperfusion injury. *Cardiovasc Res* 2002;**55**:25–37.
108. Kloner RA, Rezkalla SH. Cardiac protection during acute myocardial infarction: where do we stand in 2004? *J Am Coll Cardiol* 2004;**44**: 276–286.
109. Wijeysundera DN, Beattie WS, Rao V, Karski J. Calcium antagonist reduce cardiovascular complications after cardiac surgery: a meta-analysis. *J Am Coll Cardiol* 2003;**41**:1496–1505.
110. Costanzo P, Perrone-Filardi P, Petretta M, Marciano C, Vassallo E, Gargiulo P *et al*. Calcium channel blockers and cardiovascular outcomes: a meta-analysis of 175634 patients. *J Hypertens* 2009;**27**: 1136–1151.
111. Zucchi R, Ronca F, Ronca-Testoni S. Modulation of sarcoplasmic reticulum function: a new strategy in cardioprotection? *Pharmacol Ther* 2001;**89**:47–65.
112. Vangheluwe P, Sipido KR, Raeymaekers L, Wuytack F. New perspectives on the role of SERCA2's $Ca^{2+}$ affinity in cardiac function. *Biochim Biophys Acta* 2006;**1763**:1216–1228.
113. Ghali JK, Smith WB, Torre–Amione G, Haynos W, Rayburn BK, Amato A *et al*. A phase 1-2 dose-escalating study evaluating the safety and tolerability of Istaroxime and specific effects on electrocardiographic and hemodynamic parameters in patients with chronic heart failure with reduced systolic function. *Am J Cardiol* 2007;**99**(suppl.):47A–56A.
114. Micheletti R, Palazzo F, Barassi P, Giacalone G, Ferrandi M, Schiavone A *et al*. Istaroxime, a stimulator of sarcoplasmic reticulum calcium adenosine triphosphatase isoform 2a activity, as a novel therapeutic approach to heart failure. *Am J Cardiol* 2007;**99**(suppl.):24A–32A.
115. Yu G, Zucchi R, Ronca-Testoni S, Ronca G. Protection of ischemic rat heart by dantrolene, an antagonist of the sarcoplasmic reticulum calcium release channel. *Basic Res Cardiol* 2000;**95**:137–143.
116. Ward A, Chaffman MO, Sorkin EM. Dantrolene. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in malignant hyperthermia, the neuroleptic malignant syndrome and an update of its use in muscle spasticity. *Drugs* 1986;**32**:130–168.
117. Koehntop DE, Beebe DS, Belani KG. The safety of dantrolene in a patient with severe cardiomyopathy requiring a heart transplant. *Anesth Analg* 1997;**85**:229–230.
118. Lee C, Dhalla NS, Hryshko LV. Therapeutic potential of novel $Na^+$-$Ca^{2+}$ exchange inhibitors in attenuating ischemia-reperfusion injury. *Can J Cardiol* 2005;**21**:509–516.
119. Karmazyn M. Pharmacology and clinical assessment of cariporide for the treatment coronary artery disease. *Exp Opin Invest Drugs* 2000;**9**: 1099–1108.
120. Avkiran M, Marber MS. $Na^+/H^+$ exchange inhibitors for cardioprotective therapy: progress, problems and prospects. *J Am Coll Cardiol* 2002;**39**: 747–753.
121. Murphy E, Allen DG. Why did the NHE inhibitor clinical trial fail? *J Mol Cell Cardiol* 2009;**46**:137–141.
122. Mentzer RM, Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B *et al*. Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPEDITION study. *Ann Thorac Surg* 2008;**85**: 1261–1270.
123. Jang I-K, Pettigrew V, Picard MH, Kowey PR, Demmel V, Zile MR *et al*. A randomized, double-blind, placebo-controlled study of the safety and efficacy of intravenous MCC-135 as an adjunct to primary percutaneous coronary intervention in patients with acute myocardial infarction: rationale and design of the evaluation of MCC-135 for left ventricular salvage in acute MI (EVOLVE) study. *J Thromb Thrombolysis* 2005;**20**: 147–153.
124. Tzivoni D, Balkin J, Bär FW, Hibberd M, Reiber JHC, Cowing G. Effect of caldaret on the incidence of severe left ventricular dysfunction in patients with ST-elevation myocardial infarction undergoing primary coronary intervention. *Am J Cardiol* 2009;**103**:1–4.