Strategies for the regulation of intracellular calcium in ischemic heart disease

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‘Different noncardiomyocyte targets are also activated during the development of ischemic heart disease and are shown to promote the entry of Ca^{2+} into the myocardium.’

In view of the fact that ischemic heart disease is a complex problem having different etiologies such as atherosclerosis, thrombosis and coronary spasm [1–10], it is important to define its nature for understanding the pathogenesis and outlining therapeutic strategies. Narrowing of the coronary artery by formation of atherosclerotic plaque gradually reduces the coronary blood flow over a prolonged time period and this induces a ‘low-flow’ myocardial ischemia as a consequence of insufficient availability of both oxygen and substrates. Blockade of the coronary vessels by thrombosis or occlusion of the coronary artery by spasm may rapidly stop the blood flow and result in a ‘no-flow’ myocardial ischemia. Thus, the pattern for the development of ischemic injury under no-flow conditions can be seen to be different from that in low-flow myocardial ischemia. Although myocardial ischemia is a stimulus for the opening of the collateral vessels for supply of blood to the ischemic region [11], the timing for the opening of an existing collateral system or the formation of new vessels is considered to further complicate the type and magnitude of ischemic injury. Nonetheless, ischemic injury is commonly associated with varying degrees of arrhythmias, electrolyte imbalance, energy depletion, ultrastructural damage, apoptosis, necrosis and cardiac dysfunction [1,5–7]. Although institution of reflow is critical for salvage of the ischemic myocardium by procedures such as angioplasty, thrombolytic therapy and coronary bypass surgery, the ischemic injury is further exaggerated (reperfusion injury) if the blood flow is not restored within a certain time period of ischemic insult [2,5,6]. It should also be noted that myocardial ischemia results in cell death and the formation of scar tissue (the myocardial infarct);

however, the remaining viable myocardium (depending upon the size of the myocardial infarct), becomes hypertrophic and subsequently exhibits the signs and symptoms of congestive heart failure [12–14]. Thus, a great deal of caution should be exercised while discussing the effects of acute and chronic ischemic heart disease as well as early and late stages of myocardial infarction. This article addresses the pathophysiologic events in the ischemic–reperfused myocardium under both acute and chronic ischemic heart disease conditions.

Mechanisms of ischemic–reperfusion injury

Although risk factors such as diabetes and hypertension, which predispose the myocardium to ischemic injury, can be seen to modify the time-course as well as the pattern of ischemic changes, it is the reduction in blood flow and subsequent lack of oxygen and substrate that impair energy (ATP) production by mitochondria and produce reactive oxygen species such as superoxide radicals, hydrogen peroxide and hydroxyl radicals [15]. Several noncardiomyocyte targets (Box 1), including sympathetic and purinergic nerves, endothelium, neutrophils, leukocytes, platelets and the renin–angiotensin system, are also considered to participate in the development of ischemic injury [15].

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These targets are activated (in response to reduced blood flow to the myocardium and subsequent hemodynamic alterations) and release a wide variety of inflammatory cytokines and other mediators to produce oxidative stress [16–23]. It should be mentioned that formation of Ang II due to the activation of the rennin–angiotensin system may produce oxidative stress through its action on NADP/NADPH oxidase, whereas excessive amounts of catecholamines may produce oxyradicals during the process of

Box 1. Targets that are associated with the development of intracellular Ca^{2+} overload in the ischemic myocardium.

- Mitochondria in cardiomyocytes: superoxide radicals, hydrogen peroxide and hydroxyl radicals
- Sympathetic nerves: catecholamines and oxidation products
- Endothelium: nitric oxide, peroxynitrite and endothelin
- Neutrophils and leukocytes: hypochlorous acid and cytokines
- Platelets: serotonin
- Purinergic nerves: adenosine triphosphate
- Renin–angiotensin system: Ang II

oxidation [24–26]. Furthermore, nitric oxide produced by the endothelium upon combination with superoxide radicals will generate peroxynitrite, a highly reactive oxidant [26].

‘...intracellular Ca^{2+} overload has been shown to produce activation of proteases and phospholipases, impair energy production, alter gene expression and induce myocardial cell damage and dysfunction.’

Accordingly, it is our contention that oxidative stress caused by the excessive formation of reactive oxygen species, peroxynitrite and hypochlorous acid as well as various hormones, oxidation products of catecholamines and different cytokines sets the stage for ischemic–reperfusion injury to the myocardium. Although oxidative stress can be seen to produce cardiac dysfunction and myocardial cell damage by affecting different subcellular organelles directly, most of its effects (which simulate the ischemic–reperfusion injury) are considered to be mediated through the occurrence of an excessive amount of Ca^{2+} (intracellular Ca^{2+} overload) in the myocardium [27–28]. In fact, intracellular Ca^{2+} overload has been shown to produce activation of proteases and phospholipases, impair energy production, alter gene expression and induce myocardial cell damage and dysfunction [24,28,29].

Calcium handling by subcellular organelles in cardiomyocytes

Since intracellular Ca^{2+} overload is known to play a critical role in the development of cardiac dysfunction and myocardial cell damage in ischemic heart disease [28], it is prudent to discuss various mechanisms that are considered to maintain the intracellular Ca^{2+} at a low level (10^{-7} to 10^{-6} M) in cardiomyocytes under control conditions. As shown in Table 1, different subcellular organelles, such as the cell membrane

(sarcolemma), sarcoplasmic reticulum, mitochondria, nucleus and extracellular matrix, participate in the regulation of intracellular Ca^{2+} [7,27,30]. While sarcolemmal voltage-dependent Ca^{2+} channels and Ca^{2+} -pump ATPase are directly involved in Ca^{2+} influx and Ca^{2+} efflux, respectively, sarcolemmal Na^{+} - K^{+} ATPase, Na^{+} - Ca^{2+} exchanger, Na^{+} - H^{+} -exchanger and Na^{+} channels are involved in maintaining the intracellular concentration of Na^{+} [31–33]. The sarcolemmal Na^{+} - Ca^{2+} exchanger is also considered to be involved in Ca^{2+} efflux [30,31] whereas sarcolemmal Ca^{2+} and Mg^{2+} ecto-ATPase and superficial Ca^{2+} stores present in the extracellular matrix or glycocalyx have been proposed to participate in Ca^{2+} entry [30,34–36]. Both β -adrenoceptors and adenylyl cyclase located in the sarcolemma are also known to promote the entry as well as efflux of Ca^{2+} upon phosphorylating Ca^{2+} channels and Ca^{2+} pump ATPase in cardiomyocytes [31], whereas α -adrenoceptors are considered to enhance the entry of Ca^{2+} through the activation of phospholipase C [37]. Thus, the sarcolemmal membrane, owing to the presence of multiple Ca^{2+} handling and regulatory sites, and glycocalyx, owing to the presence of superficial Ca^{2+} storage mechanism, can be seen to maintain the concentration of intracellular Ca^{2+} at a low level in comparison with the extracellular concentration of free Ca^{2+} (1.25 mM).

There is ample evidence to suggest that the sarcoplasmic reticulum is a major intracellular organelle, which, by virtue of the presence of Ca^{2+} -release channels and Ca^{2+} -pump ATPase, is intimately involved in raising and lowering the cytoplasmic concentration of free Ca^{2+} for cardiac contraction and relaxation, respectively [30].

‘...intracellular organelles ... regulate the cytoplasmic concentration of free Ca^{2+} when cardiomyocytes are faced with intracellular Ca^{2+} overload.’

Furthermore, different mechanisms involving phospholamban, Ca^{2+} -calmodulin protein kinase and inositol-3-phosphate receptors are considered to regulate the Ca^{2+} release and uptake processes in the sarcoplasmic reticulum [30,38,39]. On the other hand, the mitochondria is considered to be a Ca^{2+} sink where an antiporter Ca^{2+} -transport system and Na^{+} - Ca^{2+} exchange system are located for dealing with high concentrations of Ca^{2+} and Na^{+} in the cytoplasm [15,30,40]. Although the nucleus has also been shown to accumulate a large amount of Ca^{2+} in

Table 1. Subcellular structures involved in the regulation of intracellular calcium in cardiomyocytes.

Structures	Sites
Sarcolemma	Ca ²⁺ channels, Ca ²⁺ -pump ATPase, Na ⁺ –K ⁺ ATPase, Na ⁺ –Ca ²⁺ exchanger, Na ⁺ –H ⁺ -exchanger, Na ⁺ -channels, β-adrenoceptors, α-adrenoceptors, adenylyl cyclase and Ca ²⁺ –Mg ²⁺ ecto ATPase
Sarcoplasmic reticulum	Ca ²⁺ -release channels, Ca ²⁺ -pump ATPase, phospholamban, Ca ²⁺ -calmodulin kinase and inositol-3-phosphate receptors
Mitochondria	Ca ²⁺ -uptake system and Na ⁺ –Ca ²⁺ exchanger
Nucleus	Ca ²⁺ -transport system
Extracellular matrix	Superficial Ca ²⁺ stores

cardiomyocytes [15,30], its Ca²⁺-transport system is not well characterized. Thus, intracellular organelles such as sarcoplasmic reticulum, mitochondria and nucleus can be seen to regulate the cytoplasmic concentration of free Ca²⁺ when cardiomyocytes are faced with intracellular Ca²⁺ overload, as in the case of ischemic heart disease.

Mechanisms for the development of intracellular calcium overload

It is now well known that both the myocardial Ca²⁺ content and cytoplasmic concentration of free Ca²⁺ are increased in ischemic heart disease [28,41–48]. It should be pointed out that unlike the irreversible stage of ischemic injury, myocardial Ca²⁺ content is not altered at early phases of ischemic injury. Furthermore, the cytoplasmic concentration of free Ca²⁺ is only elevated in cardiomyocytes from hearts subjected to irreversible ischemic injury, unlike the reversible ischemic injury [41,42,49,50]. Since ischemia produces accumulation of hydrogen, the stimulation of the sarcolemmal Na⁺–H⁺ exchanger followed by the activation of the sarcolemmal Na⁺–Ca²⁺ exchanger is considered to be a mechanism for the increase of Ca²⁺ influx as well as cytoplasmic levels of free Ca²⁺ in ischemic myocardium [7,32,51]. However, this mechanism is confounded by the fact that inhibition of the Na⁺–H⁺ exchanger with agents such as methyl-*N*-isobutyl amiloride was observed to increase the cytoplasmic concentration of hydrogen, which caused the release of Ca²⁺ from the sarcoplasmic reticulum and resulted in an increase in the cytoplasmic concentration of Ca²⁺ [32,52]. Thus, inhibitors of the Na⁺–H⁺ exchanger may not be a suitable therapy for the treatment of ischemic heart disease. Since sarcolemmal Na⁺–K⁺ ATPase is also inhibited in both ischemic and ischemic-reperfused heart [53–55],

this can be seen to increase the intracellular concentration of Na⁺ followed by the activation of the sarcolemmal Na⁺–Ca²⁺ exchanger and an increase in the intracellular concentration of free Ca²⁺. Increased entry of Na⁺ upon the opening of sarcolemmal Na⁺-channels in the ischemic myocardium can also be seen to promote the activation of the sarcolemmal Na⁺–Ca²⁺ exchanger and promote the entry of Ca²⁺. Thus, the activation of the sarcolemmal Na⁺–H⁺ exchanger and Na⁺ channels as well as the inhibition of the sarcolemmal Na⁺–K⁺ ATPase can be seen to increase the intracellular concentration of Na⁺ and thereby increase the entry of Ca²⁺ through the participation of the sarcolemmal Na⁺–Ca²⁺ exchanger. Accordingly, it appears that inhibitors of the Na⁺–Ca²⁺ exchanger reverse mode would be of great promise for reducing the intracellular Ca²⁺ overload and subsequent ischemic–reperfusion injury.

Activation of voltage-dependent Ca²⁺ channels by the accumulation of long-chain acylcarnitine and fatty acids in the sarcolemmal membrane in the ischemic heart is also considered to promote the entry of Ca²⁺ [24].

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In this regard, different types of Ca²⁺-antagonists have been shown to produce beneficial effects against ischemic–reperfusion injury by reducing the development of intracellular Ca²⁺ overload [25,56,57]. Although sarcolemmal Ca²⁺–Mg²⁺ ecto-ATPase and superficial Ca²⁺-stores in the extracellular matrix have also been suggested to participate in the development of intracellular Ca²⁺ overload in the ischemic–reperfused myocardium [35], new

agents need to be developed for the control of Ca^{2+} through these mechanisms. The participation of both β -adrenoceptors and α -adrenoceptors in the sarcolemmal membrane for promoting Ca^{2+} influx is evident from the fact that a large amount of catecholamines are released from the sympathetic nerve ending [24] and different β -adrenoceptor and α -adrenoceptor blocking agents are found to exert beneficial effects in ischemic heart disease [26].

'...control of Ca^{2+} in ischemic-reperfused myocardium cannot be achieved by any single drug affecting one site, but instead a combination therapy modifying various sites is required for reducing intracellular Ca^{2+} overload in the heart.'

Since release of catecholamines from the sympathetic nerve endings is accompanied by the release of ATP, and since the extracellular ATP has been shown to increase Ca^{2+} influx in cardiomyocytes [42,58], it is possible that ATP-induced signal transduction may be involved in the induction of intracellular Ca^{2+} -overload in ischemic heart disease. Activation of purinergic nerves and the serotonergic system as well as of platelets is also apparent in the development of intracellular Ca^{2+} overload because an ATP-receptor antagonist (pyridoxal 5'-phosphate) and a serotonin-receptor antagonist (sarpogrelate) were observed to attenuate ischemic-reperfusion injury [58–60]. In addition to the involvement of various sarcolemmal sites for promoting the entry of Ca^{2+} in the ischemic-reperfused myocardium, changes in both Ca^{2+} -release channels and the Ca^{2+} -uptake system in the sarcoplasmic reticulum have been identified to increase the cytoplasmic concentration of Ca^{2+} in cardiomyocytes [61–63]. However, no satisfactory intervention except ischemic preconditioning has yet become available for the stabilization of sarcoplasmic reticulum function in ischemic heart disease [62–64]. It should be emphasized that mitochondria are known to serve as a Ca^{2+} sink during the development of intracellular Ca^{2+} overload in the ischemic reperfused heart and this mitochondria Ca^{2+} overload impairs their ability to produce ATP [40,45]. Ischemia-reperfusion has also been shown to produce marked changes in cardiac gene expression for both sarcoplasmic reticulum and sarcolemmal proteins [53,61–63] and this can be seen to affect cardiac function in the late stages of ischemic heart disease.

Subcellular defects & oxidative stress

From the foregoing discussion, it is clear that not only is the myocardial Ca^{2+} content increased but the intracellular concentration of free Ca^{2+} is also increased during the irreversible damage caused by ischemic heart disease. This would imply that the net gain of intracellular Ca^{2+} in cardiomyocytes is from extracellular sources and this involves multisite mechanisms in the sarcolemma, whereas the increase in the cytoplasmic concentration of free Ca^{2+} may be caused by changes in the Ca^{2+} -handling abilities of the subcellular organelles such as the sarcoplasmic reticulum, mitochondria and nucleus. It should be noted that ischemia-reperfusion and hypoxia-reoxygenation have been shown to produce marked changes in the sarcolemmal Na^+/K^+ ATPase, $\text{Na}^+/\text{Ca}^{2+}$ exchange, Ca^{2+} -pump ATPase and Ca^{2+} channel [7], the sarcoplasmic reticulum Ca^{2+} -release channel and Ca^{2+} -pump ATPase [7] as well as mitochondrial function [7,40]. Such alterations favoring the development of intracellular Ca^{2+} overload appear to be due to the occurrence of oxidative stress since antioxidant therapy and ischemic preconditioning, which reduces oxidative stress, were found to attenuate these changes in ischemic-reperfused hearts [18,52,54,60,63]. Furthermore, these alterations were simulated by interventions that generate oxidative stress. In fact, the increased cytoplasmic concentration of free Ca^{2+} in cardiomyocytes following ischemia-reperfusion was not only attenuated by antioxidant therapy but was also stimulated by interventions generating oxidative stress [41,42].

'The occurrence of oxidative stress from various sources appears to account for the development of intracellular Ca^{2+} overload.'

On the other hand, catecholamine- and ATP-induced increases in the cytoplasmic concentration of free Ca^{2+} were depressed in the ischemic-reperfused hearts [41,42], which may be caused by alterations in the receptor-mediated signal transduction mechanisms located in the sarcolemmal membrane [41,42]. Since ischemia-reperfusion has also been reported to produce dramatic changes in both cardiac genes corresponding to different Ca^{2+} -handling proteins in the sarcoplasmic reticulum and sarcolemmal membrane [53,60–62], and these changes were attenuated by antioxidant therapy [53,60,63], it appears that these defects may account for

remodeling of the membrane system and, thus, may result in Ca^{2+} -handling defects in the cardiomyocytes under chronic ischemic heart disease conditions. In view of the multisite defects in ischemic heart disease for inducing intracellular Ca^{2+} overload in cardiomyocytes, it is emphasized that the control of Ca^{2+} in ischemic–reperfused myocardium cannot be achieved by any single drug affecting one site, but instead a combination therapy modifying various sites is required for reducing intracellular Ca^{2+} overload in the heart.

Conclusion

While both acute and chronic phases of ischemic heart disease are associated with cardiac dysfunction, arrhythmias, myocardial cell damage, apoptosis and necrosis, the ischemia–reperfusion injury has been observed to produce a net gain in myocardial Ca^{2+} content and an increase in the cytoplasmic concentration of free Ca^{2+} . Several cardiomyocyte-associated mechanisms, including defects in Ca^{2+} handling and regulatory proteins in both the sarcolemma and sarcoplasmic reticulum, have been identified for the development of intracellular Ca^{2+} overload following

ischemic–reperfusion injury. Different noncardiomyocyte targets are also activated during the development of ischemic heart disease and are shown to promote the entry of Ca^{2+} into the myocardium. The occurrence of oxidative stress from various sources appears to account for the development of intracellular Ca^{2+} overload. Thus, a multidrug therapy should be considered for the reduction of intracellular Ca^{2+} overload and subsequent attenuation of pathophysiological alterations associated with ischemic heart disease.

Financial & competing interests disclosure

This study was supported by a grant from the Canadian Institutes of Health Research. Harjot K Saini-Chohan was a predoctoral fellow of the Heart & Stroke Foundation of Canada. Todd A Duhamel, a postdoctoral fellow, was supported by the CIHR/Heart and Stroke Foundation of Canada IMPACT Program. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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