# Chapter 16
# Subcellular Remodeling and Cardiac Dysfunction Due to Ischemia–Reperfusion Injury

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**Abstract** Ischemic heart disease as a consequence of the blockade of coronary flow is associated with dramatic changes in cardiac function, metabolism, and ultrastructure. A wide variety of subcellular defects have been observed in ischemic and ischemia–reperfusion (I/R) hearts. There is evidence that various subcellular organelles become remodeled during the development of I/R injury and oxidative stress may be intimately involved in producing these abnormalities. In view of the direct participation of the sarcoplasmic reticulum (SR) and myofibrils in heart function, it appears that cardiac contraction and relaxation abnormalities in ischemic heart disease are due to remodeling of the SR and myofibrils, whereas remodeling of the sarcolemma membrane may determine the extent of intracellular $Ca^{2+}$ overload, subsequent proteolysis, and irreversible injury to the heart. Furthermore, the acute effects of I/R injury on cardiac function are thought to be due to changes in the activities of subcellular organelles as a consequence of functional group modification, whereas the chronic effects of I/R yielding delayed recovery of cardiac function may be the consequence of changes in cardiac gene expression and subcellular remodeling. Although female hearts are less susceptible to I/R injury, in comparison to males, the basis for this gender difference in cardiac ischemic injury and protection needs to be defined. As females lose their resistance to different cardiovascular diseases after menopause, it appears that gender differences in cardiac susceptibility

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to I/R injury may be mediated through the participation of ovarian hormones. On the other hand, it is possible that the male sex hormone, testosterone, exacerbates I/R-induced cardiac dysfunction in adult males. Notably, in comparison to males, there is very little information in the literature on subcellular remodeling or on the mechanisms which regulate cardiac function during the development of I/R injury in female hearts.

**Keywords** Ischemia–reperfusion injury • Subcellular remodeling • Oxidative stress • Intracellular $Ca^{2+}$ overload • Proteases • Gender differences

## 16.1 Introduction

Ischemic heart disease as a consequence of the blockade of coronary flow is associated with dramatic changes in cardiac function, metabolism, and ultrastructure [1, 2]; however, the exact cellular and molecular events leading to contractile dysfunction and derangement of cardiac structure are not fully understood. Although restitution of coronary flow to the ischemic heart is essential for the recovery of cardiac pump function, reperfusion after a certain period of ischemia has been shown to further aggravate the myocardial abnormalities [2–5]. As cardiac contractile defects due to I/R are almost invariably associated with situations such as angioplasty, thrombolytic therapy, cardiac surgery, and cardiac transplantation, studies on I/R injury are highly relevant for understanding the pathophysiology of an important clinical problem, namely, myocardial stunning. Both myocardial ischemia and I/R have been shown to generate different oxyradicals and oxidants such as $H_2O_2$, peroxynitrite, and HOCl, and these are suggested to be responsible for the occurrence of intracellular $Ca^{2+}$ overload due to I/R injury [5–9]. Various active oxygen species such as superoxide radicals, hydroxyl radicals, and $H_2O_2$, which are formed during the development of I/R injury, produce electrical abnormalities [10–12], ultrastructural damage [13], intracellular $Ca^{2+}$ overload [14], and cardiac dysfunction [15]. Both $H_2O_2$ and peroxynitrite have also been reported to activate some proteases and induce cardiac dysfunction [16–19]. Likewise, the intracellular $Ca^{2+}$ overload may induce cardiac dysfunction and cell damage by activating different proteases and phospholipases [20, 21] and thus may modify the activities of various subcellular organelles such as sarcolemma (SL), sarcoplasmic reticulum (SR), myofibrils, and mitochondria. While both intracellular $Ca^{2+}$ overload and oxidative stress have been shown to be involved in producing changes in cardiac gene expression as well as remodeling of subcellular organelles [20–22], oxidative stress seems to play a critical role in the genesis of intracellular $Ca^{2+}$ overload and thus may induce cardiac dysfunction by remodeling of subcellular organelles during the development of I/R injury. A schematic representation of the events involving oxidative stress and intracellular $Ca^{2+}$ overload due to I/R injury is given in Fig. 16.1. This view does not exclude the role of either lipid metabolites or oxidative stress/intracellular $Ca^{2+}$ overload in apoptosis and necrosis commonly seen in ischemic and I/R hearts.

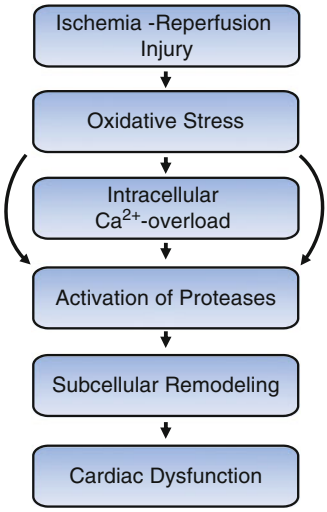


**Fig. 16.1** Involvement of oxidative stress in inducing subcellular remodeling and cardiac dysfunction due to ischemia–reperfusion injury

## 16.2 Subcellular Remodeling and Molecular Abnormalities in I/R Hearts

Over the past 30 years, a wide variety of membrane defects have been observed in both ischemic and I/R hearts [5, 20–24]. It is now clear that the SR $Ca^{2+}$ pump and associated regulatory mechanisms become defective due to changes in the molecular composition of the SR membrane as a consequence of I/R injury [25–35]. Several investigators have reported a reduction in the density of SR $Ca^{2+}$-release channels during I/R [36–39]. Various oxidants as well as hydroxyl radicals were also observed to depress the SR $Ca^{2+}$-pump activity [40, 41]. Although the efficiency of mitochondrial ATP production is impaired at the late stages of I/R injury, depression in both electron transport chain activity and $Ca^{2+}$ transport in mitochondria also occurs at moderate degree of I/R injury [42, 43]. The biochemical activities of several SL membrane proteins including the $Na^+$–$Ca^{2+}$ exchanger, $Ca^{2+}$-stimulated ATPase, $Na^+$–$K^+$ ATPase, and phosphoinositol turnover are markedly altered during myocardial I/R as well as during hypoxia–reoxygenation phases [44–55]. I/R, as well as reactive oxygen species and oxidants, have also been shown to reduce the sensitivity of myofilaments to $Ca^{2+}$ by causing proteolysis of myofibrils [56–62]. These observations provide evidence that various subcellular organelles become remodeled or altered in I/R heart and that oxidative stress may be intimately involved in producing these abnormalities. In view of the direct participation of the SR and myofibrils in heart function, it appears that cardiac contraction and relaxation abnormalities in ischemic heart disease are due to remodeling of SR and myofibrils, whereas remodeling of the SL membrane may determine the extent of intracellular $Ca^{2+}$ overload, subsequent proteolysis, and irreversible injury in the myocardium. It should also be noted that both I/R and oxidative stress have been shown to produce dramatic effects

on cardiac gene expression. We have demonstrated that mRNA levels for the SR $Ca^{2+}$ pump, $Ca^{2+}$ channels, phospholamban, and calsequestrin proteins were depressed in I/R hearts [25]. As $H_2O_2$ and I/R were observed to produce similar changes in SR gene expression, we suggested that these effects of I/R may be due to oxidative stress [25]. We have also observed that I/R produced differential changes in gene expression for SL $Na^+$–$K^+$ ATPase isoforms, and these alterations were simulated by perfusing the hearts with an oxyradical-generating system or $H_2O_2$ [63]. mRNA levels for the SL $Na^+$–$Ca^+$ exchanger were also depressed by I/R [55]. Although intracellular $Ca^{2+}$ overload was demonstrated to occur in I/R hearts [64, 65], it is not known whether alterations in cardiac gene expression are affected by both intracellular $Ca^{2+}$ overload and oxidative stress. Nonetheless, the acute effects of I/R injury on cardiac function are considered to be due to changes in the activities of subcellular organelles and proteins as a consequence of functional group modification, whereas the chronic effects of I/R including delayed recovery of cardiac function may be the consequence of changes in cardiac gene expression and subcellular remodeling.

## 16.3 Oxidative Stress and Development of Intracellular $Ca^{2+}$ Overload in I/R Hearts

Some investigators have demonstrated the generation of oxygen free radicals in I/R hearts by employing electron paramagnetic resonance spectroscopy [66–68]. Oxidative stress has been shown to result in the development of intracellular $Ca^{2+}$ overload due to I/R injury [69–73] because the activities of both SL $Na^+$–$Ca^{2+}$ exchanger and SL $Ca^{2+}$ pump were depressed following hypoxia or I/R as well as upon exposure of heart membranes to oxyradicals [74–78]. Oxyradicals were also reported to alter other SL activities such as $Na^+$–$K^+$ ATPase, $Na^+$–$Ca^{2+}$ exchanger, phospholipid methyltransferase [46, 47, 78–82], $Ca^{2+}$/$Mg^{2+}$ ecto-ATPase, superficial store of $Ca^{2+}$ [83], and ATP receptors [84], which are considered to affect $Ca^{2+}$ movements in the cell. The SL changes in different cation currents have also been observed upon exposure to oxyradicals and oxidants [85–87]. Several other defects such as changes in membrane permeability, loss of dystrophin, and alterations in phospholipases due to I/R injury have also been found in the SL membrane [88–92]. Oxidative stress has also been shown to produce marked alterations in myofibrils, mitochondria, and SR as well as induce autophagy during the development of I/R injury [62, 93, 94]. Thus, the increased formation of oxyradicals and oxidants in I/R hearts may induce a complex set of subcellular alterations with respect to their biochemical composition and functional activities related to $Ca^{2+}$ movements, and these on balance may result in the development of intracellular $Ca^{2+}$ overload and subcellular remodeling.

Oxidative stress seems to alter the subcellular activities by oxidizing different functional groups of subcellular organelles/proteins, and these changes seem to explain the development of intracellular $Ca^{2+}$ overload and cardiac dysfunction due to I/R injury [3, 5, 21]. The effects of oxidative stress favoring the development of

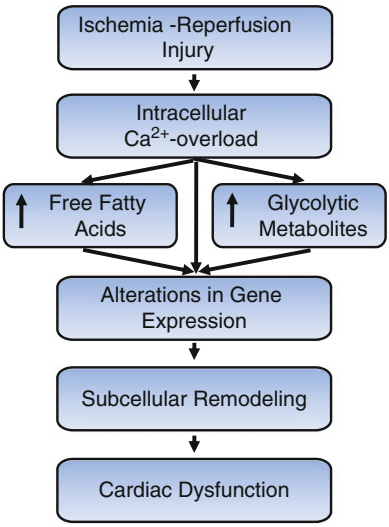


**Fig. 16.2** Role of intracellular $Ca^{2+}$ overload and changes in gene expression in inducing subcellular remodeling and cardiac dysfunction due to ischemia–reperfusion injury

intracellular $Ca^{2+}$ overload are shown to be mediated through the activation of protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and/or stress-activated protein kinase [95–97], as well as translocation of PKC in ischemic heart [98, 99]. It is also noteworthy that besides the production of oxygen-derived free radicals, changes in nitric oxide (NO) metabolism have been observed in I/R hearts [67]. Although NO is known to regulate various events, its action becomes toxic by reaction with superoxide anion forming a potent oxidant, peroxynitrite, which has also been demonstrated to impair cardiac function [100, 101]. Thus, it appears that oxidative stress generated by different sources in the I/R heart plays an important role in the genesis of subcellular remodeling and cardiac dysfunction. A general scheme involving changes in gene expression for inducing subcellular remodeling due to I/R is shown in Fig. 16.2.

## 16.4 Activation of Proteases and Subcellular Remodeling in I/R Hearts

Activation of different types of proteases including calpain I and calpain II as well as metalloproteases (MMP-2 and MMP-9) has been suggested to be intimately involved in the pathophysiology of several forms of cardiac diseases [6–19, 102–106]. These proteases are activated by $Ca^{2+}$-dependent and $Ca^{2+}$-independent mechanisms and have been shown to cleave subcellular proteins and depress or alter their activities. While calpain I and calpain II are activated by $Ca^{2+}$, both MMP-2 and MMP-9 are activated by oxidative stress as well as by proteolysis. Pretreatment of heart with calpain inhibitors, MDL-28170 and A-70523, was observed to attenuate I/R-induced

cardiac stunning and infarct size [24, 106, 107]. The activation of calpain as well as defects in the SR $Ca^{2+}$-uptake and $Ca^{2+}$-release activities due to I/R injury was also attenuated by perfusing the heart with calpain inhibitors, leupeptin and E64d, or exercise training [103, 108, 109]. Preventing the activation of calpain by nitrosylation upon perfusing the heart with L-arginine was associated with improvements of SR function and cardiac performance in I/R hearts [51, 110]. Calpain-mediated depression in the activity of SL $Na^+$–$K^+$ ATPase and the loss of cytoskeleton protein α-fodrin due to I/R injury were prevented by a calpain inhibitor, MDL-7943, as well as ischemic preconditioning [111–114]. Likewise, the activation of MMP-2 [115, 116], changes in myosin light chain, and cardiac dysfunction due to I/R injury were prevented by doxycycline, an inhibitor of MMP-2 [116]. The influence of I/R injury on MMP-2 was observed to be mediated through the phosphoinositide 3 kinase (PI3K)/protein kinase B (Akt kinase) pathway [117]. These results provide evidence that activation of both calpain and MMP-2 due to I/R injury may depress cardiac performance due to subcellular defects; however, it remains to be examined if the observed changes in the activation of metalloproteases due to I/R injury are mediated directly through oxidative stress and/or indirectly through intracellular $Ca^{2+}$ overload. It should be mentioned that both MMP-2 and MMP-9 are localized within cardiomyocytes [104, 105, 118] and their endogenous inhibitors, TIMP-2 and TIMP-1, are also found in these cells [104, 118]. Further, calpastatin serves as an endogenous inhibitor of both I and II isoforms of calpain in the heart [103]. Thus, the I/R-induced activation of MMP-2 and MMP-9 could also be due to a reduction in the TIMP-2 and TIMP-1 contents, whereas that of calpain I and II may be associated with the reduction of calpastatin content in cardiomyocytes. Nonetheless, the activation of different proteases under conditions of I/R injury would disrupt myocardial structure, remodel different subcellular organelles with respect to their protein content, and produce irreversible cardiac dysfunction.

## 16.5 I/R-Induced Subcellular Remodeling and Gender Difference

To date, the majority of work done to describe I/R-induced development of oxidative stress, occurrence of intracellular $Ca^{2+}$ overload, activation of proteases, and defects in subcellular function has employed male animals, and little information is available for the female hearts. As female hearts are less susceptible to I/R injury, the basis for gender difference in cardiac ischemic injury and protection remains to defined [119, 120]. Several epidemiological studies have revealed sex differences with respect to the incidence of coronary artery disease, atherosclerosis, apoptosis, hypertension, and heart failure [120–134]. Various experimental investigations have also reported gender differences in the development of cardiac hypertrophy and heart failure due to myocardial infarction, pressure overload, and volume overload [135–146]. Gender difference in the properties of cardiac $Na^+$–$K^+$ ATPase due to hypertension [147], SR $Ca^{2+}$ loading due to catecholamines [148], and $Na^+$–$Ca^{2+}$ exchanger due to heart failure [149] has also been observed. As females lose their

resistance to different cardiovascular diseases after menopause, it appears that the intrinsic cardioprotection observed in females may be mediated through the participation of ovarian hormones [150]. Dramatic changes in Akt and NO synthase signaling as well as protein kinase A-mediated changes in $Ca^{2+}$ handling involving SL $Ca^{2+}$ channels, $Na^{+}$–$Ca^{2+}$ exchanger, SR $Ca^{2+}$ uptake, and $Ca^{2+}$-release channels have been observed upon ovariectomy [151–154]. Furthermore, estrogen, a major ovarian hormone, has been demonstrated to affect different $Ca^{2+}$-handling proteins, β-adrenoceptors, and $Na^{+}$–$H^{+}$ exchanger as a consequence of its action on various kinase-mediated signal pathways [155–160]. However, a detailed study regarding the mechanisms of subcellular remodeling responsible for the resistance to ischemic insult in females, by employing ovariectomized animals with or without estrogen treatment, needs additional investigation before overarching conclusions can be made.

Several investigators have attempted to investigate the mechanisms of gender difference in cardioprotection against I/R injury in adult hearts. The postischemic recovery of cardiac function was greater and infarct size was smaller in female hearts in comparison to males, and these changes were attributed to differences in the Akt and PKC signal transduction [161, 162]. The loss of ischemic preconditioning effect on contractile function, infarct size, and enzyme leakage in I/R hearts was associated with impaired PKC phosphorylation [163]. Gender differences with respect to improved cardiac function in female I/R hearts were shown to be due to alterations in the regulation of $[Na^{+}]_i$ by a NO synthase-dependent mechanism [164–166]. Differences in the gender-dependent I/R-induced infarct size were associated with increased expression of SL $K_{ATP}$ channels, and in fact, blockade of these channels was observed to abolish this difference [167, 168]. Conversely, gender differences with respect to resistance of female heart to I/R injury have also been attributed to difference in mitochondrial $Ca^{2+}$ uptake [169] and tumor necrosis factor receptor signaling [170, 171]. By using ovariectomized animals, estrogen was found to attenuate I/R-induced changes in cardiac function and reduce infarct size as a consequence of changes in calpain and p38 MAP kinase activities [172–176]. Female mouse cardiomyocytes were protected against oxidative stress due to $H_2O_2$ as a consequence of Akt activation [177]. On the other hand, castration was found to decrease mRNA levels for L-type $Ca^{2+}$ channels and $Na^{+}$–$Ca^{2+}$ exchanger, and these alterations were reversed by testosterone [178–180]. Testosterone was also observed to modify I/R-induced changes in Akt signal transduction and apoptotic pathway [181–183]. Overall, there is a paucity of information on gender differences in subcellular remodeling as well as changes in mechanisms, which regulate cardiac function during I/R injury.

## 16.6 Conclusions

It is now known that the SR $Ca^{2+}$ pump and associated regulatory mechanisms are defective due to changes in the molecular composition of SR membrane as a consequence of I/R injury. Various oxidants as well as hydroxyl radicals are observed to depress the SR $Ca^{2+}$-pump activity in I/R hearts. It should be noted that ischemic

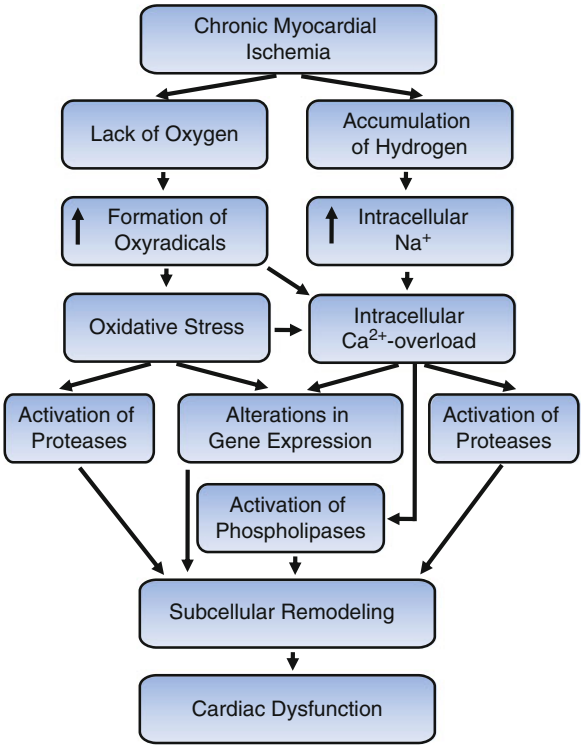


**Fig. 16.3** Mechanisms of subcellular remodeling and cardiac dysfunction involving both oxidative stress and intracellular $Ca^{2+}$ overload in ischemic heart disease

insult produces oxidative stress due to the generation of oxyradicals generating in addition to accumulating protons in cardiomyocytes. The magnitude of oxidative stress is amplified upon reperfusion of the ischemic myocardium, whereas protons are exchanged for $Na^+$ via the $Na^+$–$H^+$ exchanger. Furthermore, oxidative stress rapidly oxidizes the functional groups of $Na^+$–$K^+$ ATPase and augments the development of intracellular $Na^+$ overload. Intracellular $Na^+$ is exchanged with $Ca^{2+}$ via the $Na^+$–$Ca^{2+}$ exchanger and favors the occurrence of intracellular $Ca^{2+}$ overload in the I/R heart. Thus, it is emphasized that alterations in the activities of $Na^+$-handling proteins (SL $Na^+$–$K^+$ ATPase and $Na^+$–$Ca^{2+}$ exchanger) are critical for the net gain of $Ca^{2+}$ within the cardiomyocytes. Oxidative stress and subsequent intracellular $Ca^{2+}$ overload result in the activation of different proteases and induce dramatic changes in the composition of subcellular organelles/proteins in the I/R hearts. Accordingly, oxidative stress as well as changes in SL $Na^+$-handling proteins and protease activation plays an important role in inducing cardiac dysfunction due to I/R injury. Various events involved in subcellular remodeling during the development of cardiac dysfunction in ischemic heart disease are depicted in Fig. 16.3. It is also evident that females are more resistant to I/R-induced injury than males and the recovery of cardiac function upon reperfusing the male ischemic hearts is less

than that of the female. Although I/R-induced cardiac dysfunction in male hearts has been shown to be associated with the occurrence of oxidative stress, increase in development of intracellular $Ca^{2+}$ overload, activation of proteases, cleavage of subcellular proteins, and alterations in subcellular activities, very little information regarding these changes in female hearts is available.

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