Molecular determinants of responses to myocardial ischemia/reperfusion injury: focus on hypoxia-inducible and heat shock factors

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Abstract

During the past several years much new evidence has accumulated regarding the molecular and biochemical mechanisms underlying cardiac responses to hypoxia and to ischemia/reperfusion injury. Studies have involved cell culture, and ex vivo and in vivo preparations. This review focuses on regulation of two transcription factors that are thought to be important in these processes, hypoxia-inducible factor 1α (HIF-1α) and heat shock factor (HSF). Both of these molecules are expressed acutely and chronically in response to hypoxia and ischemia/reperfusion, and both have numerous targets that comprise part of integrated response to ischemic injury aimed at promoting cell survival. Emphasis is placed on new mechanisms of action that regulate HIF-1α, HSF, and heat shock proteins as key responses to hypoxia and ischemia, and possible approaches to therapy based on these data are discussed.

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1. Introduction

Ischemic injury to the myocardium due to the spectrum of coronary occlusion events known as acute coronary syndromes account for a large proportion of all hospital admissions and of all causes of death in western society. Despite several recent successful advances in the therapy of acute coronary syndromes based on limiting the extent of myocardial damage that ensues following occlusion of a major coronary artery by rapid restoration of blood flow, many patients are not suitable candidates for such revascularization procedures. Additionally, these approaches are often applied too late to prevent irreversible damage to the myocardium. Thus, a greater understanding of the cellular and molecular mechanisms involved in ischemic injury may foster additional improvements in clinical care.

Oxygen is one of the basic elements that supports all eukaryotic life, acting as the final electron acceptor in the respiratory chain. Intracellular O₂ concentrations are maintained within a narrow range due to the risk of oxidative damage from excess O₂ (hyperoxia), and of metabolic demise from insufficient O₂ (hypoxia). Because insufficient levels of oxygen deprive the respiratory chain of its main electron acceptor, the less efficient anaerobic glycolytic pathway supersedes mitochondrial oxidative phosphorylation as the principal source of ATP production, generating approximately a quarter of the amount of ATP normally produced during oxidative phosphorylation [1]. As a result, there are inadequate levels of high-energy phosphates to maintain normal function. If hypoxia is reversed, the subsequent reoxygenation may potentially produce damage to cells by increasing the levels of reactive oxygen species (ROS) generated during partial reduction of oxygen to water.

The heart is an organ with particular susceptibility to hypoxia since only limited reserves of high-energy phosphates are maintained [2]. The myocardium may be exposed to hypoxia or anoxia under a number of conditions such as myocardial ischemia after major coronary artery occlusion,
high altitude, and anemia. The extent as well as duration of hypoxia, in addition to the presence of other confounding factors such as tissue ischemia, determines the cardiac response to diminished oxygen supply. Both hypoxia and oxidative stress result in biochemical and functional changes as the heart attempts to maintain function in the face of perturbations in oxygen tension. Hypoxia and reoxygenation alter the cardiac protein pattern, mainly through altered gene expression, but also through changes in mRNA stability, rates of translation, post-translational protein modifications and degradation. Hypoxia also induces upregulation of specific proteins that lead to both protective as well as deleterious effects. Furthermore, increased oxygen levels occur upon reperfusion of an occluded coronary vessel (reoxygenation), either spontaneously or following treatment of acute myocardial infarction. Such reperfusion may be associated with pathophysiological levels of reactive oxygen species. Subsequently, cardiac myocytes attempt to protect themselves from the short- and long-term consequences of exposure to these harmful molecules by upregulating levels of antioxidant and stress proteins. Numerous experiments indicate that levels of superoxide increase within the hypoxic myocardium during production of $\text{H}_2\text{O}_2$ and hydroxyl radicals upon reoxygenation [3]. Under many clinical circumstances, there is a chronic reduction in coronary blood flow as occurs in the border zone surrounding a myocardial infarction. Such alterations in coronary perfusion have also been documented in patients with native vessel chronic coronary artery obstructions, bypass graft stenosis, and restenosis after coronary angioplasty/stent procedures. These alterations in coronary blood flow may lead to either global or regional myocardial ischemia, which can be repetitive, and can cause long-term homeostatic responses as outlined in Table 1.

Although the maintenance of oxygen homeostasis is an essential cellular and systemic function, it is only within the past several years that the molecular mechanisms underlying this fundamental aspect of cell biology have begun to be elucidated and their connections to physiology and pathophysiology have been established. Thus, the purpose of this review is to update the reader on the rapidly advancing knowledge concerning the regulatory mechanisms involved in the regulation of hypoxia-inducible transcription factor(s) and heat shock factor in ischemic myocardium.

### 1.1. Hypoxia-sensing mechanisms

In mammalian cells, many of the compensatory mechanisms that occur in response to changes in oxygen tension are secondary to inhibition of oxygen-dependent pathways and changes in the intracellular redox status. However, some cellular responses to hypoxia become activated before either ATP levels are depleted or the $K_m$ of the mitochondria for $O_2$ is reached [4–6]. Moreover, the mitochondrial inhibitor cyanide does not reproduce some hypoxia-induced effects. Based on previous biochemical observations, the oxygen-sensing molecule appears to be a heme-containing protein that binds to oxygen; is inhibited by carbon monoxide; and is activated by certain heavy metals, notably cobalt, nickel, and manganese, which can substitute for iron in heme proteins [7,8]. Until recently, the means by which cells sense alterations in oxygen tension remained relatively obscure. The first insight into an oxygen-sensing pathway in higher organisms came with the discovery of a family of oxygen-dependent enzymes responsible for the regulation of the hypoxia-inducible transcription factors (HIFs). Activation of the HIF system by iron chelators and cobaltous ions as well as hypoxia has led to the proposal of a ferroprotein oxygen sensor regulating mechanism for HIF transcription factors.

The HIF transcription factors are composed of two subunits: the hypoxia-regulated alpha subunit HIF-1α (or its homologs, HIF-2α and HIF-3α), and the oxygen-insensitive HIF-1β subunit (also known as the arylhydrocarbon receptor nuclear translocator, or ARNT) [9]. Under normal oxygen conditions (normoxia), HIF-1α is constitutively expressed [10–12]. However, this subunit is rapidly targeted for proteasome-mediated degradation through a protein–ubiquitin ligase complex containing the product of the von Hippel Lindau tumor suppressor protein (pVHL) [13–16]. Under hypoxic conditions, degradation of HIF-1α is prevented, and thus HIF-1α is able to accumulate within the nucleus allowing it to bind with its partner HIF-1β (see Fig. 1). Subsequently, this heterodimeric complex is able to recognize HIF-responsive elements (HREs) transactivating downstream target genes involved in the longer-term response to chronic hypoxia [17] (see Fig. 1 and Table 1).

Recently, it has been reported that degradation of HIF-1α under normoxic conditions is triggered by post-translational hydroxylation of conserved proline residues within a prolyl-peptide region known as the oxygen-dependent degradation domain (ODD) [18,19]. The hydroxylated proline residues in this sequence are recognized by pVHL, leading to subsequent HIF-1α degradation via the ubiquitin ligase pathway (Fig. 1). This modification is inherently oxygen-dependent, because the oxygen atom of the hydroxyl group is derived from molecular oxygen. Moreover, this reaction

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Direct HIF-1 target genes</th>
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<tr>
<td><strong>Erythropoiesis and iron metabolism</strong></td>
<td><strong>Ceruloplasmin</strong>, erythropoietin (EPO), transferrin, transferrin receptor, Vasculogenesis/vasomotor tone</td>
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<tr>
<td>α1B Adrenergic receptor, adrenomedullin, endothelin-1, heme oxygenase-1</td>
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<tr>
<td>Prosurvival/proliferation factors</td>
<td>Adrenomedullin, cyclin G2, EPO, heme oxygenase-1, IGF2, IGFBP-1,2,3 NOS2, NIP3, p21, TGF-β3, VEGF</td>
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<tr>
<td><strong>Metabolism</strong></td>
<td>Adenylate kinase-3, aldolase A/C, enolase 1, carbonic anhydrase-9, glucose transporter (GLUT)-1/3, 11 glycolytic enzymes</td>
</tr>
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requires cofactors such as 2-oxoglutarate, vitamin C, and iron. The requirement of this last cofactor is consistent with previous biochemical evidence suggesting the oxygen-sensing factor is iron-dependent. Thus, this critical regulatory event is carried out by a family of iron (II)-dependent dioxygenase prolyl hydroxylase enzymes that use O2 as a substrate to catalyze hydroxylation of the target proline residues[20]. Because oxygen appears to be rate limiting for prolyl hydroxylase activity, these enzymes likely represent the oxygen sensors that provide a direct link between oxygen concentrations and components of the hypoxic response pathway.

Modulation of protein stability is one pathway by which HIF activity is regulated by hypoxia. However, more recent studies have identified an additional novel mechanism by which HIF is regulated by an oxygen-dependent enzyme[21]. In addition to the ODD domain, the HIFα subunit isoforms contain two transactivation domains responsible for recruiting transcriptional coactivators essential for gene expression: (1) the N-terminal transactivation domain (N-TAD), which overlaps the ODD. Regulation of its activity is likely to be a byproduct of protein stability; and (2) the C-terminal transactivation domain (C-TAD), which is able to recruit coactivator complexes such as p300/CBP only under hypoxic conditions[22–24] (Fig. 1). Earlier studies revealed that transcriptional activation by C-TAD, when fused to a heterologous DNA-binding domain, is able to operate independently of the ODD, recruiting coactivator complexes such as p300/CBP only under hypoxic conditions. Surprisingly, the regulation of C-TAD activity also involves an oxygen-dependent hydroxylation event; however, in this case, the targeted residue appears not to be a proline but rather a conserved asparagine residue. Enzymatic hydroxylation of the conserved C-TAD asparagine residue (Asn-803) requires oxygen; this modification subsequently prevents the recruitment of coactivators such as p300 and CBP. The enzyme responsible for this modification is factor-inhibiting HIF-1 (FIH1), which has been previously demonstrated to bind to HIF-1 and to repress HIF-dependent transcription[25]. FIH1, like members of the proline hydroxylases, is a member of the 2-oxoglutarate and iron-dependent dioxygenase superfamily[26].

Thus, study of the interaction of pVHL with HIF has led to new insights into the mammalian oxygen-sensing pathway and has demonstrated an unappreciated role for enzymatic protein hydroxylation in intracellular signaling. The discovery of this modification, as well as identification of the responsible enzymes, raises the question of whether this modification occurs on other intracellular proteins. Additionally, it will be interesting to determine if additional members of the 2-oxoglutarate and iron-dependent dioxygenase superfamily and thus potential protein hydroxylases exist[21]. The answers to such questions may provide additional new insights into novel hypoxia-mediated pathways.

Additionally, recent studies have provided experimental evidence in support of the hypothesis that mitochondrial generation of superoxide and, subsequently, hydrogen per-
oxide are required for induction of HIF-1 activity and transcription of downstream target genes in hypoxic cells [27]. This model proposes that reactive oxygen species generation increases under hypoxic conditions. However, an alternative model proposes the opposite, that hypoxia results in decreased production of ROS by nicotinamide adenine dinucleotide phosphate (reduced NADPH) oxidases. It is likely that HIF-1α is a direct target of redox regulation. The redox status of cysteine residues of HIF-1α in the carboxy-terminal TAD (TAD-C) has been shown to affect its interaction with CBP/p300 coactivators, and this interaction is positively regulated by redox factor 1 (REF-1) and thioredoxin [22]. In addition, the interaction of the coactivator SRC-1 with HIF-1α is also redox regulated [28]. However, these models are supported by experimental data derived primarily from the use of redox-sensitive fluorescent compounds that measure reactive oxygen species (ROS) and from the effects of pharmacologic agents on the expression of HIF-1 and downstream genes in tissue culture. Given the lack of consensus on the role of ROS on HIF-1 activity, further in vivo genetic approaches will be required to determine the function of ROS on hypoxia-mediated signal transduction [27]. Additionally, the regulation of hypoxia by carbon monoxide (CO) and nitric oxide (NO) also is likely mediated through HIF-1 activity [29].

In one such recent report, it was found that cardiac protection induced by intermittent hypoxia is critically dependent on HIF-1 activity, further in vivo genetic approaches will be required to determine the function of ROS on hypoxia-mediated signal transduction [27]. Additionally, the regulation of hypoxia by carbon monoxide (CO) and nitric oxide (NO) also is likely mediated through HIF-1 activity [29].

Despite the recent exciting findings of oxygen-sensing proteins regulating HIF-1 activity, the regulation of HIF-1 is a complex process likely involving not only O2 concentration and oxygen-sensing proteins, but also HIF-1 protein stabilization via the PI3K/PTEN/Akt/FRAP/p70S6 kinase prosurvival pathway [31]. Multiple signaling pathways have been found to stabilize the HIF-1 transcriptional complex through the Akt pathway including nitric oxide, HER2/Neu, IGF/insulin, and erythropoietin [32–35]. Stretch-activated channels in the myocardium have also been implicated in this pathway [36]. However, the PI3 3-kinase/Akt pathway, which may also involve mTOR, is neither sufficient nor necessary to induce HIF-1 activity by itself, but may modulate HIF activity under hypoxic conditions [37]. Moreover, the cardioprotective effects of the Akt pathway may also act independently of HIF-1 activity [38]. Interestingly, many of the signaling factors which activate the Akt pathway are direct targets of the HIF-1 transcriptional regulation, such as erythropoietin and IGF2 (see Table 1 and Fig. 2). Alternatively, the Akt pathway may not directly regulate HIF-1 stability, but rather HIF-1 may act upstream of Akt to promote cardiac survival through other pathways. Although HIF-1 mRNA and protein are detected in cell culture models hours after the induction of hypoxia [39] and days after myocardial infarction [40], much earlier responses have also been documented in animal models [41]. For example, Chun et al. recently reported that HIF-1α protein accumulated in nuclei of adult rat cardiac myocytes as early as 2 h after regional ischemia [42]. The raised level of HIF-1α was related to the activation of the promoter for atrial natriuretic factor (ANF) gene, suggesting that acute increases in ANF in the left ventricle after ischemic injury is HIF-1α-dependent [42]. These observations are consistent with the virtually instantaneous hypoxic induction of HIF-1α and its reduced degradation in the presence of hypoxia [43].

Although glucose as well as GLUT1 overexpression diminished HIF-1α in rat neonatal cardiac myocytes subjected to 24 h of hypoxia [39], 2 h of reperfusion following 20 min of ischemia in isolated rat hearts perfused with a high glucose solution resulted in a marked increase in HIF-1α mRNA [44]. These reports and others cited above [40–42] indicate that HIF-1α responses are present over a broad spectrum of time following ischemia/reperfusion injury, but also suggest that acute responses could be the result of free radical generation. Future experiments will be necessary to explore this hypothesis. Thus, continued cellular and molecular research will likely reveal additional novel pathways regulating the HIF-1α response to hypoxia and ischemia.

1.2. Heat shock factors in the ischemic myocardium

The heat shock response is a highly conserved defense mechanism against tissue and cell stress injury. This system is conserved from bacteria to humans, and represents an endogenous mechanism that antagonizes protein unfolding or misfolding during stress responses. This defense mechanism requires heat shock transcription factors (HSFs), the primary mediators of the heat shock response. When cells are exposed to stress, HSFs are phosphorylated and form trimers that enter the nucleus and bind the heat shock elements (nGAAn) within the promoter/enhancer regions of heat shock proteins (HSPs) [45].

![Fig. 2. HIF-1 downstream activators and hypoxia adaptive responses.](https://academic.oup.com/cardiovascres/article-abstract/61/3/437/402834)
There are at least four known heat shock transcription factors present in mammalian cells; however, HSF1 appears to be the primary mediator of the heat shock response system. The other three heat shock transcription factor isoforms (HSF2-4) are not sufficient to preserve the heat shock response in the absence of HSF1 [46]. Disruption of the \( Hsf1 \) gene in mice reduces cardiac expression of Hsp25, \( \alpha \)-crystallin and Hsp70, but not Hsp60 and Hsp90. Consistent with the downregulation of Hsp25 was decreased activity, but not protein content, of glucose 6-phosphate dehydrogenase. Consequently, superoxide was generated at a higher rate, and several mitochondrial proteins underwent greater oxidation as a result of in vivo HSF1 deficiency. Overall, these results indicate loss of the suscepibility of HSF1-deficient cells to oxidative damage at normal (37 °C) temperature [47].

HSF1 activation during ischemia may be induced by multiple cellular stress responses. A decrease in the concentration of high-energy phosphate compounds may be sufficient to activate HSF1 [48]. Intracellular acidosis may also serve as an additional stimulus. Alterations in redox state have been documented to activate cardiac HSF1 DNA binding [49], and activate HSF1 acutely during ischemia/reperfusion [50]. However, it appears that a common consequence of stimuli that activate HSF1 is an increase in the concentrations of unfolded proteins within the cell, which may provide a common stimulus for induction of HSP gene expression [51]. Negative regulation of HSF1 may be due to several mechanisms during unstressed conditions. Interestingly, HSF4 has been suggested to negatively regulate the expression of the \( Hsf1 \) gene, and possibly, the overall heat shock response [52]. Another negative regulator of HSF1-mediated transcription is glycogen synthase kinase 3\( \beta \), which also impairs HSF1 DNA binding [53].

Reperfusion of the ischemic rat heart causes rapid activation of HSF1 [54]. A characteristic feature of the response to stress in human (but not rodent) cells is rapid and reversible relocation of HSF1 within seconds into specific subnuclear structures, termed stress granules [55], where trimerization occurs. This process also involves HSF2 and coincides with the nucleolar localization of Hsp70 [56]. The appearance of stress granules correlates with the inducible phosphorylation and transcription activation of HSF1 [55]. Upon recovery from stress such as heat shock, HSF1 rapidly dissipates from these stress granules to a diffuse nucleoplasmic distribution, typical of unstressed cells [55]. Although the majority of HSF1 phosphorylation sites are still unknown [57], it has been shown that phosphorylation of serine 230 promotes inducible transcriptional activity of HSF1 [58]. In stress granules, HSF1 undergoes post-translational modification by covalent conjugation of a small ubiquitin-like modifier 1 protein (SUMO-1) to lysine 298 [59] preceded by phosphorylation of serine 302 [57]. Negative regulators of HSF1 driven transcription include the mitogen activated protein kinase ERK and c-Jun NH2-terminal kinase [60]. These kinases recognize their substrates via a small domain (D domain) in which phosphorylation of serine 363 appears to be the major target leading to reduced transciptional activity [60].

### 1.3. Heat shock proteins in cardioprotection

Heat shock proteins (HSPs) fulfill a range of functions, including cytoprotection and the intracellular assembly, folding, and translocation of oligomeric proteins [61], and represent a rapid response to altered redox states [49]. HSPs are categorized into several families identified on the basis of their approximate molecular weights, which range from 10 to 150 kDa. Members of the HSP family are induced in response to a number of stresses, including sublethal heat, hypoxia, reoxygenation after hypoxia, and ischemia. They function to promote the folding and assembly of nascent polypeptides, and to facilitate the repair or degradation of unfolded proteins [51,62,63]. In addition to acting as cellular chaperones, HSPs mediate cytoprotection by associating with and hindering the action of key apoptotic proteins, and by facilitating the degradation of misfolded intracellular proteins by the ubiquitin/proteasome system, so-called “protein triage” [64].

Thus, numerous studies over the past decade have demonstrated that increased expression of HSPs may protect the heart from stressful environments such as ischemia and reperfusion injury [65,66]. Hearts isolated from transgenic mice that express human HSPs such as HSP70 in the myocardium have shown greatly improved functional recovery, with decreased infarct size after experimental induction of ischemia and reperfusion [67–69]. The role of HSP70 in myocardial protection has been extensively studied [70,71].

HSP70 is present in two forms: constitutive (HSP70c) and inducible (HSP70i). Both forms can be activated by stress and there is evidence in H9c2 cardiac myoblasts that the constitutive form confers protection against oxidative injury [72]. Stable overexpression of HSP70c in these cells also confers oxidative protection [73]. In other experiments, it was reported that both the inducible and the constitutive forms of this protein were activated by ethanol or heat resulting in a decrease in cytotoxicity produced by oxidative stress [74]. Previous studies had shown that overexpression of rat HSP70i increased cardiac resistance to ischemic injury [69]. In this same transgenic model, a short period of ischemia followed by reperfusion sufficient to cause regional dysfunction without infarction protected against left ventricular dysfunction compared to wild-type littermate controls [75]. Direct gene delivery by cardiac injection with recombinant adenovirus encoding HSP70i reduced infarct size in vivo after ischemia/reperfusion in the rabbit heart [76]. Delayed cardioprotection can be induced via \( \kappa \)-opioid receptor activation in adult rat ventricular myocytes. Zhou et al. [77] showed that lethal-simulated ischemia activated both HSP70c and HSP70i, but that only the antisense
oligonucleotide to HSP70i blocked delayed cardioprotection. Thus it is reasonable to hypothesize that under some experimental circumstances HSP70i is the predominant isoform of this molecular chaperone involved in conferring cardioprotection.

HSPs other than HSP70 may also provide added myocardial protection (Fig. 3). These factors include the larger heat shock proteins HSP60 and HSP90 and the small heat shock proteins HSP22, HSP27, αβ-crystallin, and HSP32. Increased expression of HSP27 in canine cardiac myocytes correlated with a greatly decreased cardiomyocyte susceptibility to metabolic or functional injury after simulated reperfusion injury [78]. Overexpression of HSP27 and αβ-crystallin protected adult rat cardiac myocytes against ischemic injury [79] and transgenic overexpression of αβ-crystallin conferred simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion [80].

Mechanisms of cytoprotection conferred by activation of αβ-crystallin include preservation of the tubulin cytoskeletal structure against ischemia-induced disruption [81], redistribution of αβ-crystallin from the cytosol to intercalated disks and Z lines of the myofibrils to provide stabilization of the myocardial contractile apparatus [82], and binding to the I-band portion of titin, an important elastic component of the myofibrils [83]. αβ-Crystallin negatively regulates cytochrome c- and caspase-8-dependent activation of caspase-3 [84] and further controls apoptosis by regulating Akt activation [85]. Like HSP70, phosphorylation plays a major role in HSP27 and αβ-crystallin regulation. Thus Akt phosphorylates HSP27 on serine-82 resulting in its dissociation from Akt. Activation of MKK6 stimulates p38 MAPK and results in the induction of αβ-crystallin mRNA and phosphorylation of αβ-crystallin on serine-59 [86]. This phosphorylation is both necessary and sufficient to provide maximal protection of cardiac myocytes from apoptosis [87]. It has also been shown that mutation of COOH-terminal lysines in overexpressed αβ-crystallin abrogates ischemic protection in cardiomyocytes [88].

HSP22 is a newly described member of the small heat shock protein superfamily and interacts with a mimic of phosphorylated HSP27; its physiologic role remains to be determined [89]. HSP32 (heme-oxygenase-1 or HO-1), which has received relatively little attention, is increased by hypoxia in rat neonatal myocytes [90] and is an effective antioxidant cardioprotective molecule [91]. HO-1 is negatively regulated by the transcriptional repressor Bach1 [92]. A physiologically regulated vector expressing the human HO-1 gene conferred protection against cardiac ischemia–reperfusion injury in a rat model [93].

HSP60 and HSP10 are heat shock proteins found predominantly in the mitochondria. Accumulation of unfolded proteins within the mitochondrial matrix results in the transcriptional upregulation of nuclear genes encoding these stress proteins via a specific transcription factor termed CHOP [C/EPB homology protein] [94]. In rat neonatal myocytes, overexpression of HSP60 and HSP10 separately or together protected cells against apoptosis induced by simulated ischemia and reoxygenation [95]. This protection was accompanied by increased ATP recovery and preservation of complex III and IV activities in mitochondria [95]. Only 15–20% of HSP60 is found in the cytosol. However, HSP60 complexes with cytosolic bax, and reduction of HSP60 by an antisense approach precipitated translocation of bax to the mitochondria and apoptosis in adult rat cardiac myocytes resulting in the release of cytochrome c, activation of caspase 3, and induction of DNA fragmentation [96]. In this cell preparation, hypoxia had a similar effect resulting in the dissociation of the HSP60–bax complex in the cytosol with translocation of cytosolic HSP60 to the plasma membrane and bax to the mitochondria, a process sufficient to trigger apoptosis [97]. These changes occur...
before reoxygenation and the concomitant generation of free radicals [97]. How ischemic preconditioning and other cardioprotective maneuvers regulate HSP60 and HSP10 has not been determined.

HSP90 is an ATP-dependent molecular chaperone involved in the folding and activation of a large number of substrate proteins that include protein kinases and transcription factors such as HIF-1α and HSF1 [98,99]. As substrate proteins interact with HSP90, multiprotein complexes are formed with a set of highly conserved partner proteins, such as HSP70, HSF1 and HSP40, and p23 [98,99]. ATP hydrolysis by an intrinsic ATPase results in a conformational change in HSP90 that is required to induce conformation change in the substrate or “client” protein [99]. The mechanisms of these interactions have recently been reviewed in detail [99]. It has been observed that p23 and HSP90 can also be involved in the disassembly of transcriptional regulatory complexes [100].

Trimeric human HSF1 associates with an HSP90–immunophilin–p23 complex through its regulatory domain resulting in transcriptional repression of trimeric HSF1 [101]. Following stress, this heterocomplex dissociates, triggering HSF1 activation and heat shock gene transcription. This response depends in part on the small GTPase Ra1 [102]. Both cardiotrophin-1, a member of the interleukin-6 peptides which mediate the action of the hypothalamic–pituitary–adrenal axis in response to stress, increase the expression of HSP90 and are cardioprotective in cardiac myocytes [106,107]. Thus, HSP90 is a multifunctional HSP that is involved in a large number of stress responses in the cardiovascular system by its interaction both with other molecular chaperones and by its regulation of both HIF-1α and HSF.

The reports cited above indicate the complexity of the mechanisms responsible for the myocardial protection provided by HSPs which remain incompletely understood. As indicated above, recent studies have proposed multiple roles for HSPs in myocardial protection ranging from direct cytoprotection of myocardial cells against reactive oxygen species (ROS) during ischemia and reperfusion to modulation of cytokine activity (Fig. 3). Thus, HSPs may favorably interfere with ROS-induced phenomena during ischemia and reperfusion because of their biologic roles as molecular chaperones. HSP levels closely correspond to the activity of antioxidant enzymes such as catalase [63]. Furthermore, cytokine production may be downregulated by HSPs through interference with the nuclear factor κB (NFκB) signalling pathway which is involved in inducing several proinflammatory genes/cytokines [108–110].

Finally, accumulating evidence from in vivo and in vitro studies strongly suggests that the heat shock response system may play an important role in regulating apoptotic events which may be part of the myocardial damage that occurs during acute ischemic injury. Overproduction of ROS from oxidative damage during acute ischemia and reperfusion may be one of the most important determinants involved in apoptotic death of myocardial cells [111]. However, the mechanisms by which HSPs protect cardiac myocytes against apoptosis remain to be fully elucidated, but it is believed that multiple levels in the apoptosis death cascade are involved. One proposed hypothesis is that HSP70 is able to inhibit apoptosis by preventing the release of cytochrome c from mitochondria through both mitochondrial-mediated pathways and receptor-mediated signaling pathways [112]. Furthermore, there is evidence suggesting that in addition to HSP70, HSP90 and low-molecular weight HSPs may provide an anti-apoptotic role by possibly acting in concert with HSP70 [95,113] (Fig. 3). Details of these proposed mechanisms have been the subject of several recent reviews [64,98,99].

1.4. Therapeutic implications

Our understanding of the cellular and molecular mechanisms involved in myocardial protection during ischemia/reperfusion injury has progressed rapidly over the past few years. Additional pathways for cellular defenses against hypoxia remain to be identified and many features of the known defense mechanisms have not been fully elucidated. Nevertheless, the plethora of knowledge currently available has provided an entry point for translating the cellular and molecular mechanical information regarding cardioprotection and hypoxia responses into clinically relevant therapeutic or preventive strategies [114,115].

One of the most exciting strategies has been to utilize the therapeutic action of HIF transcription factors prior to or during ischemic stress as a form of preconditioning stimulus or to augment the endogenous response during ischemia. Several successful strategies have been employed in experimental animal model systems involving HIF in various ingenious and novel approaches. Transgenic mice containing constitutively active HIF-1α molecule by deletion of the central oxygen-dependent degradation domain...
exhibit significantly increased activation of HIF transcriptional targets and overgrowth of blood vessels [116]. Interestingly, these vessels were not associated with increased edema and their vascular integrity appeared to be fully intact. In contrast, previous studies utilizing VEGF as a proangiogenic therapy led to leaky and nonfunctional vessels; thus, HIF activation likely provides several additional vasculogenic growth factors besides VEGF which allows for therapeutic vasculogenesis rather than inefficient angiogenesis [116]. An alternative approach has been developing a gene therapy vector containing the N-terminal angiogenesis[116]. An alternative approach has been developing a gene therapy vector containing the N-terminal DNA-binding and dimerization domain of HIF-1α fused to the strong transactivation domain of the herpes virus VP16. Administration of this naked DNA vector into the hearts of a rat myocardial infarction model resulted in an improvement in the response to hypoxia with regard to angiogenesis and reperfusion [117].

Other studies have focused on inhibiting the VHL degradation pathway of endogenous HIF-1α. Overexpression of blocking peptides against the VHL-binding prolyl hydroxylation sites in human HIF-1α has resulted in increased HIF transcriptional activity, and subsequent enhanced angiogenic responses [118]. Additionally, utilizing PR39, a macrophage-derived peptide that interacts with the proteasome and stabilizes HIF, has provided increased peri-infarct vascularization in mouse cardiac tissue [119]. However, the use of small-molecule inhibitors of the HIF hydroxylases has proven to be a most promising and exciting therapeutic pathway for preventing myocardial ischemic injury. Inhibition of the HIF hydroxylases by 2-oxoglutarate analogs stabilizes HIF, which leads to transactivation of the hypoxia response genes [19,20]. In one study, administration of a compound that inhibits EGLN1/PHD prolyl hydroxylase led to tissue preservation during myocardial infarction in rats [120].

Finally, heat shock proteins have proven to be reliable in providing myocardial protection against ROS during ischemia as well as reperfusion injury. Novel therapeutic strategies using HSPs that have been recently explored include pharmacologic interventions as well as gene transfer techniques. A previous study demonstrated that mild heat treatment, inducing expression of HSPs prior to hypothermic storage, improves the functional recovery of transplanted hearts [121]. As indicated earlier, urocortin, a member of the cardiotrophin-releasing hormone family, has been shown to protect cultured cardiomyocytes from ischemic and reperfusion injuries leading to decreased infarct size in the rat heart exposed to ischemia. It is thought that its effects may be partially mediated through the upregulation of HSP90 protein [103,107].

Furthermore, the cytoprotective hydroxylamine derivative, bimoclomol, a coinducer of heat shock proteins, especially HSP70 [122], was found to be protective in a murine model of ischemia [123]. This compound also increased the contractility of the working mammalian heart associated with increased intracellular calcium transients, and decreased the ischemia-induced depression of cardiac contractility and ST-segment elevation, as well as the occurrence of ventricular fibrillation upon reperfusion [122]. Bimoclomol elevated HSP70 in rat neonatal cardiomyocytes and protected against lethal heat stress [124]. Similarly, oral bimoclomol raised HSP70 and reduced infarct size in a rat model of ischemia and reperfusion [125]. The co-inducing effect of bimoclomol on HSP expression is mediated via the sustained activation of HSF1 via prolongation of HSF1 binding to cognate DNA elements [126]. Thus, an agent such as bimoclomol might be useful in the prevention of ischemic damage during clinical situations, such as cardiac surgery and complex vascular operations, where patients are at high risk for ischemic cardiac and other organ damage.

Recent studies also have suggested that the myocardial protection provided by angiotensin-converting enzyme inhibitors may be mediated through HSP72 and HSP73 [127]. Lastly, gene transfer techniques of HSP genes may present a promising strategy for therapeutic intervention in the ischemic heart. Delivery of HSP genes utilizing either liposome or viral vectors [76,93] may potentially be a useful strategy for increasing HSP proteins for myocardial protection.

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