Cardiac genomic response following preconditioning stimulus

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Abstract

This review focuses on the genomic response following a preconditioning stimulus. Initial studies demonstrated that classical ischemic preconditioning mediated by cyclic episodes of short durations of reversible ischemia and reperfusion could result in the reprogramming of gene expression. Some of these genes are translated into proteins during the late preconditioning or so-called “second window of protection”. Subsequent studies determined a unique similarity of the expressed gene profiles between diverse varieties of preconditioning including ischemic/hypoxic, heat shock, and oxidative stress. The most common genes that are expressed by virtually any kind of stress conditioning include antioxidants like superoxide dismutase, glutathione peroxidase and heme oxygenase and heat shock proteins such as HSP70. At a later date, differential display and subtractive hybridization techniques revealed the identities of many other genes including those belonging to mitochondrial respiratory chain such as ATPases. More recently, gene array profiles using gene chips determined several other genes triggered by preconditioning including the mitochondrial genes. The results of the studies present in the literature clearly indicate the existence of a strong resemblance between the patterns of gene expression profiles induced by diverse preconditioning stimuli, oxidative stress being situated at the cross-roads of all forms of the stresses. Redox signaling appears to be responsible for the conversion of the ischemia/reperfusion-induced “death signal” into preconditioning-mediated “survival signal”.

Keywords: Preconditioning; Second window of protection; Gene expression; Oxidative stress; Redox signaling; Heat shock protein; Ischemia; Hypoxia

1. Introduction

Cells can be protected and cell death can be delayed when a non-injurious stress precedes a lethal injury. This phenomenon has been known as “preconditioning” (PC), which provides evidence for adaptive responses to ischemia by enhancing the cell’s tolerance to stress [1–5]. The most widely studied PC is ischemic PC, which can be achieved in the heart with a variety of stress responses including cyclic episodes of short durations of ischemia/reperfusion [6], hypoxia/reoxygenation [7], hypothermia [8], hyperthermia [9] and oxidative stress [10]. Such PC stimulus renders the ischemic heart tolerant to subject lethal ischemic injury as evidenced by improved post-ischemic ventricular performance and reduced myocardial infarct size and cardiomyocyte apoptosis [1–10].

Classical ischemic PC mediated by repeated short durations of reversible ischemia and reperfusion transiently protects the myocardium, usually up to a period of not more than 2 to 3 h [11,12]. After dissipation of this early response, a so-called “second window of protection (SWOP) appears between 12 and 24h, which lasts up to 3 to 4 days [12,13]. It is generally believed that classical PC occurs through the intracellular mediators that are released in response to stress and cardioprotective abilities of several transcription factors and genes that are triggered as a result of early stress response [14]. Late PC, on the other hand, is mediated by the cardioprotective proteins that are expressed after the translation of the early PC-induced genes [12,14].

The long-lasting genomic response of PC constitutes the ultimate adaptive response, resulting from de novo protein synthesis. There are three distinct stages for such an ultimate
response: upregulation of cardioprotective genes during early preconditioning, transcription regulation and translation into proteins. Once the proteins are synthesized, the response is likely to be everlasting; however, the cardioprotective response lasts only up to a few hours. The reason for such limited response despite of the evidence of new protein synthesis remains unknown. However, recent studies have demonstrated that ischemic preconditioning potentiates angiogenesis through the upregulation of angiogenic factor VEGF and anti-apoptotic proteins Bcl-2 and survivin in myocardial infarction group when compared to the control group after 7, 14 and 21 days of LAD occlusion. In concert, there was an increase in capillary and arteriolar density followed by increased microvascular growth suggesting that PC-mediated angiogenic response can last for several days following a preconditioning stimulus. This review will focus on the early PC-mediated genomic response that ultimately leads to the synthesis of cardioprotective proteins during delayed response.

2. Gene expression

2.1. Ischemic preconditioning

The fact that cardioprotective genes are triggered in response to early PC has been known for quite some time. Earlier studies by Schaper’s group [15] and Das’s group [16] clearly demonstrated induction of the expression of several cardioprotective genes after PC. Four cyclic episodes of 5 min ischemia and 10 min reperfusion of isolated perfused rat heart resulted in the increased induction of the expression of proto-oncogenes such as c-fos, c-myc and mRNAs for heat shock protein (HSP) 27, HSP 70 and HSP89 and antioxidants MnSOD, catalase and glutathione peroxidase [16]. Increased expression of these genes was nicely correlated with the cardioprotection as evidenced by improved ventricular function and reduced infarct size. Numerous studies exist in the literature to show cardioprotective abilities of these HSPs and antioxidant proteins. Subsequent studies confirmed these early findings and further showed increased expression of HSP60 in addition to HSP 70 in the preconditioned rabbit heart [17]. However, this study showed that despite the increased expression of these HSPs, they did not appear to play a role in the early phase of adenosine receptor-dependent preconditioning in the rabbit heart. Several other studies, however, demonstrated cardioprotective abilities of HSP70 and MnSOD. Isolated rat hearts subjected to cold (5°C) or normothermic (35°C) cardioplegic solutions followed by reperfusion at normothermia, resulted in significant increase in mRNAs of c-fos, EGR-1 and c-Jun proto-oncogenes [18]. Normothermic cardioplegia with St. Thomas’II solution was without effect, whereas sequential perfusion with KHB buffer at 5°C and 35°C resulted in a similar increase in protooncogene mRNA levels. Only cold Bretschneider solution was related to a 5.2-fold induction of HSP72 mRNA levels. The pattern of differential gene expression in response to diverse preconditioning stimuli is shown in Table 1.

Gene therapy with extracellular SOD protected conscious rabbits against myocardial infarction [19]. In this study, the authors showed that Ec-SOD gene therapy markedly reduced infarct size to the extent that was comparable to that of the late phase of PC. In another related study, when a construct made with rat cDNA encoding HSP70c inserted into a mammalian expression vector, was inserted into H9c2 myocytes, stable clones were found to have a 2-fold increase in HSP70c mRNA and protein concentrations [20]. These clones were more resistant to thermal killing when compared to control cells transfected with the vector alone, implicating a functional role for the overexpressed HSP70c protein. More recently, recombinant adenoviruses expressing Cu/ZnSOD or MnSOD were utilized to modulate superoxide levels in the cytoplasmic or mitochondrial compartments, respectively, prior to coronary artery ischemia/reperfusion in the rat heart [21]. Ectopic expression of both MnSOD and Cu/ZnSOD afforded protection from I/R injury, as evidenced by a significant reduction in serum creatine kinase levels, infarct size, malonaldehyde levels and apoptotic cell death in comparison to controls. MnSOD and Cu/ZnSOD expression also significantly altered the kinetics of NFκB and AP-1 activation following ischemia/reperfusion injury, characterized by a delayed induction of NFκB and abrogated AP-1 response. Western blot analysis of Bcl-2, Bcl-xL, Bad, Caspase 3PDK1 and phospho-Akt in concert with cardioprotection and reduced apoptosis supported SOD-mediated changes in gene expression.

Subsequently, several other genes were identified in response to ischemic PC. When ischemia was induced in anesthetized open chest pigs by two 10 min occlusions of the left anterior descending coronary artery, separated by 30 min of reperfusion, insulin-like growth factor II was expressed [22]. Northern blot analysis showed constitutively expression of IGF-I, IGF-II, the type I receptor, the insulin receptor, and IGFBP-2–6. In situ hybridization further showed that IGF-I and IGF-II were mainly transcribed by myocytes. The authors concluded that a possible interaction of IGFBP-5 with other components of the IGF system might contribute to the PC response. Using subtractive hybridization technique, Maulik and Das found an increased induction of the fatty acid transport (FAT) gene in the preconditioned myocardium [23]. Out of 24 putative positive colonies screened, one clone was matched with >97% homology with FAT gene that has been implicated in binding or transport of long chain fatty acids. cDNA probe synthesized from this clone identified two major transcripts of 4.8 and 2.9 kb. This study showed that FAT gene was induced by PC and oxidative stress, but not by ischemia without reperfusion. Brief ischemia of remote preconditioning of the heart protected gastric mucosa against severe ischemia–reperfusion-induced gastric lesions as effectively.
Table 1
Identification of the genes triggered by preconditioning

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Mode</th>
<th>Up-/downregulated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncogene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-fos</td>
<td>Ischemic</td>
<td>+</td>
<td>[15,16]</td>
</tr>
<tr>
<td>c-myc</td>
<td>Ischemic</td>
<td>+</td>
<td>[15,16]</td>
</tr>
<tr>
<td>c-Jun</td>
<td>Ischemic</td>
<td>+</td>
<td>[18]</td>
</tr>
<tr>
<td>Egr-1</td>
<td>Ischemic</td>
<td>+</td>
<td>[18]</td>
</tr>
<tr>
<td>Transcription factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFkB</td>
<td>Ischemic</td>
<td>+</td>
<td>[26,28]</td>
</tr>
<tr>
<td>AP-1</td>
<td>Ischemic</td>
<td>–</td>
<td>[25]</td>
</tr>
<tr>
<td>TF-1</td>
<td>Ischemic</td>
<td>+</td>
<td>[39]</td>
</tr>
<tr>
<td>Heat shock protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSP10</td>
<td>Pharmacologic</td>
<td>+</td>
<td>[62]</td>
</tr>
<tr>
<td>HSP27</td>
<td>Ischemic, pharmacologic</td>
<td>+</td>
<td>[15,16,40,62]</td>
</tr>
<tr>
<td>HSP32</td>
<td>Ischemia</td>
<td>+</td>
<td>[15,16,40]</td>
</tr>
<tr>
<td>HSP90</td>
<td>Ischemic</td>
<td>+</td>
<td>[17]</td>
</tr>
<tr>
<td>HSP70</td>
<td>Ischemic, hyperthermic</td>
<td>+</td>
<td>[47,48,50,51]</td>
</tr>
<tr>
<td>HSP72</td>
<td>Pharmacologic</td>
<td>+</td>
<td>[55,62]</td>
</tr>
<tr>
<td>HSP89</td>
<td>Ischemia</td>
<td>+</td>
<td>[15,16]</td>
</tr>
<tr>
<td>αB-crystallin</td>
<td>Ischemia</td>
<td>+</td>
<td>[40]</td>
</tr>
<tr>
<td>Antioxidants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MnSOD</td>
<td>Ischemic, pharmacologic</td>
<td>+</td>
<td>[12,15,44,52,60]</td>
</tr>
<tr>
<td>Catalase</td>
<td>Ischemic, hyperthermic</td>
<td>+</td>
<td>[15,44]</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td></td>
<td>Pharmacologic</td>
<td>+</td>
</tr>
<tr>
<td>Heme oxygenase</td>
<td>Ischemic, pharmacologic</td>
<td>+</td>
<td>[71]</td>
</tr>
<tr>
<td>Redox-regulated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>Ischemic</td>
<td>+</td>
<td>[31,32]</td>
</tr>
<tr>
<td>Mitochondrial respiratory chain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>Ischemic</td>
<td>+</td>
<td>[29,30,40]</td>
</tr>
<tr>
<td>Adenine nucleotide translocase-1</td>
<td>Ischemic</td>
<td>+</td>
<td>[29]</td>
</tr>
<tr>
<td>ATPase 6</td>
<td>Ischemic, hyperthermic</td>
<td>+</td>
<td>[30,39,51]</td>
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<tr>
<td>Cytochrome b</td>
<td>Ischemic</td>
<td>+</td>
<td>[30,39]</td>
</tr>
<tr>
<td>Ribosomal protein L-23a</td>
<td>Ischemic</td>
<td>+</td>
<td>[39]</td>
</tr>
<tr>
<td>VEGF</td>
<td>Ischemic</td>
<td>+</td>
<td>[39]</td>
</tr>
<tr>
<td>Genes for GTP exchange factor, adenine nucleotide translocase, isoforms 1 (ANT1), β-F1-adenine triphosphatase</td>
<td>Hyperthermic</td>
<td>+</td>
<td>[51]</td>
</tr>
<tr>
<td>Mitogen-activated kinases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAPKAP kinase 3</td>
<td>Ischemic</td>
<td>+</td>
<td>[40]</td>
</tr>
<tr>
<td>Mitogen-responsive phosphoprotein</td>
<td>Ischemic</td>
<td>+</td>
<td>[40]</td>
</tr>
<tr>
<td>Nuclear-encoded genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear respiratory factor-1</td>
<td>Ischemic</td>
<td>+</td>
<td>[29]</td>
</tr>
<tr>
<td>Nitric oxide-producing genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>Ischemic, pharmacologic</td>
<td>+</td>
<td>[40,58,59,63,66]</td>
</tr>
<tr>
<td>cNOS</td>
<td>Pharmacologic</td>
<td>+</td>
<td>[58,59,65]</td>
</tr>
<tr>
<td>Growth factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor II</td>
<td>Ischemic</td>
<td>+</td>
<td>[22]</td>
</tr>
<tr>
<td>Insulin receptor</td>
<td>Ischemic</td>
<td>+</td>
<td>[22]</td>
</tr>
<tr>
<td>Type I receptor</td>
<td>Ischemic</td>
<td>+</td>
<td>[22]</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Ischemic</td>
<td>+</td>
<td>[22]</td>
</tr>
<tr>
<td>IGF-II</td>
<td>Ischemic</td>
<td>+</td>
<td>[22]</td>
</tr>
<tr>
<td>IGFBP-2–6</td>
<td>Ischemic</td>
<td>+</td>
<td>[22]</td>
</tr>
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</table>
as local preconditioning of the stomach itself via the mechanism involving prostaglandin derived from cyclooxygenase-1 and cyclooxygenase-2 and the activation of sensory nerves releasing calcitonin gene-related peptide (CGRP) combined with the suppression of IL-1β and TNFα gene expression [24]. In another study, PC was found to be effective to protect myocardium through decreasing PAI-1 mRNA expression [25].
Perhaps the most interesting finding is related to the ability of PC to induce redox-sensitive transcription factors and genes. Isolated rat hearts preconditioned by repeated reversible ischemia resulted in an increase in the mRNA of pleiotropic oxidant-sensitive nuclear transcription factor NFκB, but a decrease in AP-1 [25]. The same study showed an upregulation of Bel-2 mRNA and a downregulation of p53 indicating differential regulation of pro- and anti-apoptotic transcription factors and genes by PC. The fact that PC is regulated by redox signaling was evidenced for the first time when the DNA binding of NFκB was found to be elevated by PC [26,27]. Subsequently, Bolli’s group also found a significant role of NFκB in delayed PC [28]. In this study, a sequence of six 4-min coronary occlusion/4-min perfusion cycles induced rapid activation of NFκB, as evidenced by a marked increase in p65 content and NFκB DNA-binding activity. The PC-mediated activation of NFκB was blocked by pretreatment with N-o-nitro-L-arginine (L-NA), a nitric oxide synthase (NOS) inhibitor, N-2-mercaptopyrrolglycine (MPG), a reactive oxygen species (ROS) scavenger, chelerythrine, a protein kinase C (PKC) inhibitor and lavendustin A, a tyrosine kinase inhibitor, indicating that PC activates NFκB via formation of NO, ROS, and activation of PKC and tyrosine kinase-dependent pathways.

Redox regulation of PC was further evidenced by another study, which demonstrated activation of nuclear-encoded electron-transfer-chain gene expression in parallel with enhanced post anoxic mitochondrial respiratory recovery by delayed PC [29]. In this study, 24h after PC, infarct size was reduced by 58% and preconditioned mitochondria exhibited modest hyperpolarization of the inner mitochondrial membrane potential. Preconditioned mitochondria demonstrated improved ADP-sensitive respiration with preservation of electron transfer chain activity, which was abolished by pretreating the hearts with an antioxidant 2-mercapto-propionyl glycine (2-MPG). These biochemical modulations appeared to be regulated at the genomic level in that the expression of the genes encoding rate-controlling complexes in the electron transport chain was significantly reduced in the PC myocardium, with a concordant reduction of local tissue complement production may be one of the means by which PC protects the ischemic myocardium.

Altered Ca2+ transport protein gene has been found to play a role in PC [35]. The most extensive studies were undertaken by Dhall’s group, who eventually demonstrated that PC attenuates ischemia–reperfusion-induced remodeling of Na+/K+-ATPase in hearts [36,37]. The effect of PC induced by three 10min cycles of ischemia and reperfusion brought molecular changes in Na+/K+-ATPase subunit composition due to ischemia/reperfusion. Ischemia/reperfusion reduced the mRNA levels of α2, α3, β1 and β2 isoforms by 71%, 85%, 27% and 65%, respectively, whereas the α1 isoform was decreased by <15%. PC attenuated the reduction of these subunits induced by ischemia/reperfusion. PC also modified similar alternations in Na+/Ca2+ exchanger indicating that PC attenuates ischemia/reperfusion-mediated remodeling of Na+/K+-ATPase and Na+/Ca2+ exchanger genes in the hearts. In another related study, the same group demonstrated that PC modified ischemia/reperfusion-induced changes in gene expression for sarcoplasmic reticulum proteins such as the Ca2+ release channel, Ca2+-ATPase pump, phospholamban and calsequestrin in the rat heart [38].

PC-mediated reprogramming of gene expression was clearly demonstrated from the results of differential display and subtractive hybridization techniques. Using these techniques, Maulik et al. found upregulation and down-
regulation of a significant number of genes in the preconditioned myocardium [39]. The results documented differential patterns of gene expression after reversible ischemia, after single episode of 5 min ischemia and 10 min reperfusion and after cyclic episodes of ischemia and reperfusion. Analysis of cDNA fragments identified by differential mRNA display and Northern hybridization verified increased expression of several mitochondrial genes including ATPase 6, Cyt b and ribosomal protein L23a. The genes of ATPase 6 and Cyt b were induced even after 5 min of ischemia (3-fold and 2.5-fold, respectively) and further stimulated after 1XPC [induced by 5 min ischemia and 10 min reperfusion] (2.4-fold and 2.9-fold) and 4XPC [4 cyclic episodes of 5 min ischemia and 10 min reperfusion] (5.8-fold and 3.5-fold, respectively). Very little is known regarding mitochondrial transcription although the initiation is known to be under the control of nuclear-derived transcription factor TF-1. Both ATP6 and Cytochrome b genes are involved in oxidative ATP synthesis at the inner mitochondrial membrane and are located on the mitochondrial DNA. Upregulation of these genes during PC is likely to be the adaptive response, because synthesis of these mitochondrial proteins is essential for the heart to maintain cellular function during ischemia. However, mitochondrial gene expression is not self-sufficient, since some of the components that are essential for transcription and translation for these mitochondrial genes are encoded in the nucleus. Another gene that was found to be upregulated after PC is ribosomal protein L23a. This gene is related to *Saccharomyces cerevisiae* L25 and to *Eschericia coli* L23 ribosomal proteins that bind to a specific site in domain III of 26S and 23S rRNAs. Ribosomal protein L23a contains 156 amino acids, and participates in the initiation of the assembly of the large ribosomal subunits for the synthesis of proteins. Very little is known about the functions of this ribosomal protein; it presumably plays a role in the development and differentiation of the cells.

Subsequently Kloner’s group, using gene array technology, found 35 genes with significantly altered expression patterns [40]. This study was performed with open-chest rabbits subjected to two episodes of 5 min of ischemia and 5 min of reperfusion followed by an additional 5 h and 5 min of reperfusion. In the PC hearts, genes of MAPKAP kinase 3 and cathepsin G were upregulated, whereas in the non-ischemic area, genes for GTP exchange factor, Na⁺/K⁺-ATPase, Zn finger protein 35, Cytochrome c oxidase, mitogen-responsive phosphoprotein and a Ran-binding protein were upregulated. Cardioprotective genes triggered by PC include MAPKAPK3, HSP27, HSP70, αB-crystalline, VEGF, iNOS and plasminogen activator inhibitors 1 and 2 [41]. With permanent coronary occlusion lasting from 24 h to several weeks, and resulting in a true myocardial infarction (MI), the list of upregulated genes included those related to remodeling, e.g., collagens I and III, fibronectin, laminin and apoptosis, e.g., Bax while many downregulated genes were related to major energy-generating pathways in the heart, e.g., fatty acid metabolism [42]. In canine preconditioned myocardium, alteration of genes involved in inflammatory response was found after 16 h [43]. cDNA representational difference analysis in combination with microarray hybridization and reverse transcription PCR was used to reveal the changes in gene expression. The upregulated genes included functionally related genes for tristetraproline (TPP), selectin E, matrix metalloproteinase 9, and TNFα.

2.2. Hypoxic/hyperoxic preconditioning

A brief hypoxic episode also can precondition myocardium against a subsequent ischemic reperfusion injury. In the hypoxically preconditioned rat hearts, there was an upregulation of catalase and HSP70 gene expression, which corroborated with the improved postischemic function during prolonged hypothermic storage of the hearts [44]. In another study, hearts from rodents exposed to intermittent hypoxia were protected against ischemia–reperfusion injury [45]. Erythropoietin mRNA expression was induced in the wild-type mice subjected to intermittent hypoxia. Erythropoietin mRNA was not induced in HIF1α+/− mice suggesting that cardiac protection induced by intermittent hypoxia is critically dependent on HIF1α gene dosage. Hyperbaric oxygenation pretreatment by intermittently exposing the rats to 100% O₂ at 3ATA for 1 h daily increased myocardial resistance to ischemia–reperfusion injury [46]. Such hyperbaric oxygenation reduced myocardial infarct size and increased the catalase gene expression, which was blocked by a catalase inhibitor, 3-amino-1,2,4-triazole, implying that hyperbaric PC was mediated by an induction of catalase gene.

2.3. Hyperthermic and hypothermic preconditioning

Similar to ischemic PC, hyperthermic PC also preconditioned the heart with the upregulation of cardioprotective genes. For example, swine hearts on cardiopulmonary bypass when subjected to heat shock by continuous infusion to the globally arrested heart of warm blood cardioplegia at 42°C for 15 min; the hearts developed increased tolerance against subsequent ischemia [47]. Such tolerance to ischemia was associated with an increased expression of HSP70. In another related study, preconditioning of cultured murine cardiomyocytes with an 8 microT 60 Hz electromagnetic field [increased the temperature to 32°C] induced a 77% increase in HSP70 and resulted in cell survival [48]. In another study, local somothermal stimulation on the left median nerve territory increased myocardial HSP70 mRNA and protected the rat hearts from ischemia–reperfusion injury [49]. In a more recent study with patients undergoing open-heart surgery, intermittent warm blood cardioplegia induced an increased expression of HSP72 and it was associated with a better myocardial protection as evidenced by lower levels of CK-MB and troponin-T [50].
Studies performed in isolated perfused rabbit hearts subjected to hypothermic cardiac arrest at 31°C followed by 2h of reperfusion at 34°C, preserved the heart compared to control heart [51]. mRNA levels of the nuclear-encoded mitochondrial proteins adenine nucleotide translocase isoforms 1 (ANT1) and beta-F1-adenosine-triphosphatase (beta-F1-ATPase) decreased in control hearts, but were preserved in the hypothermically arrested hearts after reperfusion. Inducible HSP70 mRNA was elevated nearly 4-fold after ischemia in control hearts and 12-fold in hypothermic hearts.

2.4. Pharmacological preconditioning

Lipopolysaccharide (LPS) PC induces cardiac resistance to subsequent LPS or ischemia. When rats were preconditioned with a single dose of LPS (0.5mg/kg, i.p.), they acquired cardiac resistance to endotoxemic depression after 1–7 days [52]. This resistance temporarily correlated with resistance to ischemia. Similar to ischemic PC, LPS PC induced the expression of cJun and cFos mRNAs and transiently increased mRNAs encoding catalase and MnSOD. The expression of both α and β-myosin heavy chain mRNAs was upregulated, whereas the expression of cardiac α-actin mRNA was suppressed suggesting that LPS PC is associated with reprogramming of myocardial gene expression. In another study, LPS preconditioned endothelial cells by altering upregulation of E- and P-selectins [53]. More recently, LPC PC was found to occur through the attenuation of the activation of NfκB and inflammatory cytokine genes [54]. The values in PC and LPS groups were higher before ischemia/reperfusion, and then decreased from 30 min until 3 h of reperfusion. Nuclear staining of NfκB after reperfusion was less in the PC and LPS groups than in the control group. Expressions of the mRNAs of IL-1β, IL-6 and TNF-α were increased up to 3 h of reperfusion, but reduced substantially by PC or LPS suggesting that PC and LPS contributed to infarct size reduction by reducing NfκB and attenuation of cytokine gene expression.

In vitro preconditioning with a non-toxic derivative of endotoxin, monophosphoryl lipid A (MLA) in adult rat cardiac myocytes, resulted in significant resistant to ischemic injury after 20 h [55]. Western blot analysis indicated a significant accumulation of 72kDa HSP in MLA treated as compared to control cells suggesting that anti-ischemic effect of MLA is accompanied by the induction of HSP72. The same group however, found negative involvement of HSP70 MLA-mediated preconditioning of the rabbit hearts [56]. Another recent study demonstrated the ability of MLA to precondition rat heart through the downregulation of TNFα [57]. Nitric oxide, especially iNOS gene, has been implicated in pharmacologic preconditioning of MLA [58,59].

Rats pretreated with a cell wall fragment of Gram-positive bacteria (similar to LPS, which is a Gram-negative bacterium), lipoteichoic acid (LTA) for 16h, reduced the myocardial infarct size by 65% and decreased the release of troponin T into the plasma [60]. The same study demonstrated that LPS caused a time-dependent induction of TNFα, IL-1β and MnSOD mRNAs in the heart, whereas LTA failed to induce MnSOD mRNA. Interestingly, LPS caused an upregulation of the mRNAs of intracellular adhesion molecule-1 and P-selectin, whereas LTA downregulated these molecules.

Opioid-induced preconditioning can also lead to the induction of gene expression. When rats were pretreated with a delta-opioid agonist SNC-121, the hearts of the rats were resistant to ischemia–reperfusion injury [61]. Gene array data showed a significant induction of 12-lipoxygenase (12-LO) mRNA after opioid pretreatment. This induction of 12-LO mRNA was confirmed by real-time PCR, and 12-LO protein expression was enhanced by SNC pretreatment at 24 h relative to vehicle treatment. Both baicalein and phenidone (12-LO inhibitors) attenuated the protective effects of SNC pretreatment on infarct size suggesting a role of 121-LO gene expression in opioid preconditioning.

Similar to opioid-preconditioning, anesthetic preconditioning can also trigger gene expression in hearts. Using affymetrix rat U34A gene chips, 217 out of 8799 genes represented on U34A, were upregulated by anesthetic-PC as compared to 234 by ischemic PC [62]. Downregulated genes included 185 transcripts for anesthetic-PC group and 55 in the ischemic PC group. Genes commonly regulated included those associated with cell defense (HSP10, aldose reductase, Bcl-xS), whereas the genes that were differentially regulated included HSP27, HSP70, programmed cell death. In another study, morphine induced late cardioprotection through the induction of iNOS [63]. In this study, pretreatment with S-methylthiourea sulfate (SMT), a selective inhibitor of iNOS abolished morphine-induced reduction of infarct size. Morphine also failed to reduce infarct size of iNOS gene knockout mice.

Several other chemicals have been used to induce preconditioning. For example, nicorandil, a potassium channel opener, has been used for classical and delayed PC. Twenty-four hours after nicorandil treatment, expression of cyclooxygenase-2 (COX-2) and Bcl-2 was significantly elevated in myocardium of rabbits [64], indicating that nicorandil induces delayed cardioprotection against myocardial infarction through upregulation of COX-2 and Bcl-2. Bradykinin and acetylcholine-mediated PC was found to be mediated by ROS generation through sequential activation of Akt and NOS [65].

More recently, resveratrol (trans-3,4,5-trihydroxystilbene), a grape-derived polyphenolic antioxidant, has been found to pharmacologically preconditioned the heart. Resveratrol PC occurs through the upregulation of iNOS as the inhibitor of iNOS abolishes the cardioprotective abilities of resveratrol [66]. The role of iNOS gene in resveratrol PC is further supported from the studies where...
resveratrol was unable to precondition the hearts from the iNOS−/− mice [67]. A subsequent study has suggested that the coordinated upregulation of iNOS-VEGF-KDR-eNOS, is one of the mechanisms for resveratrol preconditioning of the heart [68]. Another related study has indicated that similar to ischemic PC, adenosine receptors have important function in the resveratrol preconditioning [69]. It has been suggested that adenosine A1 and A3 receptors, but not A2a or A2b receptors, play a critical role in the pharmacological preconditioning by resveratrol [67,68]. Resveratrol likely activates both adenosine A1 and A3 receptors, which phosphorylate PI-3 kinase, which in turn phosphorylates protein kinase B (Akt) and thus preconditions the heart by producing NO as well as by the activation of antioxidant transcription factor BCl-2. Das et al. showed that the activation of adenosine A3 receptors, which phosphorylate PI-3 kinase, which in turn phosphorylates protein kinase B (Akt) and thus preconditions the heart by producing NO as well as by the activation of antioxidant transcription factor BCl-2. Das et al. showed that the activation of adenosine A3 receptors, which phosphorylate PI-3 kinase, which in turn phosphorylates protein kinase B (Akt) and thus preconditions the heart by producing NO as well as by the activation of antioxidant transcription factor BCl-2. Das et al. showed that the activation of adenosine A3 receptors, which phosphorylate PI-3 kinase, which in turn phosphorylates protein kinase B (Akt) and thus preconditions the heart by producing NO as well as by the activation of antioxidant transcription factor BCl-2. Das et al. showed that the activation of adenosine A3 receptors, which phosphorylate PI-3 kinase, which in turn phosphorylates protein kinase B (Akt) and thus preconditions the heart by producing NO as well as by the activation of antioxidant transcription factor BCl-2. Das et al. showed that the activation of adenosine A3 receptors, which phosphorylate PI-3 kinase, which in turn phosphorylates protein kinase B (Akt) and thus preconditions the heart by producing NO as well as by the activation of antioxidant transcription factor BCl-2. Das et al. showed that the activation of adenosine A3 receptors, which phosphorylate PI-3 kinase, which in turn phosphorylates protein kinase B (Akt) and thus preconditions the heart by producing NO as well as by the activation of antioxidant transcription factor BCl-2.

It should be clear from the above discussion that a strong resemblance in the pattern of gene expression exists between the stress responses induced by diverse preconditioning stimuli. It appears that oxidative stress generated from the reactive oxygen species (ROS) seats at the crossroads between, ischemia, hypoxia or hyperoxia as well as hyperthermia or hyperthermia. It is not surprising that several antioxidants and redox-regulated genes are triggered in response to any preconditioning stimulus, because constitutive cellular protection is provided against environmental stress by antioxidants. The signal transduction pathway by which various stress signals are translated into oxidative stress leading to the modulation of antioxidants/HSPs are likely to be different, but the induction of the expression of their mRNAs and/or the increased translation of mRNA accumulation as a result of changes at the level of RNA transcription or stability seem to be the most plausible mechanism. This could lead to the synthesis of proteins involved in cellular protection or repair of injury. This phenomenon related to specific gene expression could be viewed as the most important line of defense for the tissue and may reflect ultimate adaptive response. Redox signaling is likely to be responsible for the conversion of ischemia/reperfusion-induced “death signal” into preconditioning-mediated “survival signal” [75].

3. Conclusion

It should be clear from the above discussion that a strong resemblance in the pattern of gene expression exists between the stress responses induced by diverse preconditioning stimuli. It appears that oxidative stress generated from the reactive oxygen species (ROS) seats at the crossroads between, ischemia, hypoxia or hyperoxia as well as hyperthermia or hyperthermia. It is not surprising that several antioxidants and redox-regulated genes are triggered in response to any preconditioning stimulus, because constitutive cellular protection is provided against environmental stress by antioxidants. The signal transduction pathway by which various stress signals are translated into oxidative stress leading to the modulation of antioxidants/HSPs are likely to be different, but the induction of the expression of their mRNAs and/or the increased translation of mRNA accumulation as a result of changes at the level of RNA transcription or stability seem to be the most plausible mechanism. This could lead to the synthesis of proteins involved in cellular protection or repair of injury. This phenomenon related to specific gene expression could be viewed as the most important line of defense for the tissue and may reflect ultimate adaptive response. Redox signaling is likely to be responsible for the conversion of ischemia/reperfusion-induced “death signal” into preconditioning-mediated “survival signal” [75].

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References


