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Forum Review Article

The Role of Nitric Oxide in Myocardial Repair and Remodeling

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Abstract

Nitric oxide (NO) plays a crucial role in many aspects of the pathophysiology of heart failure. NO is a double-edged sword; NO inhibits ischemia/reperfusion (I/R) injury, represses inflammation, and prevents left ventricular (LV) remodeling, whereas excess NO and co-existence of reactive oxygen species (ROS) with NO are injurious. The failing heart is exposed to not only oxidative stress by a plethora of humoral factors and inflammatory cells but also nitrosative stress. Activation of nitric oxide synthase (NOS) of any isoforms, [i.e., endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS)], concomitant with oxidative stress results in NOS uncoupling, leading to further oxidative/nitrosative stress. Indiscriminate removal of oxidative stress is not an effective means to prevent this detrimental process, because oxidative stress is necessary for an adaptive mechanism for cell survival against noxious stimuli. Therefore, removal of ROS in a site-specific manner or inhibition of the source of injurious ROS without affecting redox-sensitive survival signal transduction pathways represents a promising approach to elicit the beneficial effect of NO. Recent emerging pharmacological tools and regular exercise inhibit ROS generation in the proximity of NOSs, thereby increasing bioavailable NO and exerting cardioprotection against I/R injury and LV remodeling. *Antioxid. Redox Signal.* 11, 1913–1928.

Introduction

Myocardial infarction (MI) and the consequent loss of fully functional myocardium is a major etiology for heart failure. Despite aggressive primary therapy, prognosis remains serious in patients with large MI and severe left ventricular (LV) dysfunction. A large volume of functional myocardium is lost immediately after MI (within several hours) as a result of ischemia/reperfusion (I/R) injury and it is the extent of I/R injury that is a principal determinant of prognosis for MI. However, a considerable part of functional myocardium is lost within days after MI as a result of inflammation and chronic hypoxia and deficiency of nutrients in the infarction border. Remaining functional cardiomyocytes far from the site of MI undergo hypertrophy as a result of volume and pressure load, leading to continuous cell death with replacement of a fibrous tissue which is a principal mechanism for LV remodeling and heart failure. Therefore, it is imperative to develop systematic approaches to intervene this natural course of post-MI LV remodeling and heart failure. The ability of the myocardium to successfully adapt to cardiac injury ultimately determines whether the heart will decompensate and fail, or whether instead it will maintain preserved function. Thus, it would be highly desirable to influence I/R injury and healing of the cardiac wound to maintain structure and function of the heart.

Ventricular remodeling is a long-lasting reparative process following MI and also occurs after mechanical overload (for example, in hypertension or valvular heart disease), inflammation, and dilated cardiomyopathy. Despite the varying etiopathology that these different aspects of heart disease share, a similar sequence of molecular, biochemical, and mechanical events that can lead to heart failure, myocyte hypertrophy, extensive extracellular matrix production and fibrosis, even in patients who were previously unaffected by the original disease process (for example, inflammation or infarction).

Heart failure can be influenced by treatment of the underlying disease and by modification of the remodeling process including direct inhibition of cardiomyocyte death pathway, angiogenesis, and extracellular matrix organization. Ventricular remodeling can be an (early) adaptive response followed

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by a maladaptive (late) phase and involves all cells that are present in the myocardium; cardiomyocytes, interstitial cells, vascular endothelium, and immune cells. However, because the goal of cardiac repair after myocardial injury is to preserve functional myocardium, any interventions to modulate functions of these cells should culminate in protection of cardiomyocytes. There have been many interventions to protect the diseased heart from heart failure. Timely recanalization of occluded coronary arteries with the aid of mechanical and pharmacological tools are the direct and the most efficacious way to limit infarct size and preserve functional myocardium. Once the infarcted heart is revascularized, pharmacological approaches and physical therapies are taken place to inhibit the enlargement of infarct size. However, the molecular basis of these interventions to protect the diseased heart remains largely unclear.

Accumulating evidence indicates that nitric oxide (NO) plays a central role in cell death/survival and tissue repair/remodeling. NO is one of gaseous signaling molecules, which were previously considered to be toxic. However, the identification of NO as the endothelium-derived relaxing factor combined with the discovery of NO generation by nitric oxide synthases (NOS)s primed an explosion of research in this area in the 1990s (55, 94, 105). It is now apparent that NO and cognate reactive nitrogen intermediates are involved in a wide variety of pathophysiological processes in the cardiovascular system where it orchestrates a plethora of cellular activities in cardiomyocytes, endothelial cells, vascular smooth muscle cells, and circulating inflammatory cells. Therefore, NO represents one of the most valuable molecular tools in modulating cell death/survival. This article is an overview of the role of NO in myocardial I/R injury, repair and remodeling, and will discuss systematic approaches to preserve functional myocardium by modulating NO signaling.

Role of NO in Modulating Cell Death/Survival Signaling

Biosynthesis of NO

NO acts as a signaling molecule in conjunction with reactive oxygen species (ROS) by generating oxidative/nitrosative stress in the cell. NO possesses a number of key features that collectively make this molecule ideally suited to its cellular signaling functions. NO is a lipophilic diatomic gas under atmospheric conditions. It has a relatively small Stoke's radius and this, in combination with its neutral charge, facilitates rapid membrane diffusion (40). The presence of an unpaired electron in NO supports its high reactivity with oxygen (O_2), superoxide (O_2^-), transition metals, and thiols, which largely shape its cellular functions within the cell. The removal of the unpaired electron in NO generates the nitrosonium cation NO^+, while the addition of an electron forms the nitroxyl anion (NO^-). These different forms of NO exhibit distinct chemical reactivities (129). NO reacts with O_2 to produce a variety of distinct nitric oxides that each has unique reactivity profiles. In the presence of O_2^-, NO reacts with O_2^- to form peroxynitrite ($ONOO^-$), a particularly destructive molecule within biological systems (17).

Biosynthesis of NO is dependent on enzymatic activity of NOS. Three distinct NOS isoforms have been identified by molecular cloning: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS). NOS is a homodimeric oxidoreductase containing iron protoporphyrin IX (heme), flavin adenine dinucleotide, flavin mononucleotide, and tetrahydrobiopterin (BH_4) which is a cofactor essential for the catalytic activity of all three NOS isoforms (87,130). The flavin-containing reductase domain and a heme-containing oxygenase domain are connected by a regulatory calmodulin-binding domain. In the case of constitutive NOS (*i.e.*, nNOS and eNOS), binding of Ca^{2+}/calmodulin orients the other domains to allow nicotinamide adenine dinucleotide phosphate (NADPH)-derived electrons generated in the reductase domain to flow to the oxygenase domain (1), ultimately resulting in the conversion of L-arginine to NO and L-citrulline. This occurs if BH_4 is bound in the dimer interface, where it interacts with amino acid residues from both monomers to stabilize NOS dimerization and participate in arginine oxidation through the N-hydroxyl-L-arginine intermediate and the subsequent generation of NO. BH_4 depletion, because of its oxidation and/or reduced synthesis, can result in functional uncoupling of NOS. Uncoupled NOS generates more ROS and less NO, shifting the nitroso-redox balance and having adverse consequences on the cardiovascular system. Thus, reduced BH_4 and uncoupling of NOS plays an important role in I/R injury, cardiac hypertrophy, and remodeling (88).

Pathophysiology of NO

Excessive generation of NO is detrimental to cardiovascular function as exemplified in septic shock where burst generation of iNOS-derived NO causes hypotension, cardiodepression, and vascular hyporeactivity (138). An experimental study has demonstrated that NO donors increase cardiomyocyte cell death and switch the form of cell death from apoptosis to necrosis as a function of their concentration (141). The detrimental effect of excess NO is attributed to the action on mitochondria. NO inhibits the mitochondrial respiratory chain, resulting in inhibition of ATP production, increased oxidant production, and increased susceptibility to cell death (21, 26). Peroxynitrite inhibits mitochondrial respiration at multiple sites, and also causes mitochondrial permeability transition pore (MPTP) opening (20, 127), which is a critical target for cardioprotection by ischemic preconditioning (IPC), as described later (95). Inhibition of mitochondrial respiration by NO and its derivatives stimulates production of reactive oxygen and nitrogen species by mitochondria (21), which contribute to cell death in excess. NO inhibition of mitochondrial respiration is likely to be more important in the diseased heart where a large amount of iNOS-derived NO is generated under the inflammatory conditions (19).

NO Signaling

Protein nitration

On the other hand, NO acts in favor of protecting the heart from various noxious stimuli through the NO signaling. Although peroxynitrite is a destructive molecule, it may simultaneously function as a cardioprotective molecule (34). One of the important molecular targets of peroxynitrite is protein tyrosine residues, which can be modified to fairly stable 3-nitrotyrosines (3-NT) upon reacting with peroxynitrite. Protein nitration is a selective process with respect to both the proteins and the specific protein tyrosine residues that can undergo this post-translational modification (128). Nitration

of protein tyrosine residues alters the functions of a variety of proteins under physiological and pathophysiological conditions both *in vitro* and *in vivo* (27, 58). Post-translational modification of tyrosine residues has been shown to play an important role in modulating the activity of several protein kinase C (PKC) isozymes including PKC-ε (5) which has consistently been implicated in the cardioprotective signal transduction (42, 77).

Activation of guanylyl cyclase

NO also binds a variety of hemoproteins forming a nitrosyl–iron complex. For example, guanylyl cyclase (GC), which generates cyclic guanosine monophosphate (cGMP), is a key NO target (79). When NO binds to the heme group within the regulatory domain. it forms an iron–nitrosyl–heme complex that is required for GC activation and cognate cGMP formation. Subsequently, the function of this enzyme activates a cGMP-dependent protein kinase, protein kinase G (PKG), which regulates a plethora of cellular activities, including vascular tone, cell survival, endothelial permeability, and vascular homeostasis and proliferation (51).

cGMP content in the tissue is critically regulated by cGMP phosphodiesterase (PDE). In the cardiovascular system, cGMP hydrolysis is thought to be accomplished by PDE1, PDE2, and PDE5. PDE1 contains an autoinhibitory domain which maintains low activity in the absence of Ca^{2+}, and neighboring calmodulin binding domains that restore full activation in the presence of Ca^{2+}-calmodulin (81). PDE2 is not a primary PDE in vascular smooth muscle, but is expressed in cardiac myocytes, and recent data supports its role in the targeted regulation of cGMP and cyclic adenosine monophosphate (cAMP). PDE5 has been shown to play a key role in vascular smooth muscle tone, particularly in the venous system of the corpus cavernosum and the pulmonary vasculature. NO–GC-generated cGMP and PDE5 inhibition have recently been shown to suppress postischemic dysfunction in mice, and these effects are attributed to the attenuation of cardiomyocyte death due to necrosis and apoptosis by an NO-stimulated increase in the ratio of Bcl2/Bax (36). The cardioprotective effect of cGMP has been confirmed by the emergence of sildenafil and other clinically approved PDE5 inhibitors, including vardenafil and tadalafil, which have been proven to prevent several cardiovascular disorders including essential hypertension, endothelial dysfunction, I/R injury, MI, ventricular remodeling, and heart failure in animal models (67).

Protein S-nitrosylation

In addition to activating GC/cGMP/PKG-dependent signaling pathways, NO can directly modify sulfhydryl residues of proteins through *S*-nitrosylation, which has emerged as an important post-translational protein modification (46, 49, 83). Furthermore, *S*-nitrosylation of critical protein thiols protects them from further oxidative modification by ROS (49, 132, 160) that may be important in cardioprotection via protection against oxidative stress and Ca^{2+} overload in mitochondria and resultant MPTP opening (24, 131). In line with this notion, cardioprotective signal transduction mediated by tyrosine nitration, PKG, and *S*-nitrosylation may converge on inhibition of MPTP opening by inhibiting oxidative stress and Ca^{2+} overload in mitochondria. Collectively, the role of NO in cardiac injury and protection is summarized in Fig. 1.

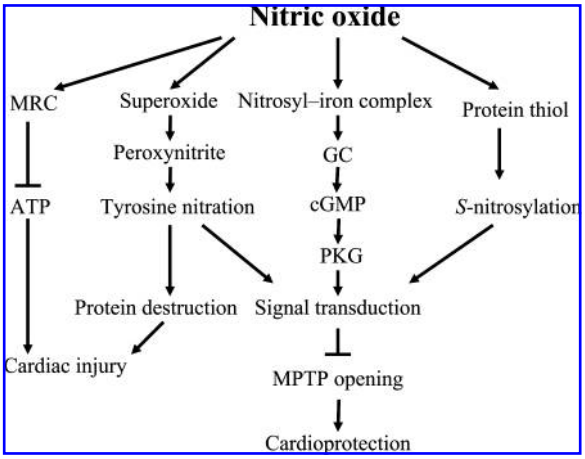

FIG. 1. The role of nitric oxide (NO) in cardiac injury and protection. Excess NO binds several components of mitochondrial respiratory chain (MRC), thereby inhibiting mitochondrial function and ATP generation, leading to cardiac injury. NO in the presence of superoxide generates peroxynitrite that is a highly reactive molecule causing tyrosine nitration and destruction of critical proteins for cell survival. On the other hand, tyrosine nitration of certain proteins is involved in cardioprotective signal transduction. NO binds hemoproteins generating nitrosyl–iron complex. Resultant activation of guanylyl cyclase (GC) generates cyclic GMP (cGMP) and activates cGMP-dependent protein kinase (PKG) which is involved in vasorelaxation and cardioprotection. Alternatively, NO binds protein thiols, causing *S*-nitrosylation of proteins involved in cardioprotective signal transduction. All the cardioprotective signal transductions promoted by NO converge on inhibition of mitochondrial permeability transition pore (MPTP) opening.

Role of NO in Modulating I/R Injury

Role of NO in ischemic preconditioning

The first step in preserving functional myocardium is to inhibit cardiomyocyte cell death. MI and the consequent loss of functional myocardium is the most frequent cause of chronic heart failure. Thus, extensive efforts have been exerted to minimize infarct size associated with MI. Permanent occlusion of coronary arteries produces as much as 80% infarction/area at risk. However, timely reperfusion can reduce infarction/area at risk to ~50% (156). Moreover, appropriate myocardial protection during I/R could further reduce infarction/area at risk by >50%. The most established approach for myocardial protection against I/R injury in animal models is IPC. IPC was first discovered by Murry *et al.* (96) and has been extensively studied by many investigators in the last 2 decades. IPC is the state-of-the-art technique for myocardial protection against I/R injury. It is now evident that IPC has two distinct phases: an early phase that lasts from a few minutes to 2–3 h, and a late phase, termed late preconditioning, which develops after 12 h, peaked between 24–48 h and lasts for 72–96 h (7, 13, 155). Thus, the memory of cardioprotection is the characteristic feature of both early and late IPC (101). Importantly, the mechanism of cardioprotection mediated by IPC is intimately related to increased generation of NO, as illustrated in Fig. 2. IPC is induced by single or repeated brief I/R before lethal periods of ischemia, during

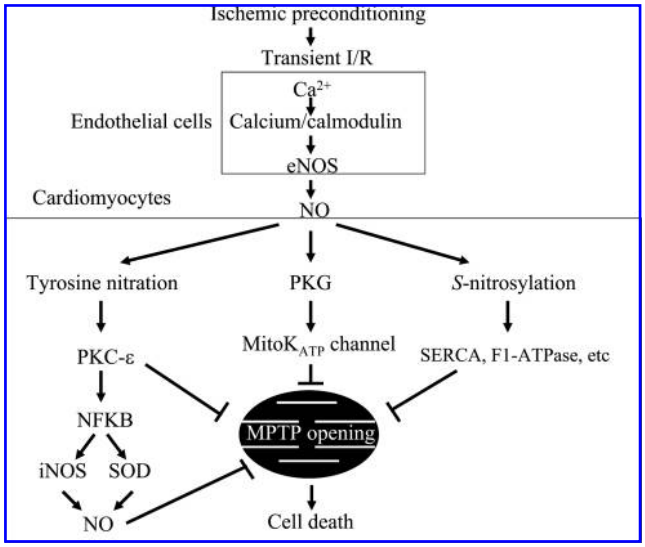

FIG. 2. The role of nitric oxide (NO) in cardioprotective signal transduction mediated by ischemic preconditioning (IPC). IPC implemented by transient ischemia (I)/reperfusion (R) results in Ca^{2+} overload in endothelial cells that activates endothelial nitric oxide synthase (eNOS) through activation of calcium/calmodulin-dependent protein kinase. Resultant increase in eNOS-derived NO promotes post-translational modifications of proteins in cardiomyocytes as described in Fig. 1. Tyrosine nitration of protein kinase C-ε (PKC-ε), cyclic GMP-dependent protein kinase (PKG) activation of mitochondrial K_{ATP} ($MitoK_{ATP}$) channels, and *S*-nitrosylation of critical protein thiols such as sarcoplasmic reticulum Ca^{2+} channels (SARCA) and mitochondrial F1-ATPase etc inhibit mitochondrial permeability transition pore (MPTP) opening wthat promotes both apoptotic and oncotic cell death. Alternatively, activation of PKC-ε activates nuclear factor-kappa B (NFKB) and increases transcriptional upregulation of inducible nitric oxide synthase (iNOS). Concomitant upregulation of the antioxidant defense system, particularly superoxide dismutase (SOD), increases bioavailable NO derived from iNOS via inhibition of conversion to peroxynitrite and uncoupling of iNOS. Resultant increase in iNOS-derived NO is an essential component of late preconditioning of which cardioprotective signal transduction also converges on inhibition of MPTP opening.

which eNOS is activated (8). The eNOS-derived NO enters cardiomyocytes and promotes NO-dependent cardioprotective signaling pathways. Tyrosine nitration of PKC-ε has been implicated in IPC-mediated cardioprotection (5). PKG activation, on the other hand, activates mitochondrial K_{ATP} ($mitoK_{ATP}$) channels that play a triggering role by generating superoxide, as described later, as well as a mediator role by inhibiting MPTP opening (102). Relatively less is known about the role of protein *S*-nitrosylation in cardioprotection afforded by IPC. A recent study demonstrated that *S*-nitrosothiols were detected in mitochondria isolated from IPC hearts, suggesting that protein *S*-nitrosylation may play an important role in IPC-mediated cardioprotection (24). Proteomic analysis have demonstrated that IPC results in *S*-nitrosylation of a number of proteins, including the cardiac sarcoplasmic reticulum Ca^{2+}-ATPase, α-ketoglutarate dehydrogenase, and the mitochondrial F1-ATPase α1 subunit (131). *S*-nitrosylation of these proteins by IPC may confer cardioprotection by preserving ATP and inhibiting intracellular Ca^{2+} overload.

Although an obligatory role of NO in mediating the early IPC remains a considerable matter of debate (78, 107, 147), NO can fully reproduce the cardioprotective effect of the early IPC in the presence of other triggers of IPC (*i.e.*, G protein-coupled rceptor (GPCR) agonists and $mitoK_{ATP}$ channel openers) and this combined pharmacological preconditioning (PPC) has been termed integrated PPC (142). The experiments performed by Uchiyama *et al*. (142) suggest that integrated PPC is equally effective as IPC in generating the memory of cardioprotection by persistently activating $mitoK_{ATP}$ channels, as illustrated in Fig. 3. The capability of integrated PPC to generate the memory of cardioprotective signal transduction was confirmed in the cultured cardiomyocyte model in which integrated PPC with adenosine (GPCR agonist), diazoxide ($mitoK_{ATP}$ channel opener), and *S*-nitroso-N-acetyl-penicillamine (NO donor) confers the memory of cardioprotection by sustained activation of PKC-ε and phosphatidylinositol 3-kinase (PI3K) (99). The memory of cardioprotection could not be achieved by simply

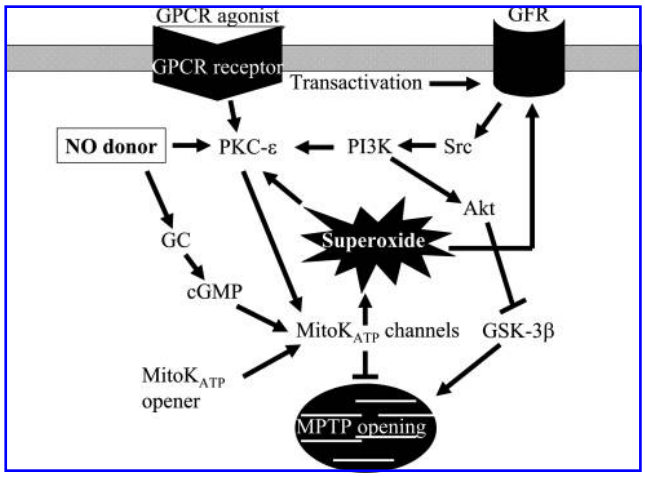

FIG. 3. The signal transduction pathways mediated by integrated pharmacological preconditioning (PPC). Nitric oxide (NO) derived from NO donors in concert with G protein-coupled receptor (GPCR) activation with GPCR agonists and mitochondrial K_{ATP} ($mitoK_{ATP}$) channel openers promotes cardioprotective signal transduction through a complex interplay between the signaling components. NO induces activation of protein kinase C-ε (PKC-ε) through tyrosine nitration in the presence of superoxide which is originated from $mitoK_{ATP}$ channels. NO also activates $mitoK_{ATP}$ channels through activation of guanylyl cyclase (GC) and increased generation of cyclic GMP (cGMP). PKC-ε activates $mitoK_{ATP}$ channels and increases superoxide generation, leading to positive feed-back activation of $mitoK_{ATP}$ channels and PKC-ε. GPCR activation results in membrane association and activation of PKC-ε. GPCR activation also promotes transactivation of growth factor receptors (GFR) that activate phosphatidylinositol 3-kinase (PI3K). PI3K then activates PKC-ε via the protein–protein interaction through the activation of Src tyrosine kinase. PI3K also activates Akt which inhibits glycogen synthase kinase-3β (GSK-3β). Activation of $mitoK_{ATP}$ channels and inhibition of GSK-3β converge on inhibition of mitochondrial permeability transition pore (MPTP) opening and mitochondrial protection. This illustration is adapted and modified from ref. 101.

increasing the dose of each drug, but was successfully reproduced by combining these drugs. These results reinforce the hypothesis that the sustained activation of PKC-ε and PI3K and the memory of cardioprotection are mediated by NO in the presence of GPCR agonists and mitoK$_{ATP}$ channel openers.

NO-mediated activation of GC and the subsequent generation of cGMP may play an additional role in amplifying the memory of cardioprotection mediated by IPC. Xu *et al*. (151) have demonstrated that exogenous NO generates superoxide and induces cardioprotection through activation of cGMP/PKG-dependent activation of mitoK$_{ATP}$ channels. PKG phosphorylates a protein on the mitochondrial outer membrane, which then causes mitoK$_{ATP}$ channels on the mitochondrial inner membrane to open, leading to increased production of superoxide by the mitochondria. Such generation of superoxide have been implicated in the redox signaling for creating the memory of cardioprotection (101).

The long-lasting nature of cardioprotection exerted by the late IPC has encouraged investigators to exploit the mechanism of this adaptive response to protect the ischemic myocardium. It has been demonstrated that the delayed protection against myocardial stunning and infarction was completely abrogated when preconditioned animals had been given a NOS inhibitor Nω-nitro-L-arginine (L-NAME) 24 h after IPC just before the second lethal ischemia (16, 133). It is now evident that the late IPC is triggered by NO, generated by the eNOS and, acting via the formation of ROS, activates a broad array of redox-sensitive transcription factors such as nuclear factor-kappa B (NFKB), activating protein-1, and signal transducers and activators of transcription families (28) which mediate late cardioprotection by increasing the synthesis of cardioprotective proteins such as manganese superoxide dismutase (MnSOD) (54), iNOS (15, 158), cyclooxygenase-2 (COX-2) (126), aldose reductase (123), and heat shock proteins (82). Of these, iNOS appears to play an essential role in the late IPC-mediated cardioprotection. Guo *et al*. (43) demonstrated that the late IPC is associated with upragulation of iNOS and that targeted disruption of iNOS gene abrogated the delayed infact-sparing effect, providing unequivocal evidence for an obligatory role of iNOS in the late IPC. Immunohistochemical and *in situ* hibridization studies have identified cardiomyocytes as the specific cell type that expresses iNOS during the late IPC (144). A growing body of evidence indicates that mitoK$_{ATP}$ channels are the distal mediator of iNOS in the late IPC (10, 134), indicating that mitoK$_{ATP}$ channels are the common distal mediator of the early as well as the late IPC. However, iNOS is not a sole mediator of the late IPC. The recent study has identified COX-2 as an obligatory co-mediator with NO to protect against myocardial stunning and infarction (125). This observation also strongly points to prostaglandin (PG) E_2 and/or PGI_2 as a likely effector of COX-2-dependent protection, indicating that COX-2 exists downstream of iNOS and COX-2 activity is increased by iNOS-derived NO.

iNOS-derived NO may also be involved in the tolerance to I/R injury in a variety of pathophysiological conditions where iNOS expression is increased as a result of oxidative stress. Kyoi *et al*. (71) demonstrated that iNOS-derived NO plays a crucial role in the tolerance to I/R injury in the cardiomyopathic hamster heart through activation of PKC and the downstream effectors, mitoK$_{ATP}$ channels. Moreover, Matsuhisa *et al*. (84) demonstrated that the angiotensin II type-1 (AT1) receptor blocker (ARB) losartan preserves the tolerance to I/R injury by activating eNOS, despite elimination of redox-sensitive upregulation of iNOS and iNOS-dependent cardioprotection in the Dhal salt-sensitive hypertension rat. These findings suggest that although iNOS is a predominant NOS isoform responsible for acquisition of tolerance to I/R injury in the hypertensive heart, site-specific elimination of ROS particularly derived from AT-1 receptors switches the cardioprotective mechanism from iNOS-dependent to eNOS-dependent. A schema of tolerance of the hypertensive heart to I/R injury is illustrated in Fig. 4.

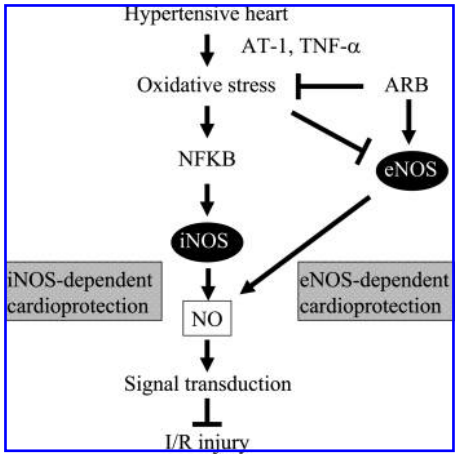

FIG. 4. The role of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) in the tolerance to ischemia/reperfusion (I/R) injury in the hypertensive heart. The hypertensive heart is exposed to oxidative stress by activation of angiotensin II type-1 (AT1) receptors and a variety of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α). Resultant activation of redox-sensitive transcriptional factors such as nuclear factor-kappa B (NFKB) promotes upregulation of iNOS. iNOS-derived nitric oxide (NO) then activates cardioprotective signal transduction as shown in Fig. 1, and exerts tolerance to I/R injury. AT1 receptor blockers (ARB) inhibit oxidative stress, while they activate eNOS by preventing oxidative stress-induced endothelial cell injury and activating Akt (9). eNOS-derived NO in turn activates cardioprotective signal transduction mediating eNOS-dependent cardioprotection.

Role of NO in myocardial inflammation after MI

There is growing recognition and experimental evidence that oxidative/nitrosative stress mediated by ROS and NO plays a role in the pathogenesis of myocardial repair after MI. Although infarct size is primarily determined by the degree of I/R injury within several hours after MI, cardiomyocyte death is a progressive event and ultimate infarct size can be reduced by appropriate interventions for post-MI myocardial repair. The sequence of events that develops early during myocardial repair is inflammation and necrotic tissue replacement with fibrotic tissue. Inflammatory cells are accumulated soon after MI and play a central role in tissue destruction and repair. However, whether inflammatory cells accelerate or inhibit cardiomyocyte death after MI has been a controversial issue. It has long been believed that ROS generated by inflammatry cells contribute to the expansion of infarct size (80, 146). Myocardial I/R causes the release of chemotactic factors

(chemokines) and overexpression of adhesion molecules increases migration of inflammatory cells. The invading inflammatory cells, particularlly polymorphnuclear leukocyte (PMN), may injure the myocardial vasculature and cardiomyocytes by generating ROS. This hypothesis led to the idea that free radical scavengers could mitigate myocardial injury after MI. The combined use of SOD and catalase to scavenge superoxide and hydrogen peroxide, respectively, was effective in limiting infarct size in a canine model of regional myocardial ischemia and reperfusion (59). In addition, ibuprofen, a representative nonsteroidal anti-inflammatory drug, limited infarct size associated with marked suppression of PMN accumulation within the ischemic myocardium (112). Furthermore, PMN depletion by antiserum resulted in similar reductions in ultimate infarct size and was accompanied by a reduction in leukocyte infiltration (111). Reduction of infarct size by free radical scavengers, ibuprofen, and PMN antiserum indicates that PMN and oxygen radicals participate in the irreversible damage to the myocardium after MI. However, subsequent animal studies could not reproduce the cardioprotective effect of free radical scavengers (12, 106, 137). Clinical trials for reperfusion therapy with SOD in patients with MI also failed to inhibit the development of infarction (37, 66). The inability of free rdical scavengers to inhibit infarct size in human does not necessarily indicate that inflammation is not involved in the expansion of infarction in human subject. Several independent studies have demonstrated that inflammation is indeed a critical event that increases cardiomyocyte death after MI and NO prevents this detrimental process by inhibiting inflammation. Corticosteroids exert beneficial effects in the treatment of acute MI, but the precise mechanisms underlying their protective effects are unknown. Hafezi–Moghadam *et al.* (44) have shown that high-dose corticosteroids exert cardiovascular protection through the rapid nontranscriptional activation of eNOS. Binding of corticosteroids to the glucocorticoid receptor (GR) stimulated PI3K and Akt, leading to eNOS activation and NO dependent vasorelaxation. Acute administration of pharmacological concentrations of corticosteroids in mice led to decreased vascular inflammation and reduced myocardial infarct size following I/R. These beneficial effects of corticosteroids were abolished by GR antagonists or eNOS inhibitors in wild-type mice and were completely absent in eNOS-deficient mice. These results indicate that corticosteroids may provide anti-inflammatory action and cardioprotection in the post-MI heart through the non-nuclear actions of GR and rapid activation of eNOS.

eNOS phosphorylation increases NO formation independent of Ca^{2+}/calmodulin, for example, after VEGF stimulation (9). Kupatt *et al*. (68) investigated whether NO formed after overexpression of VEGF or if phosphomimetic eNOS (S1177D) affects PMN-induced myocardial injury after I/R. In their study, pigs were subjected to percutaneous liposome-based gene transfer by retroinfusion of the anterior interventricular vein 48 h before LAD occlusion (60 min) and reperfusion (24 h). Thereafter, regional myocardial function was assessed as subendocardial segment shortening, and infarct size was determined. Tissues from the infarct region, the noninfarcted area at risk, and a control region were analyzed for NFKB activation, tumor necrosis factor (TNF)-α, and E-selectin mRNA and infiltration of PMN. L-NAME was applied in one group of VEGF-transfected animals. NFKB activition, PMN infiltration in the infarct region, and area at risk of infarction were reduced after transfection of VEGF or eNOS S1177D, but not after VEGF+L-NAME coapplication. Infarct size decreased and LV function improved after either VEGF or eNOS S1177D transfection, an effect inhibited by L-NAME coapplication. Thus, it was concluded that retroinfusion of liposomal VEGF cDNA reduces NFKB-dependent postischemic inflammation and subsequent myocardial reperfusion injury, an effect mediated at least in part by enhanced eNOS phosphorylation. The beneficial effect of VEGF-induiced eNOS activation on post MI inflammation and myocardial injury is intriguing and warrants further investigations.

The cardioprotective role of eNOS in inflammation-induced myocardial injury after MI was also investigated by Yin *et al*. (157) who reported that kallikrein gene delivery increased cardiac eNOS phosphorylation and NO generation, improved cardiac contractility and diastolic function, and reduced infarct size at 1 day after I/R associated with reduced macrophage/monocyte and PMN accumulation in the infarcted myocardium. The beneficial effect of kallikrein gene delivery was abolished by treatment with icatibant, a kinin B2 receptor antagonist, or L-NAME, indicating that kallikrein stimulates eNOS-derived NO generation that inhibits inflammation and myocardial injury after MI.

The anti-inflammatory action of eNOS-derived NO has been implicated in the mechanism of cardioprotection by hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors also known as statins. Statins have been established in patients with cardiovascular diseases and also more recently in patients who suffer acute coronary events, including acute MI (113). The cardioprotective effect of statins is mediated by both low density lipoprotein (LDL) lowering-dependent and -independent mechanism (75). The latter includes improvement of endothelial function, stabilization of fibrous plaques, and a decrease of vascular inflammation. Statins have been shown to modulate the immune response by inhibiting activation of immune-competent cells such as macrophages, and antigen presentation to macrophages by T cells. Moreover, treatment with statins can reduce expression, production, and circulating levels of chemokines such as monocyte-chemoattractant protein-1, and proinflammatory cytokines such as TNF-α, interleukin (IL)-6 and IL-1β, thereby inhibiting inflammation on endothelial cells. Statins enhance eNOS-derived NO bioavailability by upregulating eNOS expression via post-transcriptional mechanisms (35, 63, 69, 74) and preventing its downregulation by oxidized LDL-cholesterol (145). The role of eNOS-derived NO in inhibiting inflammation and myocardial injury after MI by treatment with statins was studied by Yamakuchi *et al*. (152) who demonstrated that simvastatin reduced infarct size after MI in mice by inhibiting endothelial exocytosis and PMN infiltration in a manner dependent on an increase in NO and *S*-nitrosylation of the N-ethylmaleimide sensitive factor, a critical regulator of exocytosis. The role of eNOS-derived NO in inflammation-induced injury was also investigated in *in vitro* anoxia/reoxygenation (A/R) preconditioning using eNOS or iNOS deficient cardiomyocytes (114). This study demonstrated that inflammation as evaluated by oxidant stress and PMN transendothelial migration was inhibited in cardiomyocytes that had undergone preconditioning A/R 24 h before the second A/R challenge. The delayed preconditioning effect was absent in eNOS- but not iNOS-deficient cardiomyocytes or in cardiomyocytes treated with

MnSOD antisense oligonucleotide, indicating that eNOS-derived NO and MnSOD act in concert in the development of delayed preconditioning and inhibition of inflammation in cardiomyocytes. This scenario seemingly contradicts the obligatory role of iNOS in the late preconditioning as described before. However, in the former study, the potential involvement of eNOS in cardioprotection is related to the anti-inflammatory effect that is necessary relatively later after reperfusion than that iNOS-dependent cardioprotection which occurs during ischemia and immediately after reperfusion. Although the role of scavenging superoxide by upregulating MnSOD in potentiating the anti-inflammatory effect of eNOS remains unclear, but may be related to the increase in the bioavailability of NO. Figure 5 depicts a proposed mechanism for cardioprotection against inflammation-induced myocardial injury after MI by enhanced generation of eNOS-derived NO.

As described before, the role of iNOS during the inflammatory phase of infarction is controversial. iNOS was significantly increased in infarcted myocardium 48 h after coronary artery ligation (150). The effect persisted for 14 days and declined thereafter. Immunohistochemical localization revealed macrophages as a major source of iNOS expression, but iNOS expression was also present in infarcted human myocardium. Increased iNOS activity appeared to be related to the induction of apoptosis in infiltrating macrophages and cardiomyocytes. Moreover, inhibition of iNOS by *S*-methylisothiourea sulfate resulted in significant improvement of LV performance and increased regional myocardial blood flow. These findings suggest that upregulation of iNOS is detrimental to the post-MI heart. However, during the inflammatory phase of MI, there are plentiful sources of ROS that increase the formation of iNOS-derived peroxynitrite instead of NO and enhance tissue destruction. Thus, as in the case for eNOS, elimination of ROS in the proximity of iNOS increases the bioavailability of NO and may convert this NOS isoform from a pro-inflammatory to an anti-inflammatory phenotype.

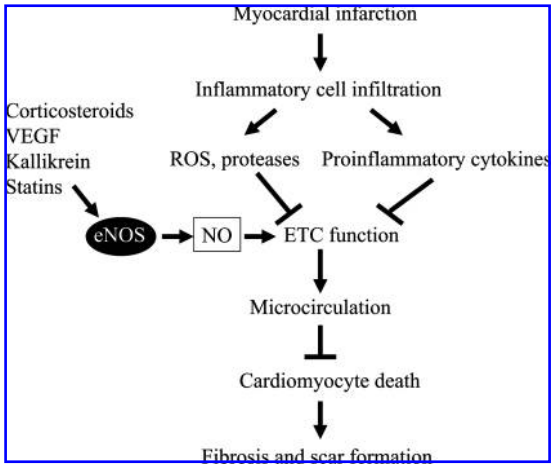

FIG. 5. A model of cardioprotection against inflammation-induced myocardial injury after myocardial infarction (MI) by enhanced generation of endothelial nitric oxide synthase (eNOS)- and inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO). MI promotes inflammatory cell infiltration. Infiltrated inflammatory cells generate reactive oxygen species (ROS), proteases, and proinflammatory cytokines. ROS and proteases can directly cause endothelial cell (ETC) injury, while proinflammatory cytokines activate death pathways within the ETCs. Resultant ETC dysfunction causes inappropriate constriction of resistance vessels distal to the site of coronary occlusion, leading to myocardial ischemia and cardiomyocyte death that further increases interstitial fibrosis and scar formation. Pharmacological tools such as corticosteroids, vascular endothelial growth factor (VEGF), kallikrein, and statins are able to activate eNOS, thereby ameliorating ETC function and inhibiting cardiomyocyte death.

Role of NO in Neovascularization After MI

Neovascularization is an important part of tissue reparative process after MI and plays a crucial role in preserving functional myocardium. Neovascularization consists of angiogenesis and vasculogenesis; the former is produced by sprouting of pre-existing vessels, and the latter is believed to be accomplished by recruitment of circulating endothelial progenitor cells (EPCs) to the site of neovascularization and differentiating into endothelial cells (93). The extent of interstitial fibrosis and scar formation after MI critically depends on neovascularization. Enhanced neovascularization and rapid development of coronary collaterals prevent cardiomyocyte death at the infarction border, reduce ultimate infarct size, and improve cardiac function. Sasaki *et al*. (117, 118) demonstrated that hypoxic preconditioning promoted angiogenesis after permanent occlusion of the left coronary artery in rats associated with significant reduction of infarct size and improvement of LV functional reserve as evaluated by a pharmacological stress test with dobutamine 1–3 weeks after MI. Further studies carried out by this group (38) have revealed that hypoxic stress triggers angiogenic signal including overexpression of VEGF, anti-death proteins Bcl-2 and surviving, and promotes endothelial cell growth within the infarcted tissue, leading to enhanced angiogenesis and inhibition of cardiomyocyte cell death. A more recent study (56) has demonstrated that IPC promotes rapid recruitment of EPCs to the heart, and NO produced in these EPCs are responsible for generation of cardioprotective cytokines including VEGF, suggesting that not only pre-exsiting endothelial cell-derived NO but also EPC-derived NO contribute to neovascularization and cardioprotection afforded by IPC. In addition, NO is involved in recruitment of circulating EPCs to the site of MI. It has been demonstrated that diabetic EPCs do not migrate in response to VEGF, but exogenous NO can reverse this impairment (121), indicating that NO is required for both migratory activity of circulating EPCs to the infarcted myocardium and differentiation and growth of these EPCs into endothelial cells to support neovascularization.

Because IPC cannot be implemented before the occurrence of MI, pharmacological approaches to enhance angiogenesis after MI have gained an increasing interest in the clinical arena. Of these, statins have been a target of extensive investigations, and a growing body of evidence suggests that the beneficial effect of statins is at least in part attributed to enhanced neovascularization in animal models of MI. Recent studies have suggested that eNOS-derived NO plays a crucial role in neovascularization in the ischemic myocardial tissue after treatment with statins. It has been demonstrated that atorvastatin therapy improves eNOS-derived NO bioavailability and increases mobilization of EPCs and neovascularization at the infarct border associated with improvement of LV function and survival after MI (72), suggesting a critical

role for restored NO production in statin-induced EPC mobilization and myocardial neovascularization. Bacause mobilization of EPCs is involved in neovascularization (3), a marked enhancement of eNOS-dependent EPC mobilization after statin treatment supports a notion that eNOS-derived NO plays a crucial role in mobilization of EPCs and improved myocardial neovascularization. In addition, statin treatment increases the expression of adhesion molecules on mobilized EPCs (143) and reduces senescence of EPCs (4) that may have important impact on their functional capacity at the site of ischemia. Other potential mechanisms whereby restored eNOS-derived NO production improves myocardial neovascularization after treatment with statins include reduced expression of growth inhibitors (*i.e.*, angiostatin) (85), and an improved local VEGF expression and activity that have been observed after overexpression of eNOS (97). These findings are consistent with the notion that preservation of eNOS-derived NO bioavailability and neovascularization is one of the beneficial effects of statin treatment after MI.

Another pharmacological tool that enhances neovascularization in the infarcted myocardium is resveratrol, a red wine and grape-derived polyphenolic antioxidant, which stimulates NO production and ROS scavenging activity, thereby increasing bioavailability of NO in the cells exposed to oxidative stress (57, 109). Such an increase in the bioavailability of NO appears to be involved in repair of infarcted myocardium by potentiating neovascularization. Kaga *et al*. (62) have demonstrated that resveratrol enhances neovascularization in the infarcted rat myocardium by increasing the expression of VEGF through the induction of thioredoxin-1 and heme oxygenase-1, both of which activities are regulated under the redox modulation by NO (32, 104, 149). Perhaps many more pharmacological tools could enhance neovascularization in the ischemic myocardium by taking advantage of NO. Further studies will be warranted to elucidate the mechanism of action by which NO enahnces recruitment of EPCs and endothelial cell differentiation and growth in the ischemic heart. A putative model of enhanced neovascularization induced by eNOS-derived NO is illustrated in Fig. 6.

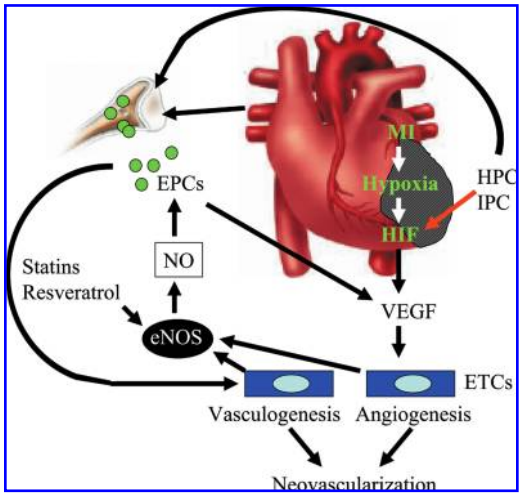

FIG. 6. A model of enhanced neovascularization induced by endothelial nitric oxide synthase (eNOS)-derived NO. Myocardial infarction (MI) causes hypoxia at the infarction border. Hypoxia activates hypoxia-inducible factor (HIF) which then promotes upregulation of vascular endothelial growth factor (VEGF) (122). VEGF stimulates growth and proliferation of endothelial cells (ETCs), leading to angiogenesis. Hypoxic preconditioning (HPC) and ischemic preconditioning (IPC) facilitate this process. On the other hand, MI stimulates mobilization of endothelial progenitor cells (EPCs) from the bone marrow and their recruitment to the site of infarction in the presence of eNOS-derived NO, leading to vasculogenesis. HPC and IPC also stimulate this process. EPCs recruited to the infarcted myocardium secrete VEGF, thereby also contributing to angiogenesis. Increased number of ETCs generates more eNOS-derived NO that promotes positive feedback regulation of angiogenesis and vasculogenesis, culminating in enhanced neovascularization. Pharmacological tools such as statins and resveratrol activate eNOS and contribute to neovascularization.

Role of NO in LV Remodeling and Heart Failure

Ventricular remodeling describes structural changes in the LV in response to chronic alterations in loading conditions, with three major patterns: concentric remodeling, when a pressure load leads to growth in cardiomyocyte thickness; eccentric hypertrophy, when a volume load produces myocyte lengthening; and myocardial infarction, an amalgam of patterns in which stretched and dilated infarcted tissue increases LV volume with a combined volume and pressure load on noninfarcted areas (100). Large infarcts induce a process of cardiac remodeling that includes gross morphologic, histological, and molecular changes of both the infarcted and the residual noninfarcted myocardium. LV remodeling is a strong prognostic determinant and is closely related to the incidence of arrhythmias and sudden cardiac death. Therefore, prevention of remodeling is of prime importance in preserving functional myocardium and preventing the development of heart failure after MI.

Because inhibition of eNOS-derived NO production results in impaired endothelium-dependent vasodilation, reduced myocardial neovascularization, and impaired mobilization of EPCs, it is hypothesized that the deficiency of eNOS-derived NO causes LV remodeling and heart failure in the diseased heart. There is evidence that impaired eNOS-derived NO bioavailability plays an important role in the pathophysiology of heart failure after experimental MI (6, 108, 148) and in patients with heart failure (30, 53, 91, 139). Furthermore, eNOS–deficient mice develop more severe LV dysfunction and remodeling after MI than do wild-type mice (120), and vice versa, endothelial overexpression of eNOS has been shown to attenuate LV dysfunction in mice after MI (61). However, the salutary effect of NO on heart failure is lost by co-existence of oxidative stress from eNOS uncoupling that stimulates cardiac pathologic remodeling from chronic pressure load. It has been shown that in a transgenic eNOS knockout model with low ROS production, severely pressure-loaded hearts developed only modest concentric hypertrophy with little fibrosis and without LV cavity dilation (136), indicating that eNOS activation becomes detrimental rather than beneficial in LV remodeling when ROS co-exists and eNOS uncoupling occurs. Consistent with this notion is the fact that administration with BH4 in mice with pressure load-induced LV hypertrophy ameliorated LV remodeling (90, 136).

The beneficial effect of NO in inhibiting LV remodeling may at least in part be mediated by the GC/cGMP system. The

inhibitory cardiovascular signals mediated by cGMP combat harmful adrenergic effects in the human heart (18). Furthermore, genetically-increased synthesis of cGMP inhibits pressure load-induced pathological remodeling (135). Via enhanced generation of cGMP, sildenafil ameliorates heart failure through systemic vasodilation and a decline in cardiac hypertrophy (50, 116). Taken together, these data strongly support the protective role of the GC/cGMP system in both developing and established heart failure. Contribution of other signaling pathways mediated by NO to inhibition of LV remodeling and heart failure remains to be investigated.

As in the case for inflammation, the role of iNOS in LV remodeling and heart failure is controversial. The prevailing hypothesis that increased NO production through overexpression of iNOS contributes to detrimental cardiac remodeling in the failing heart (31, 39, 48, 52). However, this concept appeared to be at odds with a large body of evidence indicating that iNOS-derived NO is cardioprotective against I/R injury (14). Indeed, our group has found that iNOS plays a protective role in LV remodeling in the cardiomyopathy hamster hearts (unpublished observation). More importantly, removal of oxidative stress using ARB in those hearts coverted the cardioprotective effect from iNOS-dependent to eNOS-dependent as is the case for ARB-mediated cardioprotection against I/R injury. In contrast, the genuine antioxidant N-acetylcysteine abolished ARB-mediated inhibition of LV remodeling by inhibiting redox-sensitive activation of eNOS, indicating that the site-specific rather than indiscriminate removal of oxidative stress is important in eliciting eNOS- and/or iNOS-dependent cardioprotection against LV remodeling and heart failure.

Compared to eNOS and iNOS, the mechanisms by which nNOS influences myocardial pathophysiology remain incompletely understood. However, the recent experimental studies point to an important role for myocardial constitutive NO production through nNOS in the regulation of basal and α-adrenergic cardiac function (23, 25, 76). Importantly, nNOS gene deletion has been associated with more severe LV remodelling and functional deterioration in murine models of MI (29), suggesting that nNOS-derived NO may also be involved in the myocardial response to injury. However, it seems oversimplistic to assume that all aspects of the myocardial phenotype of nNOS knockout [nNOS(−/−)] mice are a direct consequence of lack of NO production from this source. Emerging data showing co-localization of xanthine oxidoreductase (XOR) and nNOS in the sarcoplasmic reticulum of rodents, and increased XOR activity in the nNOS(−/−) myocardium (25), suggest that nNOS gene deletion may have wider implications on the myocardial redox state. Similarly, the mechanisms regulating the targeting of myocardial nNOS to different subcellular compartments and the functional consequences of intracellular nNOS trafficking have not been fully established. Whether this information could be translated into a better understanding and management of human heart failure remains the most important challenge for future investigations. Accordingly, the hypothetical mechanism for NO-mediated inhibition of LV remodeling after MI is summarized in Fig. 7.

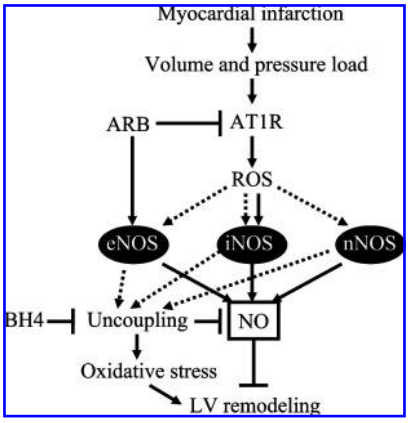

FIG. 7. The hypothetical mechanism for nitric oxide (NO)-mediated inhibition of LV remodeling after myocardial infarction (MI). MI causes volume and pressure load in remaining functional myocardium, leading to activation of angiotensin II type-1 receptors (AT1Rs). Activation of AT1Rs generates reactive oxygen species (ROS) and promotes redox-sensitive upregulation of inducible nitric oxide synthase (iNOS) that acts to inhibit LV remodeling when a sufficient amount of iNOS-derived NO remains generated, in spite of iNOS uncoupling. Angiotensin II type-1 receptor blockers (ARBs) inhibit ROS generation and iNOS expression, but in turn activate endothelial nitric oxide synthase (eNOS). Activation of eNOS in conjunction with elimination of ROS increases bioavailable NO that strongly inihibits LV remodeling. Neuronal nitric oxide synthase (nNOS)-derived NO may also contribute to inhibition of LV remodeling after MI when ROS generation is properly controled. Replenishment with tetrahydrobiopterin (BH4) that is depleted by ROS inhibits uncoupling of all isoforms of NOS. This recoupling of NOS inhibits ROS generation and oxidative stress and increases bioavailable NO, leading to powerful inhibition of LV remodeling. *Arrows with broken lines* represent the pathways toward uncoupling of NOS.

Strategies to Increase the Bioavailability of NO

As described above, pharmacological tools such as ARB, statins, corticosteroids, VEGF, kallilrein, and resveratrol could enhance the generation of NO by activating eNOS. However, activation of eNOS and perhaps other NOS isoforms (i.e., iNOS) and nNOS alone appears to be insufficient or even deleterious for cardioprotection when NOS uncoupling occurs. NOS uncoupling is the most important endogenous mechanism by which bioavailable NO is diminished in the tissue. NOS uncoupling occurs as a result of depletion of BH4 by oxidative stress in the proximity of NOS (11, 119). Recent experimental studies support an important pathophysiological role of BH4 deficiency as well as the therapeutic potential of BH4 repletion for hypertension, endothelial dysfunction, atherosclerosis, diabetes, cardiac hypertrophic remodeling, and heart failure (88–90). In addition to BH4, studies are also examining the potential role of folic acid therapy, because folic acid can enhance BH4 levels and the NOS coupling state (89). Pharmacological tools that eliminate ROS in the proximity of NOS can also increase the bioavailability of NO. It has been demonstrated that statins (60), angiotensin converting enzyme inhibitors (154), ARB (153), resveratrol (62), aldosterone blockers (22), PDE5 inhibitors (98), and the anti-diabetic thiazolidinedione (86) produce salubrious effects in experimental models of MI via their enhancement of NO bioavailability. In this context, any pharmacological tools that act as an antioxidant in a site-specific fashion in the proximity

of NOS could enhance the bioavailability of NO and confer cardioprotection. On the contrary, indiscriminate removal of ROS compromises intrinsic cardioprotective mechanism sensitive to rodox modulation (101).

Among the maneuvers that increase the bioavailability of NO without pharmacological tools, regular exercise may represent the most convenient, economical, and effective means. Growing body of evidence indicates that regular exercise upregulates and activates eNOS concomitant with upregulation of antioxidant systems. Although acute exercise activates eNOS through a redox-sensitive mechanism (2, 73), regular exercise eventually suppresses oxidative stress and inhibits eNOS uncoupling. Increased NO bioavailability by regular exercise in human was first demonstrated by Roberts *et al*. (110) who demonstrated that diet and exercise decreased oxidative stress and increased NO production associated with a decrease of blood pressure and an increase in insulin sensitivity. Subsequent experimental studies confirmed the efficacy of exercise training in endothelium-dependent vasorelaxation and NO bioavailability by upregulating eNOS and inhibiting oxidative stress (41, 92, 115). The beneficial effect of regular exercise on NO bioavailability may be associated with improvement of functional capacity and prognosis in patients with chronic heart failure (33, 45, 64). Taken together, it is imperative to address whether increasing the bioavailability of NO is a universal mechanism for cardioprotection against I/R injury, LV remodeling, and heart failure.

Concluding Remarks

Despite tremendous efforts to preserve functional myocardium after MI, only selected approaches are effective in improving the prognosis of heart failure in the clinical arena. Because development of congestive heart failure after MI passes through several critical stages with distinct and overlapping pathophysiology, it is necessary to implement systematic approaches to overcome pathophysiological problems unique at each stage. Inhibition of I/R injury is of prime importance in preserving functional myocardium at the first stage in MI. IPC and PPC are the state-of-the-art techniques that acutely activate cardioprotective signal transduction. Although application of IPC before the onset of MI may not be feacible, the late precondtioning effect can be obtained by phramacological tools and regular exercise. Caloric restriction is also cardioprotective aginst I/R injury (124). β-Blockers have long been known to inhibit I/R injury by reducing myocardial oxygen demand. A recently emerging hypothesis that myocardial reperfusion injury is produced by physical stress imposed on cardiomyocytes with fragile sarcolemmal membrane at the time of reperfusion associated with restoration of contractile activity (65, 70, 103) encourages the use of β-blockers concomitant with timely reperfusion therapies. Ischemic postconditioning is a series of brief mechanical interruptions of reperfusion following a specific prescribed algorithm applied at the very onset of reperfusion and is a realistic means to activate cardioprotective signal transduction against reperfusion injury during coronary interventions for acute MI (47, 159). After this first stage of MI, prevention from pathological remodeling becomes a therapeutic target. The approaches against LV remodeling include inhibition of inflammation, stimulation of neovascularization either by pharmacological tools or stem cell therapy that has recently been emerged as "therapeutic angiogenesis" (140). Mechanical unloading of the LV from hypertension and volume load is a fundamental approach for preventing pathological remodeling. Regular exercise not only confers cardioprotection against I/R injury through the late preconditioning effect but also improves functional capacity that ultimately decreases oxygen demand in the heart. It appears that these approaches employ NO at least in part in mediating the salutary effects on I/R injury and pathological remodeling.

NO could be a foe and friend for the failing heart depending on the bioavailability of NO. Cellular redox status is a major determinant of the bioavailability of NO. Oxidative stress decreases the bioavailability of NO by depleting BH4 and causes NOS uncoupling that further decreases bioavailable NO. Recently emerging cardioprotective drugs such as ARB and statins increase bioavailable NO by upregulating eNOS while inhibiting eNOS uncoupling by preventing NADPH oxidase activation. Supplementation with exogenous BH4 can restore normal NO biosynthesis by providing an optimal condition for NOS catalytic activity. On the contrary, indiscriminate removal of ROS or NO compromises redox-sensitive cardioprotective signal transduction. Although the exact mechanism by which NO confers cardioprotection remains to be clarified, the nitrotyrosine-, cGMP- and the *S*-nitrosylation-dependent signal transduction pathways are the potential mechanism for cardioprotection. Better understanding of the biochemistry and physiology of NO signaling will pave the way for preserving functional myocardium and rejuvenating the diseased heart by potentiating adaptation of the heart to I/R injury and postinfarction ventricular remodeling.

Acknowledgments

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Abbreviations

A/R, anoxia/reoxygenation; ARB, angiotensin II type-1 receptor blocker; AT1, angiotensin II type 1; ATP, adenosine triphosphate; BH4, tetrahydrobiopterin; cAMP, cyclic adenosine monophosphate; cDNA, complementary deoxyribonucleic acid; cGMP, cyclic guanosine monophosphate; COX, cyclooxygenase; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; GC, guanylyl cyclase; GPCR, G protein-coupled receptor; GR, gluocorticoid receptor; HIF, hypoxia-inducible factor; HMG-CoA, hydroxymethylglutaryl coenzyme A; IL, interleukin; iNOS, inducible nitric oxide synthase; IPC, ischemic preconditioning; I/R, ischemia/reperfusion; LDL, low density lipoprotein; L-NAME, Nω-nitro-L-arginine; LV, left ventricle; MI, myocardial infarction; mitoK_{ATP}, mitochondrial K_{ATP}; MnSOD, manganese superoxide dismutase; mRNA, messenger ribonucleic acid; MPTP, mitochondrial permeability transition pore; NADPH, nicotinamide adenine dinucleotide phosphate; NFKB, nuclear factor-kappa B; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; nNOS$-/-$, neuronal nitric oxide synthase knockout; NT, nitrotyrosine; PDE, phosphodiesterase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PKG, protein kinase G; PMN, polymorphnuclear leukocytes; PPC,

pharmacological preconditioning; PG, prostaglandin; ROS, reactive oxygen species; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

References

1. Abu–Soud HM and Stuehr DJ. Nitric oxide synthases reveal a role for calmodulin in controlling electron transfer. *Proc Natl Acad Sci USA* 90: 10769–10772, 1993.
2. Akita Y, Otani H, Matsuhisa S, Kyoi S, Enoki C, Hattori R, Imamura H, Kamihata H, Kimura Y, and Iwasaka T. Exercise-induced activation of cardiac sympathetic nerve triggers cardioprotection via redox-sensitive activation of eNOS and upregulation of iNOS. *Am J Physiol Heart Circ Physiol* 292: H2051–H2059, 2007.
3. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, and Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 85: 221–228, 1999.
4. Assmus B, Urbich C, Aicher A, Hofmann WK, Haendeler J, Rossig L, Spyridopoulos I, Zeiher AM, and Dimmeler S. HMG-CoA reductase inhibitors reduce senescence and increase proliferation of endothelial progenitor cells via regulation of cell cycle regulatory genes. *Circ Res* 92: 1049–1055, 2003.
5. Balafanova Z, Bolli R, Zhang J, Zheng Y, Pass JM, Bhatnagar A, Tang XL, Wang O, Cardwell E, and Ping P. Nitric oxide (NO) induces nitration of protein kinase Cepsilon (PKCepsilon), facilitating PKCepsilon translocation via enhanced PKCepsilon-RACK2 interactions: a novel mechanism of no-triggered activation of PKCepsilon. *J Biol Chem* 277: 15021–15027, 2002.
6. Bauersachs J, Bouloumie A, Fraccarollo D, Hu K, Busse R, and Ertl G. Endothelial dysfunction in chronic myocardial infarction despite increased vascular endothelial nitric oxide synthase and soluble guanylate cyclase expression: Role of enhanced vascular superoxide production. *Circulation* 100: 292–298, 1999.
7. Baxter GF, Goma FM, and Yellon DM. Characterisation of the infarct-limiting effect of delayed preconditioning: Timecourse and dose-dependency studies in rabbit myocardium. *Basic Res Cardiol* 92: 159–167, 1997.
8. Bell RM and Yellon DM. The contribution of endothelial nitric oxide synthase to early ischaemic preconditioning: The lowering of the preconditioning threshold. An investigation in eNOS knockout mice. *Cardiovasc Res* 52: 274–280, 2001.
9. Benndorf R, Boger RH, Ergun S, Steenpass A, and Wieland T. Angiotensin II type 2 receptor inhibits vascular endothelial growth factor-induced migration and *in vitro* tube formation of human endothelial cells. *Circ Res* 93: 438–447, 2003.
10. Bernardo NL, D'Angelo M, Okubo S, Joy A, and Kukreja RC. Delayed ischemic preconditioning is mediated by opening of ATP-sensitive potassium channels in the rabbit heart. *Am J Physiol* 276: H1323–H1330, 1999.
11. Bitar MS, Wahid S, Mustafa S, Al–Saleh E, Dhaunsi GS, and Al–Mulla F. Nitric oxide dynamics and endothelial dysfunction in type II model of genetic diabetes. *Eur J Pharmacol* 511: 53–64, 2005.
12. Bjorkman JA, Sutherland I, Gustafsson D, Sjoquist PO, and Abrahamsson T. Superoxide dismutase and catalase do not improve recovery of regional myocardial contractile function when given at the time of reperfusion after reversible regional ischemia in anesthetized dogs. *Basic Res Cardiol* 86: 236–244, 1991.
13. Bolli R. The early and late phases of preconditioning against myocardial stunning and the essential role of oxyradicals in the late phase: An overview. *Basic Res Cardiol* 91: 57–63, 1996.
14. Bolli R. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: An overview of a decade of research. *J Mol Cell Cardiol* 33: 1897–1918, 2001.
15. Bolli R, Dawn B, and Xuan YT. Role of the JAK-STAT pathway in protection against myocardial ischemia/reperfusion injury. *Trends Cardiovasc Med* 13: 72–79, 2003.
16. Bolli R, Manchikalapudi S, Tang XL, Takano H, Qiu Y, Guo Y, Zhang Q, and Jadoon AK. The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase. Evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning. *Circ Res* 81: 1094–1107, 1997.
17. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, and Lipton SA. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA* 92: 7162–7166, 1995.
18. Borlaug BA, Melenovsky V, Marhin T, Fitzgerald P, and Kass DA. Sildenafil inhibits beta-adrenergic-stimulated cardiac contractility in humans. *Circulation* 112: 2642–2649, 2005.
19. Borutaite V, Moncada S, and Brown GC. Nitric oxide from inducible nitric oxide synthase sensitizes the inflamed aorta to hypoxic damage via respiratory inhibition. *Shock* 23: 319–323, 2005.
20. Borutaite V, Morkuniene R, and Brown GC. Release of cytochrome c from heart mitochondria is induced by high Ca2+ and peroxynitrite and is responsible for Ca(2+)-induced inhibition of substrate oxidation. *Biochim Biophys Acta* 1453: 41–48, 1999.
21. Brown GC and Borutaite V. Nitric oxide and mitochondrial respiration in the heart. *Cardiovasc Res* 75: 283–290, 2007.
22. Brown NJ. Aldosterone and end-organ damage. *Curr Opin Nephrol Hypertens* 14: 235–241, 2005.
23. Burkard N, Rokita AG, Kaufmann SG, Hallhuber M, Wu R, Hu K, Hofmann U, Bonz A, Frantz S, Cartwright EJ, Neyses L, Maier LS, Maier SK, Renne T, Schuh K, and Ritter O. Conditional neuronal nitric oxide synthase overexpression impairs myocardial contractility. *Circ Res* 100: e32–44, 2007.
24. Burwell LS, Nadtochiy SM, Tompkins AJ, Young S, and Brookes PS. Direct evidence for S-nitrosation of mitochondrial complex I. *Biochem J* 394: 627–634, 2006.
25. Casadei B. The emerging role of neuronal nitric oxide synthase in the regulation of myocardial function. *Exp Physiol* 91: 943–955, 2006.
26. Davidson SM and Duchen MR. Effects of NO on mitochondrial function in cardiomyocytes: Pathophysiological relevance. *Cardiovasc Res* 71: 10–21, 2006.
27. Davis KL, Martin E, Turko IV, and Murad F. Novel effects of nitric oxide. *Annu Rev Pharmacol Toxicol* 41: 203–236, 2001.
28. Dawn B and Bolli R. Role of nitric oxide in myocardial preconditioning. *Ann NY Acad Sci* 962: 18–41, 2002.
29. Dawson D, Lygate CA, Zhang MH, Hulbert K, Neubauer S, and Casadei B. nNOS gene deletion exacerbates pathological left ventricular remodeling and functional deterioration

after myocardial infarction. *Circulation* 112: 3729–3737, 2005.
30. Drexler H. Endothelium as a therapeutic target in heart failure. *Circulation* 98: 2652–2655, 1998.
31. Drexler H, Kastner S, Strobel A, Studer R, Brodde OE, and Hasenfuss G. Expression, activity and functional significance of inducible nitric oxide synthase in the failing human heart. *J Am Coll Cardiol* 32: 955–963, 1998.
32. Ejima K, Layne MD, Carvajal IM, Nanri H, Ith B, Yet SF, and Perrella MA. Modulation of the thioredoxin system during inflammatory responses and its effect on heme oxygenase-1 expression. *Antioxid Redox Signal* 4: 569–575, 2002.
33. Ennezat PV, Malendowicz SL, Testa M, Colombo PC, Cohen–Solal A, Evans T, and LeJemtel TH. Physical training in patients with chronic heart failure enhances the expression of genes encoding antioxidative enzymes. *J Am Coll Cardiol* 38: 194–198, 2001.
34. Ferdinandy P and Schulz R. Nitric oxide, superoxide, and peroxynitrite in myocardial ischaemia-reperfusion injury and preconditioning. *Br J Pharmacol* 138: 532–543, 2003.
35. Feron O, Dessy C, Desager JP, and Balligand JL. Hydroxymethylglutaryl-coenzyme A reductase inhibition promotes endothelial nitric oxide synthase activation through a decrease in caveolin abundance. *Circulation* 103: 113–118, 2001.
36. Fisher PW, Salloum F, Das A, Hyder H, and Kukreja RC. Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocyte apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardiotoxicity. *Circulation* 111: 1601–1610, 2005.
37. Flaherty JT, Pitt B, Gruber JW, Heuser RR, Rothbaum DA, Burwell LR, George BS, Kereiakes DJ, Deitchman D, Gustafson N, and *et al*. Recombinant human superoxide dismutase (h-SOD) fails to improve recovery of ventricular function in patients undergoing coronary angioplasty for acute myocardial infarction. *Circulation* 89: 1982–1991, 1994.
38. Fukuda S, Kaga S, Sasaki H, Zhan L, Zhu L, Otani H, Kalfin R, Das DK, and Maulik N. Angiogenic signal triggered by ischemic stress induces myocardial repair in rat during chronic infarction. *J Mol Cell Cardiol* 36: 547–559, 2004.
39. Gilson WD, Epstein FH, Yang Z, Xu Y, Prasad KM, Toufektsian MC, Laubach VE, and French BA. Borderzone contractile dysfunction is transiently attenuated and left ventricular structural remodeling is markedly reduced following reperfused myocardial infarction in inducible nitric oxide synthase knockout mice. *J Am Coll Cardiol* 50: 1799–1807, 2007.
40. Goretski J and Hollocher TC. Trapping of nitric oxide produced during denitrification by extracellular hemoglobin. *J Biol Chem* 263: 2316–2323, 1988.
41. Graham DA and Rush JW. Exercise training improves aortic endothelium-dependent vasorelaxation and determinants of nitric oxide bioavailability in spontaneously hypertensive rats. *J Appl Physiol* 96: 2088–2096, 2004.
42. Gray MO, Karliner JS, and Mochly–Rosen D. A selective epsilon-protein kinase C antagonist inhibits protection of cardiac myocytes from hypoxia-induced cell death. *J Biol Chem* 272: 30945–30951, 1997.
43. Guo Y, Jones WK, Xuan YT, Tang XL, Bao W, Wu WJ, Han H, Laubach VE, Ping P, Yang Z, Qiu Y, and Bolli R. The late phase of ischemic preconditioning is abrogated by targeted disruption of the inducible NO synthase gene. *Proc Natl Acad Sci USA* 96: 11507–11512, 1999.
44. Hafezi–Moghadam A, Simoncini T, Yang Z, Limbourg FP, Plumier JC, Rebsamen MC, Hsieh CM, Chui DS, Thomas KL, Prorock AJ, Laubach VE, Moskowitz MA, French BA, Ley K, and Liao JK. Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. *Nat Med* 8: 473–479, 2002.
45. Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, Kaiser R, Yu J, Adams V, Niebauer J, and Schuler G. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* 98: 2709–2715, 1998.
46. Handy DE and Loscalzo J. Nitric oxide and posttranslational modification of the vascular proteome: S-nitrosation of reactive thiols. *Arterioscler Thromb Vasc Biol* 26: 1207–1214, 2006.
47. Hausenloy DJ, Tsang A, and Yellon DM. The reperfusion injury salvage kinase pathway: A common target for both ischemic preconditioning and postconditioning. *Trends Cardiovasc Med* 15: 69–75, 2005.
48. Haywood GA, Tsao PS, von der Leyen HE, Mann MJ, Keeling PJ, Trindade PT, Lewis NP, Byrne CD, Rickenbacher PR, Bishopric NH, Cooke JP, McKenna WJ, and Fowler MB. Expression of inducible nitric oxide synthase in human heart failure. *Circulation* 93: 1087–1094, 1996.
49. Hess DT, Matsumoto A, Kim SO, Marshall HE, and Stamler JS. Protein S-nitrosylation: Purview and parameters. *Nat Rev Mol Cell Biol* 6: 150–166, 2005.
50. Hirata K, Adji A, Vlachopoulos C, and O'Rourke MF. Effect of sildenafil on cardiac performance in patients with heart failure. *Am J Cardiol* 96: 1436–1440, 2005.
51. Hofmann F, Ammendola A, and Schlossmann J. Rising behind NO: cGMP-dependent protein kinases. *J Cell Sci* 113: 1671–1676, 2000.
52. Horinaka S, Kobayashi N, Mori Y, Yagi H, Onoda M, and Matsuoka H. Expression of inducible nitric oxide synthase, left ventricular function and remodeling in Dahl salt-sensitive hypertensive rats. *Int J Cardiol* 91: 25–35, 2003.
53. Hornig B, Maier V, and Drexler H. Physical training improves endothelial function in patients with chronic heart failure. *Circulation* 93: 210–214, 1996.
54. Hoshida S, Yamashita N, Otsu K, and Hori M. The importance of manganese superoxide dismutase in delayed preconditioning: Involvement of reactive oxygen species and cytokines. *Cardiovasc Res* 55: 495–505, 2002.
55. Ignarro LJ. Nitric oxide. A novel signal transduction mechanism for transcellular communication. *Hypertension* 16: 477–483, 1990.
56. Ii M, Nishimura H, Iwakura A, Wecker A, Eaton E, Asahara T, and Losordo DW. Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via "imported" nitric oxide synthase activity. *Circulation* 111: 1114–1120, 2005.
57. Imamura G, Bertelli AA, Bertelli A, Otani H, Maulik N, and Das DK. Pharmacological preconditioning with resveratrol: An insight with iNOS knockout mice. *Am J Physiol Heart Circ Physiol* 282: H1996–2003, 2002.
58. Ischiropoulos H. Biological tyrosine nitration: A pathophysiological function of nitric oxide and reactive oxygen species. *Arch Biochem Biophys* 356: 1–11, 1998.
59. Jolly SR, Kane WJ, Bailie MB, Abrams GD, and Lucchesi BR. Canine myocardial reperfusion injury. Its reduction by the combined administration of superoxide dismutase and catalase. *Circ Res* 54: 277–285, 1984.

60. Jones SP, Gibson MF, Rimmer DM, 3rd, Gibson TM, Sharp BR, and Lefer DJ. Direct vascular and cardioprotective effects of rosuvastatin, a new HMG-CoA reductase inhibitor. *J Am Coll Cardiol* 40: 1172–8, 2002.
61. Jones SP, Greer JJ, van Haperen R, Duncker DJ, de Crom R, and Lefer DJ. Endothelial nitric oxide synthase overexpression attenuates congestive heart failure in mice. *Proc Natl Acad Sci USA* 100: 4891–4896, 2003.
62. Kaga S, Zhan L, Matsumoto M, and Maulik N. Resveratrol enhances neovascularization in the infarcted rat myocardium through the induction of thioredoxin-1, heme oxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 39: 813–822, 2005.
63. Kalinowski L, Dobrucki LW, Brovkovych V, and Malinski T. Increased nitric oxide bioavailability in endothelial cells contributes to the pleiotropic effect of cerivastatin. *Circulation* 105: 933–938, 2002.
64. Katz SD, Hryniewicz K, Hriljac I, Balidemaj K, Dimayuga C, Hudaihed A, and Yasskiy A. Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. *Circulation* 111: 310–314, 2005.
65. Kido M, Otani H, Kyoi S, Sumida T, Fujiwara H, Okada T, and Imamura H. Ischemic preconditioning-mediated restoration of membrane dystrophin during reperfusion correlates with protection against contraction-induced myocardial injury. *Am J Physiol Heart Circ Physiol* 287: H81–90, 2004.
66. Klein HH, Pich S, Lindert S, Buchwald A, Nebendahl K, and Kreuzer H. Intracoronary superoxide dismutase for the treatment of "reperfusion injury", A blind randomized placebo-controlled trial in ischemic, reperfused porcine hearts. *Basic Res Cardiol* 83: 141–148, 1988.
67. Kukreja RC, Salloum F, Das A, Ockaili R, Yin C, Bremer YA, Fisher PW, Wittkamp M, Hawkins J, Chou E, Kukreja AK, Wang X, Marwaha VR, and Xi L. Pharmacological preconditioning with sildenafil: Basic mechanisms and clinical implications. *Vascul Pharmacol* 42: 219–232, 2005.
68. Kupatt C, Hinkel R, Vachenauer R, Horstkotte J, Raake P, Sandner T, Kreuzpointner R, Muller F, Dimmeler S, Feron O, and Boekstegers P. VEGF165 transfection decreases postischemic NF-kappa B-dependent myocardial reperfusion injury *in vivo*: role of eNOS phosphorylation. *FASEB J* 17: 705–707, 2003.
69. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, Sessa WC, and Walsh K. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nat Med* 6: 1004–1010, 2000.
70. Kyoi S, Otani H, Hamano A, Matsuhisa S, Akita Y, Fujiwara H, Hattori R, Imamura H, Kamihata H, and Iwasaka T. Dystrophin is a possible end-target of ischemic preconditioning against cardiomyocyte oncosis during the early phase of reperfusion. *Cardiovasc Res* 70: 354–363, 2006.
71. Kyoi S, Otani H, Matsuhisa S, Akita Y, Enoki C, Tatsumi K, Hattori R, Imamura H, Kamihata H, and Iwasaka T. Role of oxidative/nitrosative stress in the tolerance to ischemia/reperfusion injury in cardiomyopathic hamster heart. *Antioxid Redox Signal* 8: 1351–1361, 2006.
72. Landmesser U, Engberding N, Bahlmann FH, Schaefer A, Wiencke A, Heineke A, Spiekermann S, Hilfiker-Kleiner D, Templin C, Kotlarz D, Mueller M, Fuchs M, Hornig B, Haller H, and Drexler H. Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. *Circulation* 110: 1933–1939, 2004.
73. Lauer N, Suvorava T, Ruther U, Jacob R, Meyer W, Harrison DG, and Kojda G. Critical involvement of hydrogen peroxide in exercise-induced up-regulation of endothelial NO synthase. *Cardiovasc Res* 65: 254–262, 2005.
74. Laufs U, La Fata V, Plutzky J, and Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 97: 1129–1135, 1998.
75. Liao JK. Beyond lipid lowering: the role of statins in vascular protection. *Int J Cardiol* 86: 5–18, 2002.
76. Lim G, Venetucci L, Eisner DA, and Casadei B. Does nitric oxide modulate cardiac ryanodine receptor function? Implications for excitation-contraction coupling. *Cardiovasc Res* 77: 256–264, 2008.
77. Liu GS, Cohen MV, Mochly-Rosen D, and Downey JM. Protein kinase C-epsilon is responsible for the protection of preconditioning in rabbit cardiomyocytes. *J Mol Cell Cardiol* 31: 1937–1948, 1999.
78. Lochner A, Marais E, Genade S, and Moolman JA. Nitric oxide: A trigger for classic preconditioning? *Am J Physiol Heart Circ Physiol* 279: H2752–2765, 2000.
79. Lucas KA, Pitari GM, Kazerounian S, Ruiz–Stewart I, Park J, Schulz S, Chepenik KP, and Waldman SA. Guanylyl cyclases and signaling by cyclic GMP. *Pharmacol Rev* 52: 375–414, 2000.
80. Lucchesi BR and Mullane KM. Leukocytes and ischemia-induced myocardial injury. *Annu Rev Pharmacol Toxicol* 26: 201–224, 1986.
81. Lugnier C. Cyclic nucleotide phosphodiesterase (PDE) superfamily: A new target for the development of specific therapeutic agents. *Pharmacol Ther* 109: 366–398, 2006.
82. Marber MS, Latchman DS, Walker JM, and Yellon DM. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation* 88: 1264–1272, 1993.
83. Martinez-Ruiz A, and Lamas S. S-nitrosylation: A potential new paradigm in signal transduction. *Cardiovasc Res* 62: 43–52, 2004.
84. Matsuhisa S, Otani H, Okazaki T, Yamashita K, Akita Y, Sato D, Moriguchi A, Imamura H, and Iwasaka T. Angiotensin II type 1 receptor blocker preserves tolerance to ischemia-reperfusion injury in Dahl salt-sensitive rat heart. *Am J Physiol Heart Circ Physiol* 294: H2473–2479, 2008.
85. Matsunaga T, Weihrauch DW, Moniz MC, Tessmer J, Warltier DC, and Chilian WM. Angiostatin inhibits coronary angiogenesis during impaired production of nitric oxide. *Circulation* 105: 2185–2191, 2002.
86. Matsuzawa Y. The metabolic syndrome and adipocytokines. *FEBS Lett* 580: 2917–2921, 2006.
87. Mayer B and Hemmens B. Biosynthesis and action of nitric oxide in mammalian cells. *Trends Biochem Sci* 22: 477–481, 1997.
88. Moens AL and Kass DA. Tetrahydrobiopterin and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 26: 2439–2444, 2006.
89. Moens AL and Kass DA. Therapeutic potential of tetrahydrobiopterin for treating vascular and cardiac disease. *J Cardiovasc Pharmacol* 50: 238–246, 2007.
90. Moens AL, Takimoto E, Tocchetti CG, Chakir K, Bedja D, Cormaci G, Ketner EA, Majmudar M, Gabrielson K, Halushka MK, Mitchell JB, Biswal S, Channon KM, Wolin MS, Alp NJ, Paolocci N, Champion HC, and Kass DA. Reversal of cardiac hypertrophy and fibrosis from pressure

overload by tetrahydrobiopterin: Efficacy of recoupling nitric oxide synthase as a therapeutic strategy. *Circulation* 117: 2626–2636, 2008.
91. Mohri M, Egashira K, Tagawa T, Kuga T, Tagawa H, Harasawa Y, Shimokawa H, and Takeshita A. Basal release of nitric oxide is decreased in the coronary circulation in patients with heart failure. *Hypertension* 30: 50–56, 1997.
92. Moien–Afshari F, Ghosh S, Khazaei M, Kieffer TJ, Brownsey RW, and Laher I. Exercise restores endothelial function independently of weight loss or hyperglycaemic status in db/db mice. *Diabetologia* 51: 1327–1337, 2008.
93. Moldovan NI. Tissular insemination of progenitor endothelial cells: The problem, and a suggested solution. *Adv Exp Med Biol* 522: 99–113, 2003.
94. Moncada S, Palmer RM, and Higgs EA. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43: 109–142, 1991.
95. Murphy E and Steenbergen C. Preconditioning: the mitochondrial connection. *Annu Rev Physiol* 69: 51–67, 2007.
96. Murry CE, Jennings RB, and Reimer KA. Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986.
97. Namba T, Koike H, Murakami K, Aoki M, Makino H, Hashiya N, Ogihara T, Kaneda Y, Kohno M, and Morishita R. Angiogenesis induced by endothelial nitric oxide synthase gene through vascular endothelial growth factor expression in a rat hindlimb ischemia model. *Circulation* 108: 2250–2257, 2003.
98. Ockaili R, Salloum F, Hawkins J, and Kukreja RC. Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K(ATP) channels in rabbits. *Am J Physiol Heart Circ Physiol* 283: H1263–1269, 2002.
99. Okada T, Otani H, Wu Y, Uchiyama T, Kyoi S, Hattori R, Sumida T, Fujiwara H, and Imamura H. Integrated pharmacological preconditioning and memory of cardioprotection: Role of protein kinase C and phosphatidylinositol 3-kinase. *Am J Physiol Heart Circ Physiol* 289: H761–767, 2005.
100. Opie LH, Commerford PJ, Gersh BJ, and Pfeffer MA. Controversies in ventricular remodelling. *Lancet* 367: 356–367, 2006.
101. Otani H. Ischemic preconditioning: From molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 10: 207–247, 2008.
102. Otani H. Reactive oxygen species as mediators of signal transduction in ischemic preconditioning. *Antioxid Redox Signal* 6: 449–469, 2004.
103. Otani H, Matsuhisa S, Akita Y, Kyoi S, Enoki C, Tatsumi K, Fujiwara H, Hattori R, Imamura H, and Iwasaka T. Role of mechanical stress in the form of cardiomyocyte death during the early phase of reperfusion. *Circ J* 70: 1344–1355, 2006.
104. Otterbein LE and Choi AM. Heme oxygenase: Colors of defense against cellular stress. *Am J Physiol Lung Cell Mol Physiol* 279: L1029–1037, 2000.
105. Palmer RM, Ferrige AG, and Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327: 524–526, 1987.
106. Patel BS, Jeroudi MO, O'Neill PG, Roberts R, and Bolli R. Effect of human recombinant superoxide dismutase on canine myocardial infarction. *Am J Physiol* 258: H369–380, 1990.
107. Post H, Schulz R, Behrends M, Gres P, Umschlag C, and Heusch G. No involvement of endogenous nitric oxide in classical ischemic preconditioning in swine. *J Mol Cell Cardiol* 32: 725–733, 2000.
108. Qi XL, Stewart DJ, Gosselin H, Azad A, Picard P, Andries L, Sys SU, Brutsaert DL, and Rouleau JL. Improvement of endocardial and vascular endothelial function on myocardial performance by captopril treatment in postinfarct rat hearts. *Circulation* 100: 1338–1345, 1999.
109. Ray PS, Maulik G, Cordis GA, Bertelli AA, Bertelli A, and Das DK. The red wine antioxidant resveratrol protects isolated rat hearts from ischemia reperfusion injury. *Free Radic Biol Med* 27: 160–169, 1999.
110. Roberts CK, Vaziri ND, and Barnard RJ. Effect of diet and exercise intervention on blood pressure, insulin, oxidative stress, and nitric oxide availability. *Circulation* 106: 2530–2532, 2002.
111. Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, and Lucchesi BR. Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* 67: 1016–1023, 1983.
112. Romson JL, Hook BG, Rigot VH, Schork MA, Swanson DP, and Lucchesi BR. The effect of ibuprofen on accumulation of indium-111-labeled platelets and leukocytes in experimental myocardial infarction. *Circulation* 66: 1002–1011, 1982.
113. Rosenson RS. Pluripotential mechanisms of cardioprotection with HMG-CoA reductase inhibitor therapy. *Am J Cardiovasc Drugs* 1: 411–420, 2001.
114. Rui T, Cepinskas G, Feng Q, and Kvietys PR. Delayed preconditioning in cardiac myocytes with respect to development of a proinflammatory phenotype: role of SOD and NOS. *Cardiovasc Res* 59: 901–911, 2003.
115. Rush JW, Turk JR, and Laughlin MH. Exercise training regulates SOD-1 and oxidative stress in porcine aortic endothelium. *Am J Physiol Heart Circ Physiol* 284: H1378–1387, 2003.
116. Salloum FN, Abbate A, Das A, Houser JE, Mudrick CA, Qureshi IZ, Hoke NN, Roy SK, Brown WR, Prabhakar S, and Kukreja RC. Sildenafil (Viagra) attenuates ischemic cardiomyopathy and improves left ventricular function in mice. *Am J Physiol Heart Circ Physiol* 294: H1398–1406, 2008.
117. Sasaki H, Fukuda S, Otani H, Zhu L, Yamaura G, Engelman RM, Das DK, and Maulik N. Hypoxic preconditioning triggers myocardial angiogenesis: A novel approach to enhance contractile functional reserve in rat with myocardial infarction. *J Mol Cell Cardiol* 34: 335–348, 2002.
118. Sasaki H, Ray PS, Zhu L, Otani H, Asahara T, and Maulik N. Hypoxia/reoxygenation promotes myocardial angiogenesis via an NF kappa B-dependent mechanism in a rat model of chronic myocardial infarction. *J Mol Cell Cardiol* 33: 283–294, 2001.
119. Satoh M, Fujimoto S, Haruna Y, Arakawa S, Horike H, Komai N, Sasaki T, Tsujioka K, Makino H, and Kashihara N. NAD(P)H oxidase and uncoupled nitric oxide synthase are major sources of glomerular superoxide in rats with experimental diabetic nephropathy. *Am J Physiol Renal Physiol* 288: F1144–1152, 2005.
120. Scherrer–Crosbie M, Ullrich R, Bloch KD, Nakajima H, Nasseri B, Aretz HT, Lindsey ML, Vancon AC, Huang PL, Lee RT, Zapol WM, and Picard MH. Endothelial nitric oxide synthase limits left ventricular remodeling after myocardial infarction in mice. *Circulation* 104: 1286–1291, 2001.
121. Segal MS, Shah R, Afzal A, Perrault CM, Chang K, Schuler A, Beem E, Shaw LC, Li Calzi S, Harrison JK, Tran–Son–Tay R, and Grant MB. Nitric oxide cytoskeletal-induced alterations reverse the endothelial progenitor cell migratory defect associated with diabetes. *Diabetes* 55: 102–109, 2006.

122. Semenza GL, Agani F, Feldser D, Iyer N, Kotch L, Laughner E, and Yu A. Hypoxia, HIF-1, and the pathophysiology of common human diseases. *Adv Exp Med Biol* 475: 123–130, 2000.
123. Shinmura K, Bolli R, Liu SQ, Tang XL, Kodani E, Xuan YT, Srivastava S, and Bhatnagar A. Aldose reductase is an obligatory mediator of the late phase of ischemic preconditioning. *Circ Res* 91: 240–246, 2002.
124. Shinmura K, Tamaki K, Saito K, Nakano Y, Tobe T, and Bolli R. Cardioprotective effects of short-term caloric restriction are mediated by adiponectin via activation of AMP-activated protein kinase. *Circulation* 116: 2809–2817, 2007.
125. Shinmura K, Tang XL, Wang Y, Xuan YT, Liu SQ, Takano H, Bhatnagar A, and Bolli R. Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. *Proc Natl Acad Sci USA* 97: 10197–101202, 2000.
126. Shinmura K, Xuan YT, Tang XL, Kodani E, Han H, Zhu Y, and Bolli R. Inducible nitric oxide synthase modulates cyclooxygenase-2 activity in the heart of conscious rabbits during the late phase of ischemic preconditioning. *Circ Res* 90: 602–608, 2002.
127. Shiva S, Oh JY, Landar AL, Ulasova E, Venkatraman A, Bailey SM, and Darley–Usmar VM. Nitroxia: The pathological consequence of dysfunction in the nitric oxide-cytochrome c oxidase signaling pathway. *Free Radic Biol Med* 38: 297–306, 2005.
128. Souza JM, Daikhin E, Yudkoff M, Raman CS, and Ischiropoulos H. Factors determining the selectivity of protein tyrosine nitration. *Arch Biochem Biophys* 371: 169–178, 1999.
129. Stamler JS, Singel DJ, and Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. *Science* 258: 1898–1902, 1992.
130. Stuehr D, Pou S, and Rosen GM. Oxygen reduction by nitric-oxide synthases. *J Biol Chem* 276: 14533–14536, 2001.
131. Sun J, Picht E, Ginsburg KS, Bers DM, Steenbergen C, and Murphy E. Hypercontractile female hearts exhibit increased S-nitrosylation of the L-type Ca2+ channel alpha1 subunit and reduced ischemia/reperfusion injury. *Circ Res* 98: 403–411, 2006.
132. Sun J, Steenbergen C, and Murphy E. S-nitrosylation: NO-related redox signaling to protect against oxidative stress. *Antioxid Redox Signal* 8: 1693–1705, 2006.
133. Takano H, Manchikalapudi S, Tang XL, Qiu Y, Rizvi A, Jadoon AK, Zhang Q, and Bolli R. Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation* 98: 441–449, 1998.
134. Takano H, Tang XL, and Bolli R. Differential role of K(ATP) channels in late preconditioning against myocardial stunning and infarction in rabbits. *Am J Physiol Heart Circ Physiol* 279: H2350–2359, 2000.
135. Takimoto E, Champion HC, Li M, Belardi D, Ren S, Rodriguez ER, Bedja D, Gabrielson KL, Wang Y, and Kass DA. Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nat Med* 11: 214–222, 2005.
136. Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, Lazzarino G, Paolocci N, Gabrielson KL, Wang Y, and Kass DA. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. *J Clin Invest* 115: 1221–1231, 2005.
137. Tanaka M, Stoler RC, FitzHarris GP, Jennings RB, and Reimer KA. Evidence against the "early protection-delayed death" hypothesis of superoxide dismutase therapy in experimental myocardial infarction. Polyethylene glycol-superoxide dismutase plus catalase does not limit myocardial infarct size in dogs. *Circ Res* 67: 636–644, 1990.
138. Titheradge MA. Nitric oxide in septic shock. *Biochim Biophys Acta* 1411: 437–455, 1999.
139. Treasure CB, Vita JA, Cox DA, Fish RD, Gordon JB, Mudge GH, Colucci WS, Sutton MG, Selwyn AP, Alexander RW, and *et al*. Endothelium-dependent dilation of the coronary microvasculature is impaired in dilated cardiomyopathy. *Circulation* 81: 772–779, 1990.
140. Tse HF and Lau CP. Therapeutic angiogenesis with bone marrow–derived stem cells. *J Cardiovasc Pharmacol Ther* 12: 89–97, 2007.
141. Uchiyama T, Otani H, Okada T, Ninomiya H, Kido M, Imamura H, Nogi S, and Kobayashi Y. Nitric oxide induces caspase-dependent apoptosis and necrosis in neonatal rat cardiomyocytes. *J Mol Cell Cardiol* 34: 1049–1061, 2002.
142. Uchiyama Y, Otani H, Okada T, Uchiyama T, Ninomiya H, Kido M, Imamura H, Nakao S, and Shingu K. Integrated pharmacological preconditioning in combination with adenosine, a mitochondrial KATP channel opener and a nitric oxide donor. *J Thorac Cardiovasc Surg* 126: 148–159, 2003.
143. Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T, Nishimura H, Losordo DW, Asahara T, and Isner JM. Statin therapy accelerates reendothelialization: A novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. *Circulation* 105: 3017–3024, 2002.
144. Wang Y, Guo Y, Zhang SX, Wu WJ, Wang J, Bao W, and Bolli R. Ischemic preconditioning upregulates inducible nitric oxide synthase in cardiac myocyte. *J Mol Cell Cardiol* 34: 5–15, 2002.
145. Wassmann S, Laufs U, Baumer AT, Muller K, Konkol C, Sauer H, Bohm M, and Nickenig G. Inhibition of geranylgeranylation reduces angiotensin II-mediated free radical production in vascular smooth muscle cells: Involvement of angiotensin AT1 receptor expression and Rac1 GTPase. *Mol Pharmacol* 59: 646–654, 2001.
146. Werns SW and Lucchesi BR. Inflammation and myocardial infarction. *Br Med Bull* 43: 460–471, 1987.
147. Weselcouch EO, Baird AJ, Sleph P, and Grover GJ. Inhibition of nitric oxide synthesis does not affect ischemic preconditioning in isolated perfused rat hearts. *Am J Physiol* 268: H242–249, 1995.
148. Wiemer G, Itter G, Malinski T, and Linz W. Decreased nitric oxide availability in normotensive and hypertensive rats with failing hearts after myocardial infarction. *Hypertension* 38: 1367–1371, 2001.
149. Wiesel P, Foster LC, Pellacani A, Layne MD, Hsieh CM, Huggins GS, Strauss P, Yet SF, and Perrella MA. Thioredoxin facilitates the induction of heme oxygenase-1 in response to inflammatory mediators. *J Biol Chem* 275: 24840–24846, 2000.
150. Wildhirt SM, Dudek RR, Suzuki H, and Bing RJ. Involvement of inducible nitric oxide synthase in the inflammatory process of myocardial infarction. *Int J Cardiol* 50: 253–261, 1995.
151. Xu Z, Ji X, and Boysen PG. Exogenous nitric oxide generates ROS and induces cardioprotection: involvement of PKG, mitochondrial KATP channels, and ERK. *Am J Physiol Heart Circ Physiol* 286: H1433–1440, 2004.

152. Yamakuchi M, Greer JJ, Cameron SJ, Matsushita K, Morrell CN, Talbot-Fox K, Baldwin WM, 3rd, Lefer DJ, and Lowenstein CJ. HMG-CoA reductase inhibitors inhibit endothelial exocytosis and decrease myocardial infarct size. *Circ Res* 96: 1185–1192, 2005.
153. Yang B, Li D, Phillips MI, Mehta P, and Mehta JL. Myocardial angiotensin II receptor expression and ischemia-reperfusion injury. *Vasc Med* 3: 121–130, 1998.
154. Yang XP, Liu YH, Shesely EG, Bulagannawar M, Liu F, and Carretero OA. Endothelial nitric oxide gene knockout mice: Cardiac phenotypes and the effect of angiotensin-converting enzyme inhibitor on myocardial ischemia/reperfusion injury. *Hypertension* 34: 24–30, 1999.
155. Yellon DM and Baxter GF. A "second window of protection" or delayed preconditioning phenomenon: Future horizons for myocardial protection? *J Mol Cell Cardiol* 27: 1023–1034, 1995.
156. Yellon DM and Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 357: 1121–1135, 2007.
157. Yin H, Chao L, and Chao J. Nitric oxide mediates cardiac protection of tissue kallikrein by reducing inflammation and ventricular remodeling after myocardial ischemia/reperfusion. *Life Sci* 82: 156–165, 2008.
158. Zhao TC and Kukreja RC. Late preconditioning elicited by activation of adenosine A(3) receptor in heart: role of NF-kappa B, iNOS and mitochondrial K(ATP) channel. *J Mol Cell Cardiol* 34: 263–277, 2002.
159. Zhao ZQ and Vinten–Johansen J. Postconditioning: Reduction of reperfusion-induced injury. *Cardiovasc Res* 70: 200–211, 2006.
160. Zimmet JM and Hare JM. Nitroso-redox interactions in the cardiovascular system. *Circulation* 114: 1531–1544, 2006.

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1. Yixuan Zhang, Carlo G. Tocchetti, Thomas Krieg, An L. Moens. 2012. Oxidative and nitrosative stress in the maintenance of myocardial function. *Free Radical Biology and Medicine* **53**:8, 1531-1540. [CrossRef]
2. Ricardo Carnicer , Mark J. Crabtree , Vidhya Sivakumaran , Barbara Casadei , David A. Kass . Nitric Oxide Synthases in Heart Failure. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
3. Frederick A Villamena, Amlan Das, Kevin M Nash. 2012. Potential implication of the chemical properties and bioactivity of nitrone spin traps for therapeutics. *Future Medicinal Chemistry* **4**:9, 1171-1207. [CrossRef]
4. Ming-Jen Lu, Yih-Sharng Chen, Ho-Shiang Huang, Ming-Chieh Ma. 2012. Erythropoietin alleviates post-ischemic injury of rat hearts by attenuating nitrosative stress. *Life Sciences* **90**:19-20, 776-784. [CrossRef]
5. Janet R. Manning, Gregory Carpenter, Darius R. Porter, Stacey L. House, Daniel A. Pietras, Thomas Doetschman, Jo El J. Schultz. 2012. Fibroblast growth factor-2-induced cardioprotection against myocardial infarction occurs via the interplay between nitric oxide, protein kinase signaling, and ATP-sensitive potassium channels. *Growth Factors* 120206024823007. [CrossRef]
6. Mireille Bentz, Charlotte Zaouter, Qin Shi, Hassan Fahmi, Florina Moldovan, Julio C. Fernandes, Mohamed Benderdour. 2012. Inhibition of inducible nitric oxide synthase prevents lipid peroxidation in osteoarthritic chondrocytes. *Journal of Cellular Biochemistry* n/a-n/a. [CrossRef]
7. Yuxing Zhang , Yanzhi Du , Weidong Le , Kankan Wang , Nelly Kieffer , Ji Zhang . 2011. Redox Control of the Survival of Healthy and Diseased Cells. *Antioxidants & Redox Signaling* **15**:11, 2867-2908. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. B. Rinaldi, C. Di Filippo, A. Capuano, M. Donniacuo, L. Sodano, F. Ferraraccio, F. Rossi, M. D'Amico. 2011. Adiponectin elevation by telmisartan ameliorates ischaemic myocardium in zucker diabetic fatty rats with metabolic syndrome. *Diabetes, Obesity and Metabolism* no-no. [CrossRef]
9. Toru Okazaki, Hajime Otani, Takayuki Shimazu, Kei Yoshioka, Masanori Fujita, Toshiji Iwasaka. 2011. Ascorbic acid and N-acetyl cysteine prevent uncoupling of nitric oxide synthase and increase tolerance to ischemia/reperfusion injury in diabetic rat heart. *Free Radical Research* **45**:10, 1173-1183. [CrossRef]
10. Yong Pil Hwang, Hyung Gyun Kim, Tran Thi Hien, Myung Ho Jeong, Tae Cheon Jeong, Hye Gwang Jeong. 2011. Puerarin activates endothelial nitric oxide synthase through estrogen receptor-dependent PI3-kinase and calcium-dependent AMP-activated protein kinase. *Toxicology and Applied Pharmacology* . [CrossRef]
11. Hiromi Jo, Hajime Otani, Fusakazu Jo, Takayuki Shimazu, Toru Okazaki, Kei Yoshioka, Masanori Fujita, Atsushi Kosaki, Toshiji Iwasaka. 2011. Inhibition of nitric oxide synthase uncoupling by sepiapterin improves left ventricular function in streptozotocin-induced diabetic mice. *Clinical and Experimental Pharmacology and Physiology* **38**:8, 485-493. [CrossRef]
12. Xiulan Yang, Wenkuan Xin, Xi-Ming Yang, Atsushi Kuno, Thomas C Rich, Michael V Cohen, James M Downey. 2011. A2B adenosine receptors inhibit superoxide production from mitochondrial complex I in rabbit cardiomyocytes via a mechanism sensitive to Pertussis toxin. *British Journal of Pharmacology* **163**:5, 995-1006. [CrossRef]
13. Yasuhiro Maejima, Junya Kuroda, Shouji Matsushima, Tetsuro Ago, Junichi Sadoshima. 2011. Regulation of myocardial growth and death by NADPH oxidase. *Journal of Molecular and Cellular Cardiology* **50**:3, 408-416. [CrossRef]
14. Weiwei Li, Brian Olshansky. 2011. Inflammatory cytokines and nitric oxide in heart failure and potential modulation by vagus nerve stimulation. *Heart Failure Reviews* **16**:2, 137-145. [CrossRef]
15. Chiara Nediani , Laura Raimondi , Elisabetta Borchi , Elisabetta Cerbai . 2011. Nitric Oxide/Reactive Oxygen Species Generation and Nitroso/Redox Imbalance in Heart Failure: From Molecular Mechanisms to Therapeutic Implications. *Antioxidants & Redox Signaling* **14**:2, 289-331. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
16. Nwe Nwe Soe, Bradford C. Berk. 2011. Cyclophilin A: A Mediator of Cardiovascular Pathology. *Journal of the Korean Society of Hypertension* **17**:4, 133. [CrossRef]
17. Jee-In Kim, Hee-Seong Jang, Kwon-Moo Park. 2010. Endotoxin-induced renal tolerance against ischemia and reperfusion injury is removed by iNOS, but not eNOS, gene-deletion. *BMB Reports* **43**:9, 629-634. [CrossRef]
18. Junya Kuroda, Junichi Sadoshima. 2010. NADPH Oxidase and Cardiac Failure. *Journal of Cardiovascular Translational Research* **3**:4, 314-320. [CrossRef]
19. Angeles Garcia-Pascual, Alicia Labadía, Marta Garcia-Flores, María Sancho, Domingo Triguero. 2010. Refractoriness of urethral striated muscle contractility to nitric oxide-dependent cyclic GMP production. *Nitric Oxide* **23**:1, 26-33. [CrossRef]

20. An L. Moens, Ronghua Yang, Vabren L. Watts, Lili A. Barouch. 2010. Beta 3-adrenoreceptor regulation of nitric oxide in the cardiovascular system. *Journal of Molecular and Cellular Cardiology* **48**:6, 1088-1095. [CrossRef]