Relevance of Nitric Oxide for Myocardial Remodeling

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Endogenous myocardial nitric oxide (NO) may modulate the transition from adaptive to maladaptive remodeling leading to heart failure. In rodent models of pressure overload or myocardial infarction, the three NO synthase (NOS) isoforms were shown to play a neutral, protective, or even adverse role in myocardial remodeling, depending on the quantity of NO produced, the location of each NOS and their regulators, the prevailing oxidant stress and resultant NO/oxidant balance, as well as NOS coupling/dimerization. Beside neuronal NOS and—in specific conditions—inducible NOS isoforms, endothelial NOS (eNOS) exerts cardioprotective effects on pressure-overload, ischemia/reperfusion, and myocardial infarction–induced myocardial remodeling, provided the enzyme remains in a coupled state. Besides its effects on excitation-contraction coupling in response to stretch, eNOS acts as an "endogenous β-blocker" by restoring the sympathovagal balance, opposing excessive hypertrophy as well as promoting vasodilatation and neoangiogenesis, thereby contributing to tissue repair. As eNOS was also shown to mediate the beneficial effects of cardiovascular drugs commonly used in patients with heart failure, strategies to increase its expression and/or coupled catalytic activity in the myocardium offer new therapeutic avenues for the treatment of this disease.

Introduction

Left ventricular (LV) remodeling was described in 1985 as the observed LV dilation and deterioration of performance in rats with chronic myocardial infarction (MI) that was attenuated by angiotensin-converting enzyme inhibitor treatment [1]. More than 20 years later, cardiac remodeling is defined by all molecular, cellular, interstitial, and myocardial changes that occur in response to any direct myocardial stress potentially leading to heart failure (HF), irrespective of its etiology. Cardiac hypertrophy and/or cardiac dilation are the two main patterns of remodeling leading to HF [2], and the signaling pathways governing this evolution now represent crucial targets for the treatment of this disease [3]. The main cardiac stresses leading to remodeling and HF are mechanical stretch from pressure or volume overload, oxidative stress with reactive oxygen species (ROS) [4], hypoxia, viral infection, and primary contractile or cytoskeletal gene mutation [2]. These initial cellular insults are inflicted to the myocardium in corresponding clinical settings well-known to produce adverse remodeling (ie, aortic stenosis or hypertension, mitral regurgitation, ischemia, and reperfusion MI, viral myocarditis, and genetic dilated cardiomyopathy, respectively).

Cardiac remodeling is developed in two phases [5]. The acute phase (within minutes, maximal after 24 h, up to 2 wk) is characterized by early stimuli-induced cytokine release (mainly tumor necrosis factor [TNF]-α, interleukin [IL]-1β, and IL-6) and inflammatory cell recruitment. Cytokines lead either to myocyte survival and wound healing with some degree of myocyte elongation and hypertrophy or to myocyte apoptosis and necrosis with calcium desensitization and decreased contractility, according to the intensity of cytokine release and oxidant stress. A subsequent chronic phase (within weeks to years) occurs in response to a second, sustained wave of cytokines production and is characterized by several hallmarks in both stressed and remote myocardium (eg, transition towards concentric or eccentric ventricular hypertrophy); matrix metalloproteinase (MMP) activation (mainly MMP1, MMP2, and MMP9) responsible for collagen degradation; transforming growth factor (TGF)-β–induced fibroblast proliferation; integrin dysregulation and interstitial fibrosis; vascular endothelial growth factor (VEGF)-induced angiogenesis; and progenitor cell mobilization and possibly some myocyte regeneration.

Importantly, chronic cardiac remodeling is called "adaptive" when concentric hypertrophy and limited diastolic dysfunction compensates wall stress and preserves systolic function. By contrast, "maladaptive" remodeling consists in excessive matrix remodeling, eccentric dila-

tion, geometry disruption, interstitial fibrosis leading to progressive HF, and premature death. Adaptive and maladaptive cardiac hypertrophy are driven by differential protein kinase signaling cascades [6]. "Reverse remodeling" designates all inverse cellular processes able to restore cardiac function.

Mechanisms responsible for transition from adaptive to maladaptive remodeling are incompletely characterized and represent the highest focus of interest and controversies in HF research [7]. Several mechanisms responsible for this transition have recently been proposed, most of which correspond to a deregulated response to the stress factors mentioned above, with excessive activation of MMPs [8,9], production of ROS, neurohumoral activation (β-adrenergic and renin-angiotensin-aldosterone systems), resulting in altered intracellular calcium handling [10], apoptosis, and cardiac fibrosis [11].

In this review, we propose to dissect the potential modulatory role of nitric oxide (NO) on this transition from adaptive to maladaptive remodeling. Indeed, cardiac endogenous NO is a well-known modulator of systolic contractility, diastolic relaxation, sympatho-vagal balance, coronary vasodilation, and angiogenesis as well as myocyte apoptosis and mitochondrial respiration [12,13]. Furthermore, NO and other reactive nitrogen species (eg, S-nitrosothiols [SNO]) critically modulate the redox status of cardiac cells by controlling the production, concentration and signaling of reactive oxygen species (ROS, eg, superoxide anion O_2^-, H_2O_2, OH^-). NO/oxidants disequilibrium with resultant oxidative and nitrosative stresses are now recognized as central features in the pathophysiology of HF [14].

Which NOS?

All three isoforms of NO synthases (NOS) are widely expressed in the heart and, in variable proportion, in cardiomyocytes. Neuronal NOS (nNOS or *NOS1*) and endothelial NOS (eNOS or *NOS3*) are both constitutively expressed and produce small amounts of NO in a tightly regulated fashion, whereas the production of larger amounts of NO by inducible NOS (iNOS or *NOS2*) is mainly regulated by the transcription of the *NOS2* gene and the availability of enzyme cofactors.

All NOS enzymes function as dimers. However, in absence of tetrahydrobiopterin (BH4) and substrate, L-arginine, the NOS lose their dimeric structure, and electron transfer from the reductase to oxygenase domain in the monomeric NOS may become "uncoupled" from catalytic NO formation resulting in the production of superoxide anions (O_2^-). This uncoupled NOS-derived O_2^- rapidly combines with NO to form peroxynitrite ($ONOO^-$), further enhancing oxidant stress. BH4 is highly sensitive to oxidation by peroxynitrite [15], leading to aggravated uncoupling. Using mice deficient in eNOS or the NADPH oxidase subunit p47(phox), Landmesser et al. [16] showed that hypertension induces superoxide-induced BH4 oxidation and subsequent eNOS uncoupling, ROS overproduction, and exacerbated hypertension that can be reversed by BH4 supplementation. Similar mechanisms are likely to occur under increased oxidant stress for the myocardial NOS.

Conversely, the cytosolic chaperone protein, heat shock protein (Hsp) 90 promotes calmodulin association to eNOS, recruits Akt on the eNOS-calmodulin complex, prevents protein phosphatase PP2A-mediated dephosphorylation of Akt, and promotes sustained eNOS activation [17]. Of interest, liposomal transfection of Hsp90 was shown to protect the myocardium against ischemia/reperfusion injury in pigs and is also associated with eNOS threonin (Thr)-495 dephosphorylation and serine (Ser)-1177 phoshorylation [18], suggesting that enhanced "chaperoning" by Hsp90 promotes a sustained production of NO by a "coupled" eNOS despite the prevailing oxidant stress. Statins also promote eNOS activation through Akt-mediated Ser-1177 phosphorylation of eNOS together with increased interaction with Hsp90 [19,20]. Therefore, all molecular interventions that protect NOS from uncoupling are likely to prevent excessive ROS production by these enzymes and the transition to maladaptive remodeling.

nNOS Exerts Cardioprotective Effects Against Myocardial Remodeling

nNOS knockout (nNOS-/-) mice develop hypertrophy with aging [21] with concentric LV remodeling, which is exacerbated upon combined nNOS and eNOS genetic deletion [22]. After MI, nNOS-/- mice developed a faster and more severe LV dilation with a hyporesponsiveness to dobutamine at 8 weeks compared with wild-type, infarcted mice [23]. In another study, infarcted nNOS-deficient mice had increased mortality and a persistent increase in xanthine oxidoreductase activity [24], further suggesting a protective role of nNOS after MI. Of note, cardiac tissue from nNOS-/- mice (but not eNOS-/-) produce 60% higher cardiac superoxide levels at basal state and fourfold greater amounts compared with wild-type in response to xanthine, suggesting a direct antioxidant mechanism for nNOS perhaps through direct inhibition of xanthine oxidoreductase, at least in mice [25]. Myocardial nNOS expression and activity are increased in patients with end-stage congestive HF secondary to idiopathic dilated cardiomyopathy [26], possibly corresponding to an adaptive, albeit insufficient mechanism of protection. Whether nNOS remains "coupled" in these conditions is an open question.

A main caveat from these studies is their exclusive use of a systemic knockout of nNOS. From older studies, we know that nNOS-derived NO presynaptically facilitates cardiac vagal control, so that nNOS deletion may adversely affect remodeling through an imbalance of the

sympathovagal equilibrium, regardless of direct effects on the myocytes. More insights are awaited from the phenotype of cardiac-specific nNOS conditional deletion or overexpression.

iNOS May Exert Deleterious Effects on Myocardial Remodeling

iNOS does not play a critical role in pressure overload–induced hypertrophic cardiomyopathy, as observed in a mouse model of transverse aortic constriction with similar LV hypertrophy between iNOS-/- and wild-type mice [27]. By contrast, increased myocardial iNOS activity contributes to depressed contractility [28] and β-adrenergic hyporesponsiveness [29] in various rat models of volume overload–induced HF.

After MI, myocardial upregulation of iNOS may lead to LV dysfunction and remodeling as well as increased 30-day [30] or 4-month [31] mortality, as suggested by the preserved cardiac function and improved survival in iNOS-/- mice compared with wild types [32]. This deleterious influence of iNOS was not observed in similar model of more severe MI-induced HF [33]. Again, the use of systemic knockout leaves an open question on the respective participation of iNOS-expressing inflammatory versus parenchymal cells for the observed phenotype.

Some discordant results in transgenic mice with cardiac specific iNOS-overexpression [34,35] have also cast some doubt on the functional relevance of iNOS for the development of HF. A consensual view, though, is that upon high levels of cardiomyocyte-specific expression and in the absence of myoglobin (that "buffers" excessive NO), these models recapitulate all the features of ventricular remodeling with nitrosative stress, cardiac hypertrophy, progressive ventricular dilatation, interstitial fibrosis, and HF. Notably, isolated myocytes from human failing hearts of various etiologies also exhibit iNOS-dependent β-adrenergic hyporesponsiveness [36].

iNOS May Have Some Beneficial Effects

Intramyocardial iNOS gene therapy provides long-term protection of mouse hearts against 1- to 2-months differed MI [37]. Earlier work has also led to propose iNOS as the cardioprotective mediator of late (day 2) preconditioning [38]. Likewise, iNOS has been proposed as a critical mediator of the pharmacologic preconditoning by the phosphodiesterase-5 (PDE-5) inhibitor, sildenafil [39]. Nondiabetic iNOS-/- mice have higher infarct size and apoptotic index compared with nondiabetic wild-type mice, although the opposite is observed in diabetic mice, suggesting a protective role for iNOS specifically in the context of low or absent oxidant/hyperglycemic stress [40].

Overall, the iNOS isoform may play a dual role, probably deleterious upon production of high amounts of NO that will overflow physiologic intracellular buffers and exacerbate oxidant/nitrosative stress but cardioprotective at moderate intracellular levels with compartmentalized physiologic signaling in the absence of oxidant stress [41].

Role of eNOS in Ventricular Remodeling

Coupled eNOS exerts cardioprotective effects on pressure overload–induced myocardial remodeling

In isolated cardiac myocytes, NO exerts anti-hypertrophic effects mediated by cGMP [42] and downstream activation of protein kinase G (PKG). Among several targets, PKG-I was shown to inhibit the pro-hypertrophic calcineurin nuclear factor of activated T cells (NFAT) signaling pathway [43–45], possibly through inhibition of L-type calcium currents and entry.

eNOS is also a central modulator of myocyte contractility, relaxation and rate in both basal and β-adrenergic stimulated conditions [46,47]. Cardiomyocyte-restricted eNOS overexpression attenuates β-adrenergic stimulation and reinforces vagal inhibition of cardiac contraction both in vitro and in vivo [48], thereby providing protection of the myocardium against the toxicity of excessive catecholaminergic stimulation. Similar antiadrenergic effects of sildenafil depend on the upstream activation of an eNOS-soluble guanylate cyclase (sGC) axis and downstream activation of PKG-1 that require z-band colocalization of PDE5A and eNOS-derived cGMP formation [49]. The same group showed that sildenafil [50••] can prevent chamber, cellular, and molecular remodeling in mice submitted to chronic transverse aortic constriction (TAC) and can even reverse pre-existing hypertrophy through cGMP-dependent deactivation of classic pro-hypertrophic pathways (ie, calcineurin/NFAT, phosphoinositide-3 kinase (PI3K)/Akt, and ERK1/2 signaling pathways). PDE5A is preferentially compartmentalized near the z-band of myocytes, corresponding to T-tubular structures rich in caveolae where the eNOS isoform is colocalized [20].

Of note, the positive inotropic response of mouse cardiomyocytes to stretch also requires PKB-mediated eNOS phosphorylation, with direct NO-mediated activation of the ryanodine receptor and SR calcium release [51]. The molecular mechanism transducing mechanical stretch to eNOS activation is unknown. A likely candidate might be melusin, also a z-band, muscle-specific protein interacting with the β1-integrin that prevents adverse cardiac remodeling in response to chronic TAC [52]. Transgenic cardiac overexpression of melusin allows prolonged concentric compensatory hypertrophy and prevents transition towards failure in response to 12 weeks pressure overload [53]. Interestingly, myocardial tissue from melusin TG mice exhibit increased phosphorylation of PKB/Akt, a well-established upstream eNOS activator through Ser-1177 phosphorylation [54,55]. eNOS (and downstream cGMP) could mediate some of the cardioprotective effects of melusin, provided it remains in a "coupled" state.

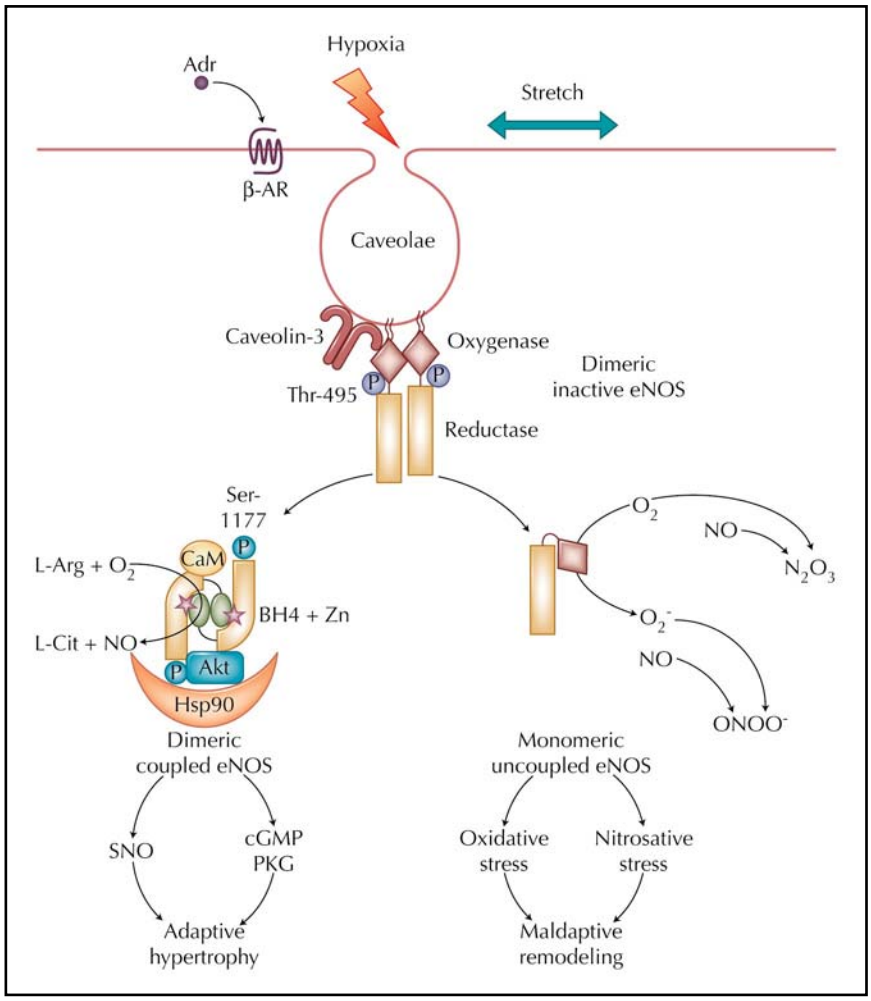

Figure 1. Modulatory role of cardiac endothelial nitric oxide synthase (eNOS) on adaptive versus maladaptive remodeling. In resting state, dimeric inactive eNOS is localized at the caveolar membrane and inhibited through interaction with caveolin and threonin-495 (Thr-495) phosphorylation. After calcium-calmodulin activation, caveolin dissociation, heat shock protein (Hsp)90-mediated recruitment and stabilization of a multiprotein complex (including phosphatases and kinases), protein kinase B (PKB)-induced serine-1177 (Ser-1177) phosphorylation and in the presence of adequate substrate and cofactors, dimeric coupled eNOS with its oxygenase domain produces regulated amounts of NO, exerting cardioprotective effects towards adaptive remodeling. By contrast, in an excessive oxidant environment, monomeric uncoupled eNOS with its oxygenase domain produces superoxide anions instead of NO, precipitating maladaptive remodeling and heart failure. Adr—adrenalin; β-AR—β-adrenergic receptor; BH4—tetrahydrobiopterin; CaM—calmodulin; L-Arg—L-arginine; L-Cit—L-citrulline N_2O_3—dinitrogen trioxide; ONOO—peroxynitrite; P—phospho; SNO—S-nitrosothiol.

According to the evidence cited above, eNOS in the cardiomyocyte may be particularly suited to protect against adverse remodeling, both through its ability to sustain excitation-contraction coupling and contractility in response to mechanical stretch and to maintain the sympathovagal balance as an "endogenous β-blocker" [13,48,56].

Indeed, several observations suggest the specific implication of eNOS in the antihypertrophic effects of NO. As previously mentioned, mice with nonconditional, systemic eNOS deletion develop hypertrophy by age 5 months [21], in part attributable to hypertension secondary to vascular eNOS deficiency. At age 20 months, eNOS-/- mice have a 25% increase in cardiac mass compared with wild-type controls, with 47 differentially expressed genes [57]. Systemic eNOS-/- mice also developed more LV hypertrophy and dysfunction in response to moderate transverse aortic constriction compared with controls in two studies [58,59], one of which included a group of eNOS-/- mice treated with hydralazine to obviate the confounding effect of systemic hypertension [58]. Preliminary evidence from eNOS cardiac-specific overexpressors would support the concept of eNOS-mediated protection of the cardiomyocytes against TAC-induced hypertrophy (but not fibrosis), with preserved LV systolic function (Janssens, personal communication).

However, another study by Takimoto et al. [60••] emphasized the dual, opposite roles of eNOS depending on the enzyme's catalytic ("coupled") state (Fig. 1). They showed that 3 to 9 weeks of severe (> 65 mmHg) TAC in wild-type (eNOS+/+) mice triggers eNOS uncoupling, leading to enhanced production of myocardial ROS, worse dilatory remodeling, and cardiac dysfunction. Accordingly, eNOS-/- mice displayed only modest concentric hypertrophy, with less fibrosis, myocyte hypertrophy, fetal gene re-expression, ROS, and $ONOO^-$ production compared with eNOS+/+ mice. In the latter, severe TAC-induced eNOS uncoupling was associated with a lower content of reduced BH4, less eNOS dimer detection and lower Ca^{2+}-dependent NOS activity, as well as more ROS production, all prevented by BH4 supplementation. Whether uncoupled vascular or cardiomyocyte eNOS predominantly accounts for the phenotype cannot be determined in this comparison using the systemic eNOS knockout. It is possible (but still not demonstrated) that the adverse outcome in the eNOS+/+ resulted from vascular (ie, coronary endothelial) eNOS uncoupling whereas the cardiomyocyte eNOS catalytic activity may be more resistant (perhaps due to higher antioxidant capacity in the myocytes), as suggested from the preliminary data in the myocyte-specific overexpressors.

Another confounding factor in the context of systemic eNOS deletion is the increased level of atrial natriuretic peptide (ANP), because ANP restores myocardial cGMP contents, providing compensatory antihypertrophic mechanisms. In another study, combined deletion of eNOS and of guanylyl cyclase A receptor for ANP led to marked cardiac hypertrophy [61].

eNOS protects against ischemia/reperfusion

Studies using transgenic mice overexpressing eNOS provided further support for a cardioprotective role of eNOS in this setting. Endothelial-restricted eNOS overexpression afforded protection with reduced MI and better diastolic function at day 7 after reperfusion compared with nontransgenic mice [62]. Specifically, cardiomyocyte-restricted eNOS overexpression protects against myocardial ischemia/reperfusion injury with decreased infarct size and preserved LV function [63,64]. Accordingly, transfection of the myocardium with constructs to increase eNOS activity either with eNOS cDNA [65], a phosphomimetic Ser-1177D eNOS, or Hsp90 (a chaperone for eNOS increasing its activity) [18] improved ischemia/reperfusion injuries. The beneficial effect of granulocyte colony stimulating factor in rats submitted to ischemia/reperfusion has also been attributed to activation of the Akt-eNOS pathway [66]. The benefit of eNOS in ischemia/reperfusion probably stems from protective effects of NO both on the endothelium (eg, protection from apoptosis, decreased activation and leucocytes adhesion) and cardiomyocytes; contrary to long-term hemodynamic stress (as produced in chronic aortic banding), the short-term injury is probably insufficient to produce persistent uncoupling of the enzyme, thereby maintaining its protective properties.

eNOS attenuates post-MI remodeling and HF

Whereas in one study [67] eNOS-/- infarcted mice had similar infarct size but worse systolic and diastolic basal function, decreased capillary density, increased myocyte hypertrophy, and mortality at 4 weeks, eNOS deletion did not alter the development of HF after large MI in another [68]. The more severe insult in the latter may have resulted in higher and prolonged oxidant stress and abrogated the protective effect of eNOS through uncoupling, although this was not specifically measured. Mice with cardiomyocyte-restricted eNOS overexpression, on the other hand, showed similar infarct size at 1 week but improved LV function and reduced hypertrophic remodeling at 4 weeks after MI, despite similar degree of fibrosis [69•].

eNOS may promote cardiac regeneration

Several lines of evidence suggest that the benefit of eNOS activation after an ischemic insult may result from NO's potentiation of tissue repair (eg, through endothelial progenitor cells). Indeed, eNOS was identified as a critical mediator of endothelial progenitor cells' mobilization from the bone marrow to the ischemic hindlimb [70] as well as to infarcted hearts (in response to estradiol) [71] through NO-mediated MMP-9 activation. The protective effect of atorvastatin against remodeling and mortality after MI in mice through endothelial progenitor cell mobilization also involved eNOS activation, as it was lost in eNOS-/- animals [72]. Whether eNOS may similarly promote cardiac regeneration from stem/progenitor cells in cardiac niches is an equally attractive hypothesis that deserves further research.

eNOS mediates the beneficial effects of cardiovascular drugs

The use of eNOS-/- mice allowed the identification of the critical role of eNOS to mediate antiremodeling effects of a variety of drugs currently used in the clinic for the treatment of ischemic or hypertrophic cardiac diseases, ie, the angiotensin convertase inhibitor, enalapril [68], or the angiotensin receptor type 1 antagonist, losartan [68]. More recent studies showed that angiotensin-converting enzyme inhibitors increase eNOS expression and activity in atrial myocardium of patients before elective coronary artery bypass grafting [73] and that captopril reduces superoxide levels and prevents eNOS uncoupling in cardiomyopathic hamsters [74].

Of interest, celiprolol, a third generation vasodilatory β1-blocker, was shown to prevent pressure overload–induced LV remodeling, with less myocyte and myocardial hypertrophy and fibrosis, and to prevent the transition to HF in a NO-dependent manner [75]. Celiprolol induced myocardial eNOS overexpression and activation through Akt-dependent Ser-1177 phosphorylation and also reduced the expression of the protein inhibitor of neuronal NOS, a known inhibitor of eNOS as well.

Nebivolol, another third generation β1-blocker with eNOS-dependent vasodilatory properties [76], vasodilates human coronary microvessels, promotes neoangiogenesis, and preserves coronary reserve through β3-adrenoreceptor-mediated eNOS activation [77]. Nebivolol (but not metoprolol) inhibits endothelial superoxide formation by reducing NADPH oxidase expression and activity and preventing eNOS uncoupling [78]. It was also shown to reduce O_2^- and $ONOO^-$ release from dysfunctional endothelial cells from black patients [79].

Conclusions

All NOS isoforms may play a cardioprotective role against adverse myocardial remodeling, depending on the quantity of NO produced, on the location of each NOS and their regulators, on the prevailing oxidant stress, and resultant NO/oxidant balance, as well as NOS coupling/dimerization. A coupled eNOS acts as an "endogenous β-blocker," able to restore the sympathovagal balance and opposes excessive hypertrophy as well as promotes vasodilatation and neoangiogenesis, thereby contributing to tissue repair. Further understanding of the post-translational regulation

of eNOS in the myocardium should pave the way for the development of therapeutic agents which would enhance eNOS expression while preserving its coupled state through additional antioxidant properties.

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