Redox Signaling in Cardiac Physiology and Pathology

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Abstract: Redox signaling refers to the specific and usually reversible oxidation/reduction modification of molecules involved in cellular signaling pathways. In the heart, redox signaling regulates several physiological processes (e.g., excitation-contraction coupling) and is involved in a wide variety of pathophysiological and homoeostatic or stress response pathways. Reactive oxygen species involved in cardiac redox signaling may derive from many sources, but NADPH oxidases, as dedicated sources of signaling reactive oxygen species, seem to be especially important. An increasing number of specific posttranslational oxidative modifications involved in cardiac redox signaling are being defined, along with the reactive oxygen species sources that are involved. Here, we review current knowledge on the molecular targets of signaling reactive oxygen species in cardiac cells and their involvement in cardiac physiopathology. Advances in this field may allow the development of targeted therapeutic strategies for conditions such as heart failure as opposed to the general antioxidant approaches that have failed to date. (Circ Res. 2012;111:1091-1106.)

Key Words: myocardial contractility ■ cardiac hypertrophy ■ NAD(P)H oxidase ■ oxidant signaling

The concept that oxidative stress—a pathologically high level of oxidant species in cells—may drive cardiovascular disease progression has led to many clinical trials of interventions, such as antioxidant vitamins. These, however, failed to show efficacy in reducing disease risk and progression. Part of the reason may be the focus on oxidative stress as detrimental neglected the wider role of redox balance and reactive oxygen species (ROS) and reactive nitrogen species in cellular (patho)physiology. Redox signaling—defined as the specific, usually reversible, oxidation/reduction modification of cellular signaling pathway components by a reactive species—is increasingly appreciated as centrally important in many physiological and pathological processes. The main ROS involved in redox signaling are the superoxide anion (O$_2^-$) and the more stable nonradical hydrogen peroxide (H$_2$O$_2$) to which it dismutates, whereas more powerful oxidants such as hydroxyl are so reactive they are unlikely to be specific or reversible. Redox signaling also involves reactive nitrogen species such as NO and peroxynitrite, the latter being formed from the reaction of O$_2^-$ with NO.$^2$

In the heart, redox signaling is involved in physiological processes (e.g., excitation-contraction coupling [ECC], cell differentiation), homoeostatic and stress response pathways (e.g., adaptation to hypoxia/ischemia), and pathology (e.g., adverse cardiac remodeling, fibrosis). This review covers recent advances in understanding the regulation of production of signaling ROS, their mechanisms of action and molecular targets in cardiac cells, and their involvement in cardiac physiopathology. We focus mainly on cardiomyocytes but redox signaling in other cells (e.g., fibroblasts, endothelial cells), and functional cross talks among these are also important. Reactive nitrogen species–dependent regulation has been reviewed elsewhere.$^2$

ROS Sources

ROS are generated as a by-product of cellular respiration and metabolism or by specialized enzymes that seem to be centrally involved in redox signaling. The signaling effects of ROS are influenced by their site of production, precise species, local concentration, and cell compartment–specific antioxidant pools. Major ROS sources in the heart and other tissues include the mitochondrial electron transport chain (ETC), other mitochondrial and metabolic enzymes, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Noxs), and uncoupled NO synthases (NOS).

Mitochondrial ETC

Electron leakage from the ETC causes 1-electron reduction of O$_2$ to O$_2^-$ (instead of reduction to H$_2$O). Although considered to be because of an electron leak, such ROS may nevertheless contribute to homoeostatic redox signals. ROS levels increase significantly during mitochondrial dysfunction. They can trigger the mitochondrial permeability transition (MPT) and lead to further ROS release—termed ROS-induced ROS release$^3$—which propagates and amplifies ROS production and effects. ROS from non-ETC sources (e.g, Noxs) may also stimulate ETC-dependent ROS production. Interestingly, transient MPT pore (MPTP) opening in unstressed cells can couple to ETC-dependent ROS production, termed superoxide flashes,$^4$ and...
Mitochondrial ROS levels are influenced by ROS scavenging systems, such as superoxide dismutase, catalase, glutathione peroxidase, and peroxiredoxin. NADPH-regenerating reactions that maintain reduced pools of glutathione, glutaredoxin, and thioredoxin (Trx) are particularly important and depend on a sufficient level of NADPH being present, influencing mainly the cytosolic pentose phosphate pathway and the activity of mitochondrial Ca-dependent Krebs cycle dehydrogenases.

Oxidases Involved in Metabolism

Oxidases and oxygenases convert O₂ to reactive species during biosynthetic reactions in diverse metabolic pathways. In many cases, the ROS are involved in enzyme catalysis reactions and consumed, but oxidases that metabolize glucose, α-amino acids, monoamines, and xanthine use O₂ as the terminal electron acceptor and generate O₂⁻ or H₂O₂. Among these, xanthine oxidase (XO) and monoamine oxidases (MAO) are implicated in cardiac pathology. XO generates H₂O₂ or O₂⁻, the former predominating under hypoxia, and may contribute to oxidative stress in ischemia-reperfusion and heart failure. Neuronal NOS (nNOS) is reported to limit XO-derived ROS production, although the pathophysiological relevance of this observation remains to be fully established. MAO catabolize neurotransmitters such as norepinephrine and serotonin, generating H₂O₂, and are important ROS sources in mice with pressure overload–induced heart failure. The mitochondrial localization of MAO raises the intriguing possibility that they interact with other mitochondrial ROS sources. Although ROS generated by the above enzymes contribute to oxidative stress, it seems unlikely that they are involved in regulated redox signaling.

Uncoupled NO Synthases

Cardiomyocytes constitutively express nNOS and endothelial NOS (eNOS), whereas inducible NOS is expressed in pathological situations. NOS enzymes switch from NO to O₂⁻ production when they become uncoupled due to depletion of the cofactor tetrahydrobiopterin (BH4) and, in the case at least of eNOS, due to S-glutathionylation of specific cysteine residues in the reductase domain. Both events occur with increased oxidative stress so that ROS generation by NOS acts as an amplifying mechanism. If NOS are partially uncoupled, O₂⁻ and NO may be concomitantly produced and generate peroxynitrite, which has its own distinct effects. The switch from NO to O₂⁻ production is considered a crucial event in the pathogenesis of many cardiovascular diseases.

NADPH Oxidases: Dedicated Sources of Signaling ROS

In contrast to other oxidases, Nox family enzymes have no known biosynthetic or catabolic function but rather catalyze generations of ROS and protons as their primary property. This occurs via electron transfer from NADPH to flavin and heme moieties and then molecular O₂. Seven oxidase family members are known, each based on a distinct catalytic subunit (ie, Nox1–5 and dual oxidase 1–2) and with differing requirements for additional partner subunits. Noxs are crucial regulators of redox signaling in multiple body systems and organisms, generally through tightly regulated and spatially confined production of low ROS levels in the vicinity of target proteins. Such redox signaling is involved in regulating cell differentiation, proliferation, migration, and survival. However, some Noxs have quite different cell-specific functions, eg, phagocyte Nox2 and epithelial dual oxidase in non-specific host defense against microbes and Nox3 in otoconia morphogenesis in the inner ear vestibular system.

Nox2 and Nox4 are expressed in cardiac cells, including cardiomyocytes, endothelial cells, fibroblasts, and inflammatory cells. Both exist as a heterodimeric flavocytochrome with a p22phox subunit but otherwise exhibit significant differences in structure, function, and localization. Nox2 is acutely activated by stimuli such as angiotensin II (AngII), endothelin-1, growth factors, cytokines, and mechanical forces. These stimuli induce posttranslational modifications in cytosolic subunits (p47phox, p67phox, p40phox, and Rac1), which promote binding to the flavocytochrome at the plasmalemma to initiate O₂⁻ production. Nox4, however, is constitutively active and has no obligatory requirement for additional subunits (apart from p22phox) but may be regulated mainly by changes in...
abundance. Stimuli that increase Nox4 levels include hypoxia, ischemia, pressure overload, endoplasmic reticulum stress, and transforming growth factor-β (TGF-β). The interaction of Nox4 with other proteins (eg, Poldip2, Tks5) may modulate activity, but there are no data on such interactions in the heart. In contrast to Nox2, Nox4 seems to generate predominantly H₂O₂, a property related to specific structural features in the protein. This may be particularly important for interactions with NO signaling, because H₂O₂ can enhance eNOS activity, whereas O₂⁻ disrupts NO signaling. Indeed, in vivo evidence that Nox4 may enhance eNOS signaling has been reported. Nox4 is intracellularly located in cardiomyocytes and endothelial cells and was found at several sites, including the endoplasmic reticulum, nucleus, and mitochondria. More information is necessary on the precise intracellular locations of Nox4 and how they relate to specific functions. There are isolated reports of Nox1 expression in endothelial cells and cardiomyocytes but as yet no published evidence of a function in the heart in vivo. Nox5 differs from Nox1/2/4 in that it is Ca-regulated and only found in higher mammals. The absence of Nox5 in mice/rats probably accounts for the lack of information on any important cardiac functions for this isoform.

Nox activity might also be regulated by NADPH and flavin adenine dinucleotide availability, and it was suggested that increased NADPH levels fuel ROS production. However, NADPH is also critical for regeneration of reduced glutathione and maintenance of antioxidant defense. Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme for NADPH production through the pentose phosphate pathway, and glucose-6-phosphate dehydrogenase activity is critical for cardiac Ca homeostasis and contractile function during ischaemia-reperfusion. On the other hand, increased glucose-6-phosphate dehydrogenase-NADPH pathway activity leads to detrimental reductive stress associated with increased glutathione levels in a mouse model of mutant cB crystallin cardiomyopathy. Therefore, the relationship between NADPH and ROS levels may be complex and may depend on the subcellular compartment and Nox isoform.

**Mechanisms of Redox Regulation**

Protein sensors that respond to alterations in local redox state with a change in conformation, stability, molecular interactions, and activity serve as signal transducers. Such proteins include ion transporters, receptors, kinases, phosphatases, transcription factors, and structural proteins. Conversion of the redox signal into a different output typically involves specific posttranslational modifications such as amino acid oxidation, hydroxylation, or nitration.

Perhaps the most susceptible targets of signaling ROS are protein cysteine thiols. Although we do not discuss reactive nitrogen species–mediated signaling, it should be noted that NO-dependent modification of protein cysteine residues may modulate function in an analogous manner to cysteine oxidation. Redox-sensing protein thiol cysteines tend to have a low pKa, meaning that they are substantially deprotonated and exist as thiolate anions at physiological pH. This ionization markedly enhances reactivity with oxidants such as H₂O₂, providing a basis for selective redox signaling because only select thiols exist in this state. Further selectivity is achieved by colocalization of the protein sensor and ROS source. Protein thiolates react with H₂O₂ to form a sulfenic acid, which is stable in some proteins but usually rapidly reacts with thiol-reducing equivalents to yield a disulfide before being recycled back to the reduced state. Protein sulfenates transition to different types of disulfide, depending on the specific protein. Some proteins react with low-molecular-weight thiols, such as free cysteine or glutathione, to yield S-cysteinylated or S-glutathionylated states, respectively. In proteins where a sulfenate forms adjacent to a vicinal thiol, interprotein or intraprotein disulfides can form (see examples later). Protein sulfenates can further oxidize to sulfenic and sulfonic acids, events that are often considered damaging. For example, protein tyrosine phosphatase 1B (PTP1B) and GAPDH undergo oxidation to the sulfenic acid state, which is likely irreversible. PTP1B forms other reversible oxidation states, such as sulfide and sulfenyl amide, which may provide protection from sulfination/sulfonation and terminal inactivation of the phosphatase. However, at least in the case of the 2-Cys peroxiredoxin enzymes, sulfination is reversed by the ATP-dependent sulfiredoxin enzyme.

In the above scheme, H₂O₂ reacts with low pKa target thiols (eg, in PTP1B) to induce sulfenation as a first step to other oxidation states. However, despite PTP1B having a pKa of ~6.8 and being reported to be an oxidation target, it is questionable whether direct oxidation by H₂O₂ occurs in cells. This is because cells are highly abundant in very low pKa (~5.5) peroxiredoxins endowed with additional structural features that enable an immensely greater reaction rate with H₂O₂ than targets like PTP1B. It is, therefore, difficult to understand how PTP1B or other targets can compete for the oxidant (or indeed that H₂O₂ is freely diffusible as is often stated). One explanation is that PTP1B oxidation only occurs after inactivation of the 2-Cys peroxiredoxins. Peroxiredoxins, which have a Kᵣ for H₂O₂ of around 10 to 20 μmol/L, may serve as gatekeepers of oxidant signaling, with higher local H₂O₂ concentrations inducing sulfination of their peroxidatic cysteine and inhibiting peroxidase activity. Sulfination allows local peroxide levels to rise and oxidize proteins (eg, PTP1B) that would not normally be oxidized in the vicinity of active peroxiredoxin—the floodgate hypothesis. This floodgate is subsequently closed when sulfiredoxin reactivates peroxiredoxin, but in the interim there is a window of oxidant signaling during which less sensitive protein thiols are oxidized. Against this hypothesis is the fact that cells have additional antioxidant enzymes such as 1-Cys peroxiredoxins. An alternative or complementary mechanism involves the initial reaction of oxidants with highly reactive protein thiolates in peroxiredoxins or Trx to yield sulfenates or disulfides, which then react with the less reactive protein sensor to induce oxidation. Therefore, oxidant sensing would occur through one protein, whereas the resulting transduction (signal-changing) event involves another protein. In such a scenario, redox target proteins such as PTP1B might be passed the oxidant via a professional peroxide sensor protein.

**Redox-Sensitive Targets in the Heart**

The redox-sensitive modulation of phosphatase activity (eg, PTP1B, PTEN), small G proteins (eg, Ras), and various
transcription factors (eg, activator protein 1, nuclear factor-κB [NF-κB], hypoxia-inducible factor [HIF] 1) is well established in many different body systems and is not discussed in detail here. Instead, we consider examples of redox-regulated molecular targets that may be especially important for cardiomyocyte function, before discussing their physiopathological relevance.

**Ca/CamModulin-Dependent Kinase II**

Ca/calmodulin-dependent kinase II (CaMKII) phosphorylates several cardiac Ca-handling proteins to modulate excitation-contraction coupling, apoptosis, and gene transcription. CaMKII is a multimeric complex with multiple catalytic domains, therefore providing a basis for a graded Ca response. Ca-dependent activation leads to intersubunit autophosphorylation at Thr-287 within the autoinhibitory domain, preventing its reassociation with the catalytic domain and sustaining kinase activity even when Ca levels decline. CaMKII activity is enhanced by pro-oxidant conditions, and the Anderson laboratory showed that oxidation of 2 redox-active regulatory domain methionine residues (Met-281 and 282) sustained CaMKII activity even after removal of Ca/calmodulin (Figure 1). H$_2$O$_2$-induced CaMKII activation initially requires Ca, because this promotes access to the redox-active methionine residues. Nox2-derived ROS may be upstream of CaMKII oxidation in the heart. Using an antibody that recognizes dual-oxidized Met281/282 CaMKII, chronic AngII treatment or myocardial infarction (MI) was shown to promote oxidation and apoptosis in wild-type but not p47$^{phox}$-null (ie, Nox2-deficient) hearts. Transgenic mice expressing a CaMKII inhibitory peptide or mice expressing Met281/282Val CaMKII were resistant to these effects. Sulfoxidized methionine residues can be reversed to the reduced state by methionine sulfoxide reductase A. Methionine sulfoxide reductase A null mice have heightened sensitivity to AngII- and infarction-induced CaMKII oxidation and cardiomyocyte apoptosis, providing further evidence that oxidative kinase activation is detrimental.

**cAMP-Dependent Protein Kinase A**

cAMP-induced protein kinase A (PKA) activation after β-adrenergic stimulation is a major regulator of myocardial function, especially in response to acute and chronic stress. PKA activation involves a complex series of events, including protein phosphorylation, subcellular localization, and interaction with other proteins. PKA activation is modulated by Ca and ROS, which can alter kinase activity and substrate specificity. Understanding the molecular mechanisms of PKA activation is crucial for developing therapeutic strategies to counteract heart failure and other cardiac disorders.
ECC, whereas PKA activation in vessels supplying exercising muscle contributes to vasodilation. Recent work shows that PKA is redox regulated through the formation of 2 interprotein disulfides in the regulatory R1α subunits (Figure 1).26 R1α oxidation is associated with cAMP-independent enhancement of PKA catalytic activity and kinase translocation from cytosol to membrane fractions. The latter is related to increased kinase affinity for A-kinase anchoring proteins because the disulfides that form in R1α flank the A-kinase anchoring protein docking site.27 Oxidation may, therefore, localize the kinase to substrates that colocalize with A-kinase anchoring proteins, a process that promotes dissociation of the catalytic and regulatory subunits (ie, kinase activation), explaining cAMP-independent PKA substrate phosphorylation. PKAR1α disulfide formation is also induced by transnitrosylation, which stimulates cAMP-independent vasodilation.28 The in vivo physiological relevance of these mechanisms remains to be established. Other oxidative modifications can also alter PKA activity. Intramolecular disulfide formation between Cys-199 and Cys-343 in the catalytic subunit or glutathionylation at Cys-199 inhibits PKA activity.29 The R2 and C2 subunits of PKA can also disulfide dimerize, which inactivates the kinase.30

cGMP-Dependent Protein Kinase G
Protein kinase G (PKG) regulates vascular tone, as well as cardiomyocyte contraction and hypertrophy. PKG1 predominates in the cardiovascular system and is a homodimer held together by a leucine zipper interaction at the N-terminus; allosteric binding of cGMP activates the kinase. PKG1α can be oxidized to form an intermolecular disulfide between its 2 Cys42 residues within the dimerization domain (Figure 1), activating the enzyme independently of the NO-cGMP pathway.25 Disulfide activation enhances PKG affinity for substrates, leading to kinase translocation to subcellular compartments containing substrates. The trans-nitrosylating agent, nitrosothiols, also induces PKG1α disulfide formation and vasorelaxation, independent of guanylate cyclase activity.28 Recent work has demonstrated the physiological relevance of this oxidative activation mechanism in vivo (see later).32

Cardiac Ryanodine Receptor
The ryanodine receptor (RyR) 2 mediates Ca release from sarcoplasmic reticulum (SR) stores and is phosphorylated by PKA and CaMKII, kinases that are themselves redox regulated (see above). However, RyR2 is itself subject to cysteine thiol oxidation, perhaps not surprising as the tetrameric complex contains 364 cysteines with ≈84 being basally reduced.31 RyR is activated by NO-induced S-nitrosylation at multiple cysteines, independently of cGMP formation.34 In general, oxidizing conditions also increase RyR2 open probability but may lead to irreversible activation and Ca leak (Figure 2). There may be a close interrelationship between the effects of NO and ROS because RyR2 S-nitrosylation is thought to protect against detrimental oxidation.

SR Ca-ATPase
Similar to RyR, cardiac SR Ca-ATPase (SERCA) 2a is indirectly redox regulated because its regulatory protein phospholamban can be phosphorylated by PKA (at Ser16) or CaMKII (at Thr17). However, it may also be directly regulated by thiol oxidation (Figure 2). Low oxidation levels reversibly increase SERCA activity, whereas higher levels cause irreversible inactivation.33 Peroxynitrite-induced S-glutathionylation of Cys-674 directly activates SERCA2 in smooth muscle and contributes to vasorelaxation.34 In a rabbit model of atherosclerosis with depressed vasorelaxation, a reduction in SERCA S-glutathionylation was noted, which was associated with partial sulfination at several cysteines, including Cys674.35 It is likely that Cys674 sulfonation prevents SERCA activation by S-glutathionylation and may target it for degradation. Nitroxyl can also activate cardiac SERCA2a.37 Adult rat ventricular myocytes treated with the nitroxyl donor Angeli’s salt showed increased SERCA glutathionylation and activity.38

Other Proteins Involved in ECC and Contraction
The Na+-K+ ATPase (sodium pump) is phosphoregulated by PKA and protein kinase C (PKC) but is also redox modulated via direct oxidation of sensitive cysteines (Figure 2). AngII-induced inhibition of pump activity involved PKCe-dependent Nox activation and subsequent inhibitory S-glutathionylation of Cys46 in the β1 subunit, which also occurred in an in vivo sheep MI model.39 Other channels and pumps that may be redox regulated include L-type Ca channels, the plasmalemmal Ca-ATPase, the Na/Ca exchanger, K+ channels, and Na+ channels.40

Histone Deacetylases
Histone acetylation promotes and deacetylation inhibits gene expression, these processes being regulated by histone acetyltransferases and histone deacetylases (HDACs), respectively. A Trx1-sensitive oxidation of the class II HDAC, HDAC4, was implicated in α1-adrenoceptor–induced cardiomyocyte hypertrophy.41 Class II HDACs normally inhibit the transcription of prohypertrophic myocyte enhancer factor-2-dependent genes. It was shown that phenylephrine induced intramolecular disulfide formation between Cys-667 and Cys-669 in HDAC4 as well as between Cys-274 and Cys-276 in a partner protein, DnaJb5, resulted in phosphorylation-independent nuclear export of HDAC4 and the induction of hypertrophy (Figure 3). Recently, isoproterenol-induced export of HDAC5 from cardiomyocyte nuclei was also found to be mediated by oxidation independent of phosphorylation.42 These results highlight an important mechanism, whereby oxidation regulates HDAC localization and subsequent cardiomyocyte hypertrophy. This mechanism differs from the well-known redox regulation of transcription factor binding to DNA (Figure 3).

Sirtuins (SIRTs) are NAD+-dependent class III HDACs that deacetylate not only histones but many nonhistone proteins that may have important extranuclear effects. SIRT1 is activated by the natural cardioprotective polyphenol resveratrol and is also sensitive to oxidants. S-nitrosoglutathione was shown to S-glutathiolate SIRT1, and although this had no effect on basal deacetylase activity, it attenuated resveratrol-induced SIRT1 activation.43 This suggests that S-glutathiolation may modulate the effects of SIRT1. Other SIRT isoforms may also undergo redox regulation.
Glyceraldehyde 3-Phosphate Dehydrogenase

GAPDH is a multifunctional enzyme that is also redox regulated. Its classic role in glycolysis is redox regulated because its catalytic thiol is subject to reversible and irreversible forms of inhibitory oxidation, which have been observed during myocardial ischemia-reperfusion. GAPDH can be S-nitrosylated to trigger nuclear translocation and apoptosis. Another redox-regulated function of GAPDH is to control mRNA stabilization. GAPDH binds the 3′ untranslated region of endothelin-1 mRNA and enhances degradation by destabilizing it. However, when GAPDH is S-glutathionylated during oxidant stress at its catalytic Cys-152, this decreases mRNA binding to promote stabilization and enhance protein expression.

Thioredoxin

A key regulator of cellular redox signaling is the small thiol-containing Trx protein family, Trx1–3. Each contains 2 principal reactive cysteines in its active site, which can form an intramolecular disulfide bond. Oxidized disulfide Trx is catalyzed to the reduced form by Trx reductase in an NADPH- and flavin adenine dinucleotide-dependent reaction. Reduced Trx directly reduces disulfides in substrate proteins by disulfide exchange leading to Trx oxidation, typically via specific protein-protein interaction. Trx also reduces other thiol oxidation states, such as S-nitrosylation, back to P-SH. Trx itself is subject to S-nitrosylation at Cys-69, which is crucial to its antiapoptotic function. A proteomic analysis of oxidized cardiac proteins reduced by Trx showed many metabolic proteins, suggesting that Trx may play an important role in preventing metabolic dysfunction. The importance of Trx in the heart was investigated using transgenic mice overexpressing a cardiac-specific dominant-negative (Cys32Ser/Cys35Ser) Trx1 mutant. These mice had increased oxidative stress and left ventricular (LV) hypertrophy (LVH) compared with wild-type animals under basal conditions, which was further potentiated after chronic pressure overload, whereas overexpression of wild-type Trx1 had the opposite effect. Such studies have led to suggestions that recombinant Trx could be a therapy to combat detrimental oxidative stress and redox signaling in heart disease.

Physiological Roles of Cardiac Redox Signaling

Cardiomyocyte Differentiation and Proliferation

Cellular redox balance is an important regulator of differentiation and proliferation in many cell types, including cardiomyocytes developing from embryonic stem cells. The proportion of beating cardiomyocytes within embryoid bodies is increased by mechanical strain or electric stimulation via an increase in intracellular ROS levels, which may reflect increased cardiomyocyte differentiation and proliferation. The redox activation of phosphatidylinositol 3-kinase-Akt

Figure 2. Modulation of excitation-contraction coupling proteins. A, Oxidation of the Na’/K’ ATPase β1 subunit decreases pump activity by dissociating the α from the β subunit, which can be reversed by β3 adrenoceptor stimulation. B, Ryanodine receptor (RyR) 2 oxidation to the S-nitrosylated or glutathiolated state enhances channel open probability. C, Glutathiolation of Cys674 on SR Ca-ATPase (SERCA) augments its activity but is prevented by the hyperoxidation of this residue under pathological conditions. ROS indicates reactive oxygen species; ONOO, peroxynitrite; PLM, phospholamban; SNO, S-nitrosothiol.
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signaling downstream of β1-integrin, and leading to changes in β-catenin signaling, has been implicated in cardiomyogenesis.50 It was reported that Nox4 knockdown reduced the potential of mouse embryonic stem cells to form cardiomyocytes and involved the p38 mitogen-activated protein kinase (MAPK)–dependent nuclear translocation of myocyte enhancer factor-2C.51 Inhibition of Nox2 with a dominant-negative Rac mutant did not alter beating embryoid body formation, suggesting that Nox effects are isoform specific.52 In Xenopus embryos, mitochondrial ETC-derived ROS were suggested to be involved in heart formation through Ca-dependent activation of the transcription factor, nuclear factor of activated T-cells.53 Recently, it was reported that in the embryonic heart, closure of the MPTP leading to a reduction in ROS levels is important in the maturation and differentiation of mitochondria and cardiomyocytes, although the precise redox mechanism remains unclear.54 More detailed information on the molecular mechanisms through which redox signaling affects cardiomyocyte growth and differentiation is required.

**Excitation-Contraction Coupling**

The redox regulation of different proteins involved in ECC was discussed earlier. Although the pathological contribution of such mechanisms to heart disease is increasingly recognized, whether such redox signaling has an important physiological role has been less clear. However, recent studies suggest that low-level ROS production has reversible physiological effects on RyR2 activation. Sánchez et al55 provided indirect evidence that Nox2 contributes to RyR2 S-glutathionylation during tachycardia, which was suggested to augment Ca release and contractile force during exercise. Recently, Prosser et al56 reported an important physiological role for acute stretch-induced activation of Nox2 in the mechanotransduction of Ca release and therefore contractile force in cardiomyocytes. Using an innovative single cell–based apparatus for studying cardiomyocyte stretch, these authors found that Nox2 located in the sarcolemma and T tubules generated local ROS in a microtubule-dependent process and increased Ca spark release, thereby tuning RyR2 Ca signaling sensitivity. This may be an important physiological mechanism involved in stretch-induced augmentation of contractile activity.

**Regulation of Blood Flow**

The physiological regulation of vascular tone by NO is well recognized. Recent studies also suggest an important physiological role for ROS in the regulation of vessel tone and blood pressure. We investigated the in vivo relevance of oxidative PKG1 activation by generating Cys42Ser PKG1α knock-in mice that cannot form an intramolecular disulfide because of a single-atom substitution of the redox-sensitive sulfur by oxygen.32 Cys42Ser PKG1α redox-dead mice had deficient resistance vessel dilation to H2O2 or acetylcholine, along with an elevated basal blood pressure, which was related to impaired endothelial-derived hyperpolarizing factor activity. This study suggests that oxidative PKG1α activation is an important mechanism contributing to endothelial-derived hyperpolarizing factor–dependent vasodilation and blood pressure regulation.32 Oxidant-induced PKG1α disulfide activation is reported during human coronary arteriolar vasodilator responses to flow.57 H2O2 can also induce vasodilation through other endothelial-derived hyperpolarizing factor mechanisms such as the modulation of K+ channel–dependent hyperpolarization. Using a novel mouse model with endothelial-targeted overexpression of Nox4, we found that Nox4-derived H2O2 enhanced endothelium-dependent relaxation and reduced blood pressure in vivo,58 an effect not observed in endothelial-targeted Nox2 transgenic mice.59,60 Whether Nox4 is a physiological ROS source for oxidative PKG1α activation is, however, not yet clear. The importance of the above mechanisms for myocardial function also requires further study.
Pathological Roles of Cardiac Redox Signaling

Redox signaling is implicated in the development of many components of the failing heart phenotype, such as contractile dysfunction, Ca dysregulation, cardiomyocyte hypertrophy, cell death, arrhythmia, fibrosis, and chamber dilation. ROS are also involved in ischemia-reperfusion injury predisposing to heart failure, as well as signaling pathways that protect against such injury. Most of the current literature on pathological redox signaling and the ROS sources that are responsible comes from experimental animal studies. However, it is known that the levels and activity of ROS sources that are especially important in signaling, such as Nox2, are increased in the failing human heart, and correlations are reported between Nox2 levels and MAPK activation.

Abnormal Ca Regulation, Contractile Dysfunction, and Arrhythmia

Abnormalities of Ca homeostasis are a fundamental feature of the failing heart, contributing to contractile and energetic dysfunction, arrhythmia, transcriptional changes, and mitochondrial ROS production. Redox signaling impacts significantly on myocyte Ca homeostasis.

Diastolic Ca leak from the SR through dysfunctional RyRs decreases SR Ca content and the Ca transient and contributes to increased diastolic Ca and arrhythmia as well as reduced systolic force. Mechanisms implicated in these abnormalities include RyR2 hyperphosphorylation by PKA and CaMKII and direct redox modification of RyR2. Oxidation-enhanced activation of PKA/CaMKII could potentially contribute to such RyR2 dysfunction.

Evidence for direct oxidation-dependent RyR2 dysregulation is reported in experimental heart failure. nNOS colocalizes with RyR2, and it was reported that deficient nNOS-mediated RyR2 S-nitrosylation promotes thiol oxidation via XO-dependent ROS, leading to increased diastolic Ca and arrhythmia.

In the mdx model of Duchenne muscular dystrophy, which is deficient in nNOS activity, the Nox2-mediated mechanotransduction mechanism for RyR2 Ca release discussed earlier became hyperactive and led to aberrant Ca release and arrhythmogenic waves; the same was found under chronic AngII stimulation. The precise molecular mechanism that is involved downstream of Nox2 (eg, kinase or RyR2 oxidation) remains to be established.

Increased SERCA2a oxidation may contribute to contractile dysfunction. In Gqγ, overexpressing transgenic mice that develop heart failure, sulfonation of SERCA2a at Cys674 and nitration at Tyr294/295 were noted, which correlated with decreased maximal Ca-stimulated SERCA activity and marked abnormalities of Ca transients and contractile function. These abnormalities were ameliorated by crossing Gqγ transgenic mice with mice overexpressing catalase in cardiomyocytes. Increased oxidation is also implicated in contractile dysfunction associated with obesity, hyperglycemia, and ischemia-reperfusion. Oxidative CaMKIIβ activation was recently reported to enhance the Na current and thereby lead to cellular Na and Ca overload.

Contractile dysfunction may also result from oxidative modification of contractile proteins, although the ROS sources that are involved and the functional impact of specific modifications require more investigation. Disulfide bridge formation in the cardiac-specific N2-B segment of titin increases muscle passive stiffness, an effect reversible by Trx.

Increased atrial oxidative stress is involved in the pathophysiology of experimental and clinical atrial fibrillation (AF), and Nox2 is implicated in this process. Nox2-derived ROS production was increased in right atrial appendages of patients undergoing cardiac bypass surgery who developed postoperative AF and was independently associated with increased AF risk. These authors showed in a goat rapid-pacing model and in patients who developed postoperative AF that Nox2 is important mainly for AF initiation, whereas uncoupled NOS and mitochondrial ROS may be more important in longstanding AF. AF also develops in mice with cardiac-specific overexpression of a constitutively active Rac1, which activates Nox2. The molecular mechanisms downstream of Nox2 that promotes AF remain unclear. Interestingly, it was recently reported that Nox2-dependent CaMKII oxidation promotes sinus node dysfunction by causing apoptosis of sinoatrial cells in a mouse model of AngII-induced dysrhythmia.

It should be noted that other redox-sensitive mechanisms, such as effects on the sodium pump, Na/Ca exchanger, and K+ and Na+ channels, may also contribute to arrhythmia (as discussed in the section on Other Proteins Involved in ECC and Contraction earlier).

Cell Death

Cardiomyocyte apoptosis and necrosis contribute to acute ischemia-reperfusion injury, as well as the development of chronic heart failure. Both the extrinsic apoptotic pathway (activated by death receptor superfamily ligands such as tumor necrosis factor-α) and the intrinsic pathway regulated by Bel-2 family proteins and leading to mitochondrial MPT opening or outer membrane permeabilization may be subject to redox regulation. It should be noted that this redox regulation is distinct from the subsequent deleterious effects of mitochondrial ROS release after induction of the MPT discussed earlier.

ROS can activate the intrinsic pathway through multiple mechanisms, including direct or Ca-dependent induction of MPT opening or promotion of Bax/Bad translocation to mitochondria because of DNA damage–induced activation of the p53 transcription factor. p53 induces the E3 ubiquitin ligase, MDM2, which promotes degradation of an antiapoptotic regulator, apoptosis repressor with caspase recruitment domain, that interacts with Bax to inhibit apoptosis involving the mitochondrial pathway. Apoptosis repressor with caspase-deficient mice develop more rapid pressure overload–induced heart failure and larger infarct size after ischemia-reperfusion. Excessive G-protein–coupled receptor signaling induces apoptosis through several mechanisms. In adult cardiomyocytes, β-adrenoceptor–induced apoptosis leads to ROS-dependent activation of c-Jun N-terminal kinase and activation of the mitochondrial death pathway. The MAPKKK, apoptosis signaling kinase-1 (ASK-1), is strongly ROS activated downstream of G-protein–coupled receptors through dissociation of Trx-1 and, in turn, activates p38MAPK/c-Jun N-terminal kinase and the mitochondrial pathway. ASK-1 overexpression induces cardiomyocyte apoptosis, whereas ASK-1−/− mice exhibit attenuated ventricular remodeling after pressure overload through a decrease in...
Apoptosis. AngII-stimulated Nox2-derived ROS may be involved in activating the ASK-1/p38MAPK proapoptotic pathway. Gqα-mediated cardiomyocyte apoptosis was shown to involve PKC-dependent transcriptional upregulation of the Bcl-2 family member Nix, which activates the mitochondrial death pathway, although whether this is redox sensitive is unclear. Recently, Nox2-dependent oxidative CaMKII activation was established as an important proapoptotic mechanism after MI or chronic AngII infusion, as discussed earlier. CaMKII activation was found to be upstream of p38MAPK in the AngII-induced death pathway in cardiomyocytes. It should be noted that CaMKII can also activate ASK-1.

Responses to Myocardial Ischemia-Reperfusion

Mitochondrial ROS production associated with MPT was discussed earlier. Additional sources of ROS production during ischemia-reperfusion include Noxs, XO, and inflammatory cells. Cardiomyocyte and inflammatory cell Nox2 levels are increased early after acute MI in both humans and animal models. The early phase after acute MI involves clearance of dead cells and repair of the infarct, with significant inflammatory infiltration, tissue remodeling, and fibrosis. It was shown that S-nitrosylation of the RyR2 related to increased mitochondrial ROS production and NO production early after MI in rats resulted in diastolic SR Ca leak and contributed to ventricular arrhythmia, increased infarct size, and greater delayed remodeling. These effects were reduced by prevention of calstabin 2 depletion from the RyR2 complex using the Ca channel stabilizer S107. Acute infarct size is unaltered in p47phox−/− or Nox2-null mice, but it was shown that aldosterone-induced Nox2-dependent CaMKII oxidation mediates early cardiac rupture after MI, by promoting an increase in matrix metalloproteinase (MMP)-9 activity. This severe complication was prevented in p47phox−/− mice after CaMKII inhibition.

After the initial phase of infarct healing, there is a much slower phase of infarct expansion, which is accompanied by progressive hypertrophy and fibrosis in the remote myocardium, together with LV dilation and functional deterioration. Both p47phox−/− and Nox2-null mice have reduced adverse post-MI remodeling during a 4-week time period, indicating an involvement of Nox2 in this process. Nox-derived ROS generation in the brain paraventricular nucleus may also contribute to post-MI remodeling by facilitating sympathoexcitation. Cardiac-specific mineralocorticoid receptor deletion decreases post-MI remodeling, at least, in part, by reducing Nox2 activation, indicating that at least some of the Nox effect is mediated at the level of the heart. Multiple ROS sources may contribute to post-MI remodeling because XO inhibition with allopurinol also attenuates maladaptive post-MI LV remodeling.

Cardioprotection

Cardioprotection induced by ischemic preconditioning or other stimuli may involve ROS signaling. Several studies suggest that ROS signaling is involved in the initial preconditioning period, which eventually leads to MPT inhibition during prolonged ischemia. ROS originating from the mitochondria may activate PKC that acts as an important upstream preconditioning signal or may cause transient MPTP openings. Nox2-derived ROS can also trigger preconditioning through PKC activation. In late ischemic preconditioning, there is evidence for the involvement of HIF targets such as heme oxygenase-1 and inducible NOS.

Redox signaling during ischemia-reperfusion contributes to protective or adaptive responses too. One of the most important pathways is the facilitation of protective HIF signaling through redox-sensitive posttranslational, translational, and transcriptional mechanisms. Both mitochondrial and Nox-derived ROS can enhance HIF activation. HIF drives the transcriptional activation of genes involved in angiogenesis, survival pathways, antioxidant defense, and metabolism and induces effects that are beneficial both in the short and medium term. Evidence of increased HIF and HIF-dependent proteins after acute MI is found in both human myocardium and animal models, whereas mouse models of enhanced HIF signaling show a reduction in infarct size and contractile dysfunction as well as improved longer-term remodeling.

Cardiac Hypertrophy and Failure

Multiple overlapping signaling pathways are involved in the development of cardiomyocyte hypertrophy, and ROS modulate many of these pathways (Figure 4). We focus here on evidence for the modulation of hypertrophic signaling pathways by endogenously generated ROS and, where known, the molecular mechanisms and ROS sources that are involved.

G-protein–coupled receptor agonist–induced hypertrophy (eg, with AngII, endothelin-1, α-adrenoceptor agonists) involves redox-modulated extracellular-signal-regulated kinase-1/2 and NF-κB activation, with Nox2 being the likely ROS source. AngII-induced increases in ROS and Ca may act synergistically to induce hypertrophy. A key mechanism that causes activation of the Ras-Raf-MEK1/2-extracellular-signal-regulated kinase-1/2 signaling pathway during α-adrenoceptor–mediated hypertrophy is the oxidation of specific cysteine thiols in Ras protein, which is activated downstream of ASK-1, which is itself activated in a Ca- and Rac-dependent process that probably involves Nox2. Direct evidence for the involvement of Nox2 in AngII-induced hypertrophy comes from cultured cardiomyocyte studies and experiments in Nox2 knockout mice, where LVH induced by supressor AngII infusion was abrogated. Similarly, AngII-induced in vivo LVH was inhibited in mice with cardiomyocyte-specific Rac1 deletion and deficient Nox2 activation or in ASK-1−/− mice. The involvement of class II HDAC oxidation in phenylephrine-induced hypertrophy was discussed earlier, although the relevant ROS source remains to be established.

Pressure-overload hypertrophy involves multiple stimuli, including mechanical strain and the activation of G-protein–coupled receptors, gp130, receptor tyrosine kinase, integrin, natriuretic peptide, and other receptors. Mechanical strain induces Rac1/Nox2-dependent extracellular-signal-regulated kinase-1/2 and p38MAPK activation and hypertrophy in cultured cardiomyocytes, and ventricular Nox2 levels and activation increase with chronic pressure overload. Enhanced Nox2 activation contributes to LVH and contractile dysfunction/dilation based on studies in cardiomyocyte-specific
Rac1−/− mice and Nox2-null mice, respectively. Similarly, angiotensin-converting enzyme 2–deficient mice that have enhanced susceptibility to pressure overload–induced LVH and failure are significantly improved on a p47phox−/− background. However, Nox2 activation is not obligatory for the development of pressure-overload LVH because Nox2-null animals develop a similar degree of hypertrophy to wild-type littermates after aortic constriction or in models of renin-angiotensin-aldosterone system activation associated with hypertension, indicating that there are other pathways to hypertrophy. Components of several other signaling pathways implicated in pressure-overload LVH are potentially subject to redox regulation, eg, the activation of phosphatidylinositol 3-kinase/Akt, protein kinase D, PKA, JAK-STAT signaling, and calcineurin/nuclear factor of activated T-cells, but whether this is indeed important and, if so, what ROS sources are responsible remain to be established.

Some redox-activated pathways have a protective function during chronic pressure overload. The level of myocardial capillarization is an important determinant of functional cardiac compensation during pressure overload, and an insufficient capillary density relative to cardiomyocyte mass promotes pathological LV remodeling. It was shown that an HIF1-dependent increase in paracrine secretion of prosurvival factors (eg, vascular endothelial growth factor) from cardiomyocytes was a major driver of this process. We recently found that an increase in myocardial Nox4 levels during chronic pressure overload enhances HIF1-vascular endothelial growth factor signaling and preservation of capillary density, with beneficial effects on LV remodeling and dysfunction. This study demonstrated that mice with cardiac Nox4 overexpression exhibited protection against pressure overload–induced LVH, dilation, and contractile depression, whereas Nox4-null mice had a worse phenotype than wild-type littermates, effects accounted for at least, in part, by an increase in paracrine angiogenic activity. Nox4 may increase HIF1 levels and angiogenesis by inhibiting prolyl hydroxylase enzymes that normally cause O2-dependent degradation of HIF, increasing HIF1 transcription and enhancing eNOS activity.

The development of heart failure after prolonged, severe pressure overload may involve an increase in mitochondrial ROS, protein oxidation, and mitochondrial DNA damage. This was reported to be an important heart failure mechanism in mice treated with chronic pressor AngII infusion for 4 weeks or transgenic mice with cardiomyocyte-specific G protein-coupled receptor overexpression, whereas mice with mitochondrial-targeted catalase overexpression showed significant protection. In vivo administration of a mitochondrial-targeted antioxidant peptide ameliorated chronic pressure overload–induced failure. Mitochondrial ROS sources in addition to the ETC could contribute to these effects. In mice with pressure overload–induced heart failure, pharmacological inhibition of mitochondrial MAO-A reduced ROS levels and apoptosis and improved LV dilation and contractile dysfunction. Similar beneficial effects were found in mice expressing a dominant-negative MAO-A, implicating MAO-A as an important detrimental ROS source in this setting. Recently, it was reported that mice with cardiomyocyte-targeted overexpression of MAO-A exhibited protection against pressure overload–induced LVH, dilation, and contractile depression, whereas mice with mitochondrial-targeted catalase overexpression showed significant protection. In vivo administration of a mitochondrial-targeted antioxidant peptide ameliorated chronic pressure overload–induced failure. Mitochondrial ROS sources in addition to the ETC could contribute to these effects. In mice with pressure overload–induced heart failure, pharmacological inhibition of mitochondrial MAO-A reduced ROS levels and apoptosis and improved LV dilation and contractile dysfunction. Similar beneficial effects were found in mice expressing a dominant-negative MAO-A, implicating MAO-A as an important detrimental ROS source in this setting. Recently, it was reported that mice with cardiomyocyte-targeted overexpression of MAO-A exhibited protection against pressure overload–induced LVH, dilation, and contractile depression, whereas mice with mitochondrial-targeted catalase overexpression showed significant protection. In vivo administration of a mitochondrial-targeted antioxidant peptide ameliorated chronic pressure overload–induced failure.
that mitochondrial-located Nox4 may contribute to increased ROS during chronic severe pressure overload and promote the development of heart failure through a similar mechanism. These authors found that cardiomyocyte-targeted increase in Nox4 levels promoted pressure overload–induced failure (but with no effect on hypertrophy), whereas Nox4-deficient mice developed less severe heart failure after transverse aortic constriction. These results suggesting detrimental effects of Nox4-derived ROS during pressure overload contrast with our own work described in the previous paragraph, where we found beneficial proangiogenic effects of Nox4. The reasons for these divergent data remain yet to be fully elucidated, but possible explanations could be methodological differences between the 2 studies, such as site and severity of aortic constriction and the mouse background strain, or that the effects of Nox4 might depend on the level to which it is upregulated.

**Interstitial Fibrosis and Extracellular Matrix Remodeling**

The development of cardiac fibrosis involves not only fibroblasts but also interactions with cardiomyocytes, endothelial cells, inflammatory cells, and other circulating cells. Fibrosis triggered by increased mechanical load or local tissue injury typically involves increased renin-angiotensin-aldosterone system and inflammatory pathway activation, with TGF-β signaling and changes in redox balance thought to play a critical role. Redox regulation in multiple cell types may impact on fibrosis. TGF-β promotes transformation of interstitial fibroblasts into myofibroblasts. ROS activate latent TGF-β and also promote the transcription of profibrotic factors such as connective tissue growth factor. These factors are produced not only within fibroblasts but also by cardiomyocytes under stress. AngII-stimulated myofibroblast transformation involves Nox2-dependent redox regulation of c-Jun N-terminal kinase and calcineurin/nuclear factor of activated T-cells. TGF-β–induced mesangial cell proliferation also involves ROS-dependent calcineurin activation, raising the possibility that a similar mechanism may operate in cardiac fibroblasts. AngII-induced fibroblast expression of endothelin-1, which is profibrotic, involves redox activation of AP-1. Nox4 is reported to participate in TGF-β–induced cardiac myofibroblast differentiation through enhanced Smad2/3 signaling, consistent with effects in other tissues.

Inflammatory cells may be strongly profibrotic through the modulation of local cytokine signaling. ROS upregulate chemokines such as monocye chemoattractant protein-1/CCL2. Nox2 is involved in inflammatory cell activation and may modulate inflammatory cell recruitment and consequent fibrosis. Redox signaling in microvascular endothelial cells promotes the expression of surface adhesion molecules that facilitate recruitment of inflammatory cells and possibly circulating fibroblast progenitors. Endothelial-mesenchymal transition, a process whereby endothelial cells transform into fibroblasts, may be an important contributor to cardiac fibrosis during tissue repair, and TGF-β signaling and endothelial endothelin-1 are reported to promote endothelial-mesenchymal transition. However, it is not known whether this process is redox regulated.

Direct evidence for an involvement of Nox proteins in cardiac fibrosis comes from studies in gene-modified mice. Nox2-null mice showed significantly reduced interstitial fibrosis compared with wild-type littermates after subpressor AngII infusion, pressor AngII infusion, aldosterone infusion, chronic renin-angiotensin-aldosterone system activation, aortic banding, or MI. In the in vivo aldosterone model, Nox2 enhanced NF-κB activation, connective tissue growth factor levels, and MMP-2 activation. A similar antifibrotic effect was found in p47 phosph−/− mice and Nox2-deficient cardiomyocyte-specific Rac1−/− mice. These data provide strong evidence for a pivotal involvement of Nox2 in cardiac fibrosis. Nox4 is also reported to be profibrotic in many cellular models but may not be an essential component of profibrotic signaling in the heart in vivo because global Nox4 knockout mice developed more (not less) fibrosis than wild-type littermates after chronic pressure overload.

MMP activation is commonly associated with fibrotic remodeling in the failing heart but also contributes to ventricular dilation after chronic pressure overload or MI. MMP activation may release profibrotic growth factors from the extracellular matrix or have antifibrotic effects because of the release of matricellular proteins such as thrombospondin-1 so that the effects depend on context. Both the expression and activation of MMPs are redox sensitive and occur in several cardiac cell types. Nox proteins induce MMP expression and activation in many noncardiac cell types, and aldosterone increased MMP-2/MMP-9 levels and activity in cardiomyocytes via a Nox-dependent pathway. β-adrenergic stimulation induces the H₂O₂/c-Jun N-terminal kinase-dependant expression of the extracellular MMP inducer in cardiomyocytes, which in turn increases MMP-2 activity.

**Clinical and Translational Implications**

Nonspecific antioxidant approaches (such as vitamin supplementation) have generally proven unsuccessful in clinical trials that aimed to reduce cardiovascular morbidity and mortality. Based on the data discussed in this article, more targeted strategies against specific ROS sources and downstream pathways modulated by such ROS could be a better approach. Indeed, it has been suggested that part of the beneficial effects of agents such as angiotensin-converting enzyme inhibitors and statins could be attributable to antioxidant actions resulting from inhibition of Nox enzymes. The development of specific Nox inhibitors is currently an area of intense activity. A combined Nox1 and Nox4 inhibitor (GKT137831, developed by Genkyotex) was found to have beneficial effects in a model of liver fibrosis and is currently in phase 1 clinical trials for diabetic nephropathy. To target detrimental signaling in cardiac hypertrophy and failure, it would seem important to inhibit Nox2, but a similar specific Nox2 inhibitor is not yet available to our knowledge. An alternative class of Nox inhibitors has been developed by Vasopharm, although the exact mechanism of action and isoform specificity of these agents are debated. VAS2870, which is thought to inhibit Nox1, Nox2, and Nox4, was reported to prevent endothelial dysfunction in the aorta of aged spontaneously hypertensive rats.
and to protect against stroke-induced damage. This agent has not so far been tested in cardiac disease, and one important question is whether beneficial effects of inhibiting Nox2 might be counteracted by Nox4 inhibition. ROS production by uncoupled NOS could potentially be targeted by the oral administration of BH4 to recouple NOS, and this approach was shown to reverse cardiac hypertrophy and fibrosis in mice that had undergone transverse aortic constriction. However, it is not clear that this can readily translate to humans because a recent study in patients undergoing cardiac surgery found that although biopterin levels could be increased by oral BH4 supplementation, the BH4 was oxidized to BH2, which is ineffective in recoupling NOS. An alternative strategy may be to directly inhibit NOS. An allosteric NOS inhibitor (VAS203, Vasopharm), which interacts with the BH4-binding site, was reported to be beneficial in a model of traumatic brain injury but is yet to be tested in cardiac disease. MAO inhibition was found to be beneficial in a mouse model of pressure overload–induced heart failure as discussed earlier and could be clinically tested because MAO inhibitors are available.

A different strategy is to specifically target mitochondrial oxidants, an approach that seems promising in various animal models. For example, a mitochondria-targeted antioxidant peptide SS-31 was found to reduce heart failure in mice with AngII-induced chronic pressure overload, as discussed earlier. A different agent, MitoQ (Antipodean Pharmaceuticals), is currently being evaluated in phase 2 clinical trials for its ability to protect against liver injury, following promising results in animal studies (http://www.antipodeanpharma.com/). This agent was reported to have beneficial effects in the settings of cardiac ischemia-reperfusion injury and hypertension-induced cardiac hypertrophy, and it will be of interest to see whether such results translate to human cardiovascular disease.

In addition to preventing oxidant formation, one may try to boost endogenous antioxidant and cytoprotective capacity through more specific approaches than antioxidant vitamins. Gene therapy with Trx-1 reportedly reduces adverse cardiac remodeling post-MI in diabetic rats, although how easily such an approach would translate to humans remains to be seen. A promising small molecule strategy is to target the transcription factor nuclear factor (erythroid-derived 2)-like 2, which regulates multiple antioxidant and phase 2 defense genes. A recent randomized clinical trial of a small molecule nuclear transcription factor nuclear factor (erythroid-derived 2)-like 2 activator, Bardoxolone methyl, showed very promising results in preserving glomerular filtration rate in patients with chronic kidney disease, and such an approach merits testing in cardiac disease. Finally, there is scope to develop strategies to selectively alter the activity of specific downstream redox-sensitive targets discussed in this article (eg, CaMKII, PKA, PKE), depending on the context.

Conclusions

Redox signaling is being found to be important in an increasing number of physiological cardiac processes and plays a role in most pathological processes. Although nonspecific effects of increased ROS levels are important in certain settings (eg, acute ischemia-reperfusion, advanced heart failure), it is increasingly evident that quite subtle ROS source–specific regulation is also involved in the integrated cellular signaling that underlies cardiac responses to stress in many pathologies. Such redox regulation mediates not only detrimental processes but also important protective or adaptive pathways. The Nox proteins—the only known dedicated sources of signaling ROS—seem to be especially important and act through a wide variety of specific molecular mechanisms that rely on spatially restricted ROS generation and defined posttranslational oxidative modifications of target proteins. Antioxidant systems, such as Trx and peroxiredoxin, are of major importance in restricting and perhaps mediating spatially confined redox signaling. Information regarding the in vivo relevance of specific ROS sources and specific redox-regulated pathways is rapidly being built up, aided by an increasing number of gene-modified models that are available for such studies. We still need to better understand how compartment-specific redox regulation and signaling is achieved, how different ROS sources interact, and how redox signaling is integrated with other signaling pathways. Technological advances in ROS detection and quantification, molecular imaging, and systems biology, among others, will be important in this regard. A more detailed understanding of the roles of redox signaling in cardiovascular disease may facilitate the development of targeted therapeutic strategies as opposed to the discredited general antioxidant approaches of the past.

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Disclosures

None.

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