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Comprehensive Invited Review

NADPH Oxidases in Cardiovascular Health and Disease

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ABSTRACT

Increased oxidative stress plays an important role in the pathophysiology of cardiovascular diseases such as hypertension, atherosclerosis, diabetes, cardiac hypertrophy, heart failure, and ischemia–reperfusion. Although several sources of reactive oxygen species (ROS) may be involved, a family of NADPH oxidases appears to be especially important for redox signaling and may be amenable to specific therapeutic targeting. These include the prototypic Nox2 isoform-based NADPH oxidase, which was first characterized in neutrophils, as well as other NADPH oxidases such as Nox1 and Nox4. These Nox isoforms are expressed in a cell-

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and tissue-specific fashion, are subject to independent activation and regulation, and may subserve distinct functions. This article reviews the potential roles of NADPH oxidases in both cardiovascular physiological processes (such as the regulation of vascular tone and oxygen sensing) and pathophysiological processes such as endothelial dysfunction, inflammation, hypertrophy, apoptosis, migration, angiogenesis, and vascular and cardiac remodeling. The complexity of regulation of NADPH oxidases in these conditions may provide the possibility of targeted therapeutic manipulation in a cell-, tissue- and/or pathway-specific manner at appropriate points in the disease process. *Antioxid. Redox Signal.* 8, 691–728.

I. INTRODUCTION

All aerobic organisms generate reactive oxygen species (ROS), oxygen-based molecules that are characterized by their high chemical reactivity. ROS include free radicals (species with one or more unpaired electrons) such as superoxide ($O_2^{\bullet-}$) and hydroxyl radicals ($OH^{\bullet}$), and nonradical species such as hydrogen peroxide (H_2O_2). In health, ROS generation is counteracted by the activity of enzymatic and nonenzymatic antioxidant systems that scavenge or reduce ROS levels, thereby maintaining an appropriate redox balance in cells and tissues. Perturbation of this normal balance due to increased ROS production and/or reduced antioxidant reserve leads to a state of oxidative stress, namely an enhanced susceptibility of biological molecules and membranes to reaction with ROS.

Increased oxidative stress is recognized to play an important role in the pathophysiology of numerous diseases, which in the cardiovascular system include hypertension, atherosclerosis, diabetes, cardiac hypertrophy, heart failure, and ischemia–reperfusion. A huge number of experimental studies have defined mechanistic pathways through which oxidative stress impacts on these diseases, and have shown that manipulation of oxidative stress may have therapeutic potential. Plentiful evidence also implicates a role for oxidative stress in human cardiovascular disease, and oxidative stress has been shown to be an independent risk marker for future cardiovascular disease (172, 299). Nevertheless, clinical trials of antioxidant vitamins in patients at risk of cardiovascular disease have not shown benefit in reducing cardiovascular events or mortality (163). It is increasingly evident, however, that the situation is much more complex than might initially be imagined. ROS have a wide range of potential actions that are influenced by the specific moiety generated, its localization, amount, and proximity to other radicals, enzymes, and signaling molecules. A key determinant of the biological consequences of cellular ROS generation in specific biological settings is likely to be the enzymatic source of ROS generation, particularly with regard to redox signaling (*see later*). Potential sources of ROS in the cardiovascular system include mitochondria, NADPH oxidases, uncoupled nitric oxide (NO) synthases, xanthine oxidase, cytochrome P450-based enzymes, and infiltrating inflammatory cells. This article focuses on the roles of NADPH oxidases, a family of enzymes first described in phagocytes but now known to be expressed much more widely. NADPH oxidases appear to be especially important for redox signaling and may be amenable to specific therapeutic targeting as opposed to the nonspecific 'antioxidant' approaches utilizing vitamin E and/or vitamin C, which have been disappointing in clinical trials to date. Several recent studies have provided confirmatory evidence of important pathophysiological roles for NADPH oxidases in human cardiovascular disease (128, 137, 224, 316).

II. REACTIVE OXYGEN SPECIES IN CARDIOVASCULAR BIOLOGY

Traditionally, oxidative stress was considered to be universally deleterious as a result of free radical-induced oxidation and damage of macromolecules, membranes, and DNA. For example, the restoration of O_2 supply during myocardial reperfusion after prolonged ischemia is accompanied by a burst of free radical production that has damaging consequences such as the acceleration of cell death through apoptosis and necrosis. More recently, however, it has been appreciated that ROS can exert more subtle modulatory effects. First, tightly regulated ROS production can modulate the activity of diverse intracellular molecules and signalling pathways and thereby induce highly specific acute and chronic changes in cell phenotype—a mechanism commonly termed "redox signaling." Second, the interaction of $O_2^{\bullet-}$ with the signaling molecule nitric oxide (NO) leads both to a reduction in NO bioavailability and the generation of another reactive species, peroxynitrite ($ONOO^{\bullet-}$), which itself has biological activity. The inactivation of NO by ROS is a key mechanism underlying the development of endothelial dysfunction, which in turn is an important contributor to cardiovascular disease pathophysiology. Therefore, ROS can exhibit a wide spectrum of biological activity with at one extreme being signaling molecules that may subserve useful physiological functions and at the other being harmful species responsible for oxidative damage.

As an example, consider the $O_2^{\bullet-}$ radical (generated by a one-electron reduction of molecular O_2) which is quite unstable and has a half-life of only a few seconds in aqueous solution. It is poorly cell membrane-permeable and therefore usually restricted to the cell compartment in which it is produced. When $O_2^{\bullet-}$ is produced in relatively low amounts (picomolar range) it is rapidly dismutated to H_2O_2, especially in the presence of superoxide dismutase (SOD) enzymes. H_2O_2 is considerably more stable, diffusible, and cell membrane-permeable than $O_2^{\bullet-}$ and may therefore be responsible for redox signaling effects attributed to $O_2^{\bullet-}$ in many settings. The reaction of $O_2^{\bullet-}$ with NO (rate constant ~7×10^9 $mol^{-1}.L.s^{-1}$) occurs at a significantly faster rate than with SOD (125), so that in the presence of high nanomolar NO there may be significant generation of $ONOO^{\bullet-}$. When $O_2^{\bullet-}$ levels are higher still, it may react with iron–sulfur centers in proteins and release iron which reacts with H_2O_2 to produce highly reactive $\cdot OH$ radicals.

III. REDOX SIGNALING

Transduction of the chemical ROS signal into a biologically relevant event is mediated by posttranslational covalent modification of specific amino acid residues on proteins, resulting in a change in protein function. This can be an acute alteration (over seconds to minutes) in function of the target molecule (an ion channel or contractile protein), or may result in chronic changes in cell phenotype (over hours and days) when the target protein is a signaling molecule such as a protein kinase or a redox-sensitive transcription factor. For the ROS-mediated posttranslational modification to succeed in biologically relevant signaling, the modification should proceed at a physiologically significant rate, be chemically reversible under physiological conditions, and/or be enzymatically catalyzed. A classical example is the progressive oxidation of thiol residues by, for example H_2O_2, to give rise to reaction products such as sulfenic acid, sulfinic acid, and sulfonic acid derivatives (265). Alternatively, oxidation may promote the formation of cysteine disulfide bonds within a protein or mixed disulfide bonds between a cysteine-containing protein and a low molecular weight thiol such as glutathione (265). Interestingly, different modifications to cysteine residues within a protein, in terms of either the source of ROS or the oxidation form, can deliver discrete and diverse regulatory outcomes. Once formed, intramolecular and mixed disulfide linkages can be removed by thiol disulfide exchange reactions and the activities of protein disulfide reductase, glutaredoxin, and thioredoxin reductase. A large number of proteins are known to be regulated by S-thiolation, including structural proteins (43, 83), metabolic enzymes (252), ion translocators (82), DNA isomerases (345), and signaling proteins (182). The specificity that is essential for pathophysiologically relevant redox signaling is effected through several mechanisms, including ligand-dependent stimulation of ROS production, the colocalization of ROS with specific substrates or downstream targets, and stimulus-coupled regulation of thiolyation within the confines of a signaling molecule [for a detailed discussion of this topic, see recent reviews (102, 106, 265)]. Within the above general scheme, NADPH oxidases have several attributes that position them as prime candidates to be enzymes specifically designed to facilitate cellular redox signaling.

IV. PHAGOCYTIC AND NONPHAGOCYTIC NADPH OXIDASES

The NADPH oxidase was first described in professional phagocytes of the innate immune system (e.g., neutrophils and macrophages) where it is responsible for generating a large burst of $O_2^{\bullet -}$ (the "oxidative burst"), using NADPH as an electron donor, during the process of phagocytosis (185). This high level ROS generation is largely generated within phagocytic vacuoles (i.e., within the "extracellular" compartment) and is pivotally involved in the killing of ingested pathogens, although not necessarily directly. The significance of phagocytic NADPH oxidase in host defence is clearly demonstrated in a rare disorder known as chronic granulomatous disease (CGD), in which genetic defects in essential oxidase components result in an inactive enzyme and a predisposition to recurrent life-threatening infections in affected children (76, 327). Considerable information on the structural requirements for a fully functional phagocyte NADPH oxidase derives from studies in CGD patients. The phagocyte NADPH oxidase comprises a membrane-associated low-potential heterodimeric flavocytochrome b_{558} composed of one 22 kDa $p22^{phox}$ (for phagocyte oxidase) subunit and one $gp91^{phox}$ subunit which has a core molecular weight of ~65 kDa but migrates on SDS-PAGE with an apparent mass of ~91 kDa due to its heavy glycosylation state. Interaction between $p22^{phox}$ and $gp91^{phox}$ appears to be necessary for stability of the flavocytochrome complex. Although the flavocytochrome contains all the catalytic machinery required for electron transfer from NADPH to molecular O_2, activation of the phagocyte oxidase requires the translocation of several cytosolic regulatory subunits ($p47^{phox}$, $p40^{phox}$, $p67^{phox}$, and the small G protein Rac1 or Rac2) to the membrane and their association with cytochrome b_{558} (Fig. 1).

Over the last 10–15 years, it became evident that a rather similar, albeit lower-level, NADPH or NADH-dependent ROS-generating activity exists in numerous nonphagocytic cell types. In the cardiovascular system, these include vascular smooth muscle cells (VSMC) (118, 336), endothelial cells (EC) (24, 25, 111, 166), adventitial and cardiac fibroblasts (44, 263), and cardiomyocytes (29, 203, 355, 362). In general, nonphagocytic cells appear to generate low-level ROS continuously even in the absence of extrinsic stimulation (unlike neutrophils) but could increase their ROS production in response to specific stimuli. The use of relatively specific inhibitors (e.g., diphenylene iodonium [DPI] and apocynin) suggested that the source of this activity might be an NADPH oxidase enzyme, whereas other studies found that the $p22^{phox}$ oxidase subunit, but not $gp91^{phox}$, was expressed in almost all cell types. These observations prompted a search for homologues of $gp91^{phox}$, and resulted in the identification of a new family of homologous $gp91^{phox}$ isoforms, each encoded by distinct genes. These are now termed Noxs (for NADPH oxidase), with $gp91^{phox}$ known as Nox2 in the new terminology.

The first new member of the Nox family, Nox1, was originally cloned from a human colon cDNA library, and was shown to be expressed additionally in prostate, uterus, and cultured vascular smooth muscle cells (VSMC) (318). Subsequently Nox3, -4, and -5 were all cloned from human fetal kidney cDNA (49). Nox3 is primarily expressed in fetal tissues and the adult inner ear (18, 49). Nox4 (also known as Renox) was independently cloned by three separate groups, and is widely expressed in many adult tissues including pancreas, placenta, heart, vessels, ovary, testis, skeletal muscle, and, in particular, kidney (49, 103, 303). Nox5 is highly expressed in fetal tissue, and also in adult testis, spleen, ovary, placenta, and pancreas (49). All the novel Noxs encode predicted proteins of around 65 kDa, and show 21%–59% identity to Nox2, with Nox3 being the most similar and Nox5 the most divergent; all catalyze electron transfer from a reduced

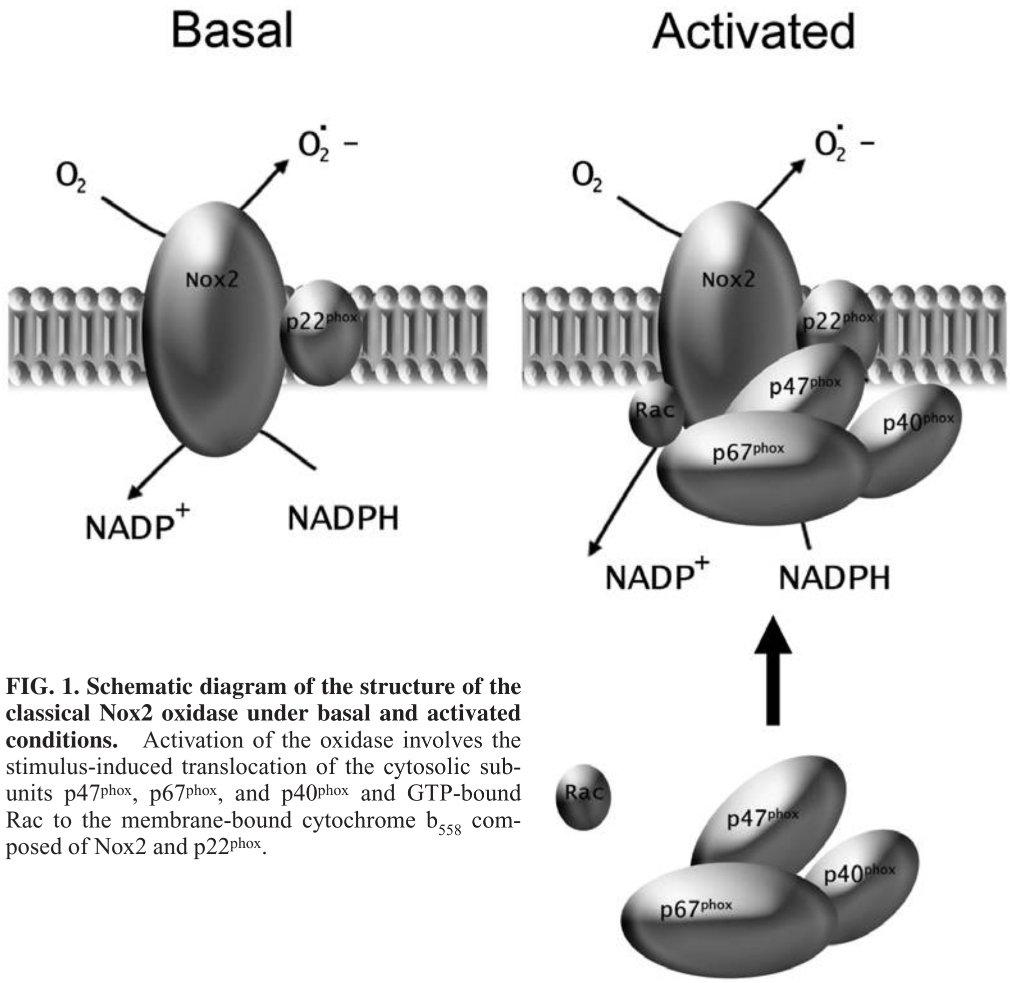

FIG. 1. Schematic diagram of the structure of the classical Nox2 oxidase under basal and activated conditions. Activation of the oxidase involves the stimulus-induced translocation of the cytosolic subunits p47phox, p67phox, and p40phox and GTP-bound Rac to the membrane-bound cytochrome b_{558} composed of Nox2 and p22phox.

substrate to molecular O_2 in a similar manner to Nox2 although their requirements for other subunits may differ (49). Two longer proteins with predicted molecular weights of ~177 kDa, namely Duox1 and Duox2, were cloned from human thyrocyte cDNA libraries and show 53% and 47% homology, respectively, to Nox2 within their C-terminal regions (64). However, the Duoxs also contain an N-terminal extension with no counterpart within the other Nox isoforms (see Fig. 2). Strong expression of both Duoxs was initially identified in the thyroid, with additional weak expression of Duox2 observed in the stomach (64). The extended family of Nox isoforms can be classified into three groups, according to the presence of specific domains: (a) Noxs1–4 have similar predicted general structures with six transmembrane α-helices, containing conserved histidines implicated in heme binding, and putative flavin- and NADPH-binding domains towards the carboxyl termini (184) (Fig. 2); (b) Nox5 builds on the basic structure of Nox2 with an additional N-terminal calmodulin-like EF domain that contains four Ca^{2+}-binding sites, allowing its activation by elevated cytosolic Ca^{2+} (20, 21), and demonstrates similarities with some plant oxidases (20, 21, 49); (c) The Duox enzymes further extend the Nox5 structure to include an N-terminal peroxidase-homology domain that is separated from the calcium-binding domain by an additional transmembrane segment (64, 81, 84, 185).

Recently, isoforms of the regulatory subunits p47phox and p67phox have also been discovered in some nonphagocytic cells although in cardiovascular cells the classical isoforms appear to be more important. Colon epithelial cells express an ~41 kDa p47phox isoform termed NoxO1 (for Nox Organizer 1) and an ~51 kDa p67phox isoform termed NoxA1 (for Nox Activator 1). NoxO1 and NoxA1 substitute for p47phox and p67phox respectively in some cell types and may specifically function to activate Nox1 *in vivo* (17, 50, 105, 326). NoxO1 differs from p47phox in that it lacks phosphorylation sites that disinhibit an autoinhibitory region in the latter molecule, and therefore appears to be capable of supporting constitutive Nox1 activity (unlike p47phox which generally requires phosphorylation to facilitate oxidase activity). NoxA1 seems to be broadly similar to p67phox apart from lacking an N-terminal SH3 domain and a p40phox binding site. NoxO1 and NoxA1 have also been detected in liver, pancreas, and testis (326). Finally, the expression of Rac isoforms also varies among different cell types with Rac2 being the main isoform found in phagocytes, whereas Rac1 is the predominant isoform in most nonphagocytic cells.

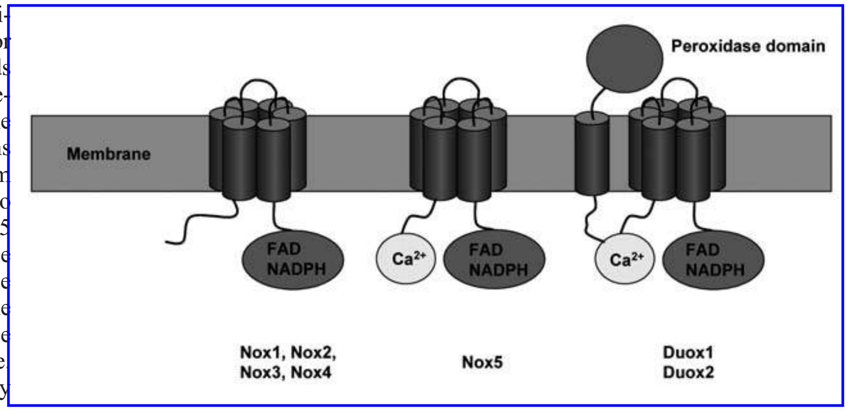

FIG. 2. Transmembrane topology of Nox and Duox enzymes. The predicted transmembrane α-helices contain conserved histidine residues which comprise binding sites for haems. The carboxyl-terminal domain folds within the cytoplasm and binds to flavin adenine dinucleotide (FAD) and NADPH. The enzymes catalyze the transfer of electrons from NADPH to molecular oxygen, to form superoxide across the membrane. The amino terminal calcium-binding domain of Nox5 and the Duox enzymes are also predicted to be on the cytosolic side of the membrane, while the additional transmembrane α-helix of the Duox enzymes would localize the peroxidase domain to the opposite side of the membrane. This could therefore utilize ROS generated by the transmembrane catalytic core of the enzyme to generate other oxidant species.

V. INTERACTIONS BETWEEN NADPH OXIDASES AND OTHER ROS SOURCES

While the current article focuses on NADPH oxidase-derived ROS, it is increasingly clear that there are complex interactions among different ROS sources such that in many pathological settings multiple sources may be involved. In many cases, NADPH oxidase-derived ROS may promote ROS production by other sources thereby amplifying the total levels of ROS (Fig. 3). Several studies have found that NADPH oxidase-derived ROS can promote the oxidative degradation of the essential NO synthase cofactor, H_4B, thereby leading to NO synthase uncoupling and $O_2^{\bullet-}$ (rather than NO) generation. This phenomenon has been termed amplification via "kindling radicals" (188, 195). Secondly, the

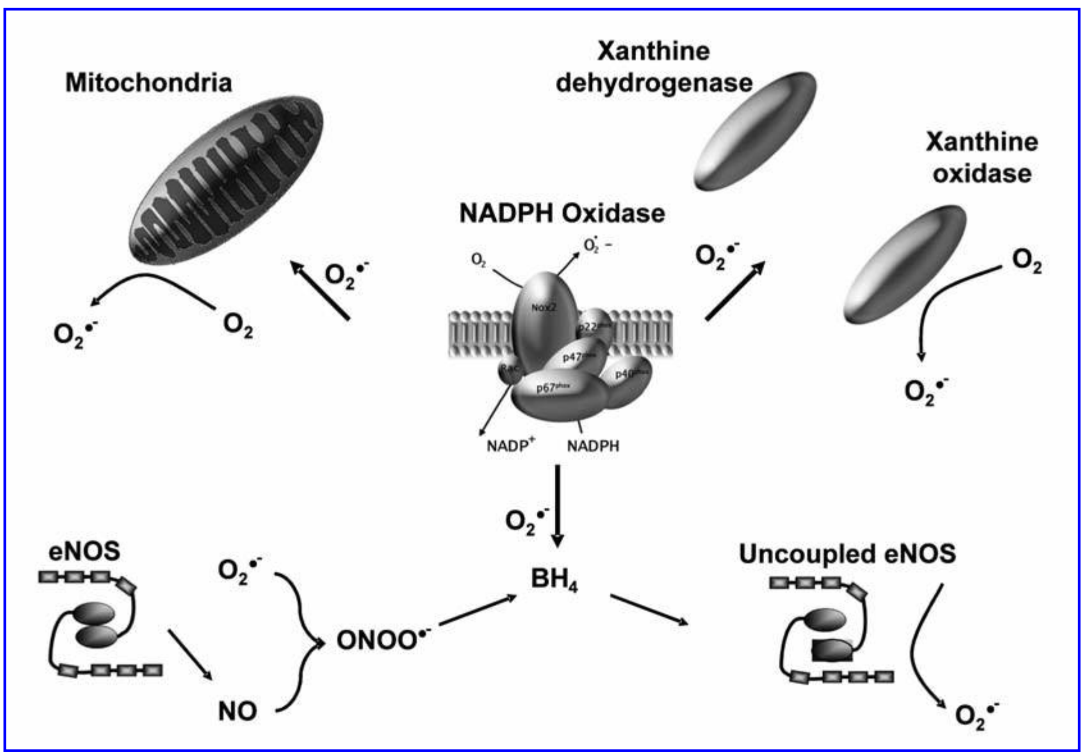

FIG. 3. Interplay between NADPH oxidase and other ROS sources. $O_2^{\bullet-}$ generated from NADPH oxidase can potentially influence ROS production by other enzymatic sources of $O_2^{\bullet-}$. For example, xanthine dehydrogenase is converted to $O_2^{\bullet-}$-generating xanthine oxidase through oxidation. Similarly, mitochondrial ROS generation can be increased by ROS derived from other sources. Finally, $O_2^{\bullet-}$ or $ONOO^-$ can degrade the essential NO synthase co-factor H_4B, hereby promoting NOS uncoupling and further $O_2^{\bullet-}$ production (reproduced from Ref. 285 with permission).

oxidative conversion of xanthine dehydrogenase to xanthine oxidase (238) may also serve to increase $O_2^{\bullet-}$ levels. This has been reported to be an important mechanism contributing to EC $O_2^{\bullet-}$ production in response to oscillatory shear stress (237). Thirdly, mitochondrial ROS generation can be increased by ROS derived from other sources (381), while a recent study suggested that mitochondrial ROS generation in turn may lead to NADPH oxidase activation in EC (298).

In addition to the above interactions, NADPH oxidase activity is itself potentially subject to feedback or feedforward regulation. For example, in VSMC or fibroblasts, exposure to exogenous H_2O_2 caused NADPH oxidase activation and endogenous $O_2^{\bullet-}$ generation, thereby amplifying the vascular injury process (214). On the other hand, Rac1-dependent EC NADPH oxidase activation and subsequent $O_2^{\bullet-}$ production mediates a feedback loop leading to increased proteosomal degradation of Rac1, which may then downregulate enzyme activity (179).

VI. NADPH OXIDASE SUBUNIT EXPRESSION IN CARDIOVASCULAR CELLS AND TISSUES

The p22^{phox} subunit is readily detected at both mRNA and protein level in cardiovascular cells (i.e., VSMC, EC, cardiomyocytes, fibroblasts) of most species studied to date. The expression of the catalytic Nox subunits, however, varies among the different cell types with distinct and tissue-restricted expression patterns (Table 1). Individual cell types can coexpress more than 1 Nox subunit, implying distinct functions of different Nox subunit-based oxidases. VSMC in culture have generally been reported to express significant levels of Nox1 and Nox4 with isolated reports also of Nox5 expression (20, 111, 167, 191, 313, 356), and one report of Duox1 in human aortic media (167). Significant Nox2 rather than Nox1 expression was found in human resistance artery VSMC (330), while low levels of Nox2 are also detectable in rat VSMC. In EC, a large number of studies in several different species have reported the expression of Nox2 mRNA and protein (192). Nox4 appears to be expressed at higher level than Nox2 in EC (7, 8, 338). Cardiomyocytes are generally reported to express both Nox2 and Nox4, but not Nox1 (38). Nox2 expression is also documented in adventitial fibroblasts (217), whereas cardiac fibroblasts reportedly express Nox4 rather than Nox2 (55). Nox5 was reported to be present in EC and cardiac fibroblasts in some studies (8, 20, 57, 111, 348) but there are no reports to date of either Nox3 or Duox2 expression in cardiovascular cells.

It should be noted that currently available data regarding the expression patterns of the Nox isoforms (see Table 1) are often contradictory, at least in part due to lack of suitable antibodies, species differences, and differences between cultured cells and tissue *in situ*. Data regarding *in vivo* expression in cardiovascular tissues and Nox isoform-specific functions remains extremely limited at present. Furthermore, the level of Nox mRNA expression does not necessarily correlate with oxidase activity. For example, recently, novel Nox4 splice variants have been discovered including two that have dominant negative characteristics for ROS generation (116). A novel Nox2 splice variant was also identified which is predicted to give rise to a truncated protein comprising only two transmembrane domains, together with a new C-terminal sequence, although the functional characteristics of this variant have not yet been established (133). A previously described Nox1 splice variant, however, subsequently proved to be an artifact (19, 104).

The cytosolic components of the classical NADPH oxidase (i.e., p47^{phox}, p40^{phox}, p67^{phox}, and Rac1) have generally been detected at both mRNA and protein level in most cardiovascular cells (reviewed in Ref. 192), apart from p67^{phox} which could not be detected in cultured VSMC (271). The cardiovascular expression of NoxO1 and NoxA1 has not been systematically studied but there is a report of low levels in rat basilar artery EC (7).

VII. NADPH OXIDASE ACTIVATION

A. Acute activation of phagocyte Nox2 NADPH oxidase

The vast majority of available information on the biochemical and molecular mechanisms underlying NADPH oxidase activation relates to the classical Nox2 oxidase of neutrophils, which we therefore consider first. Electron transfer in Nox2 occurs from NADPH, which binds to Nox2 at the cytosolic C-terminus, via FAD and two heme moieties (one towards the inner face and one towards the outer face of the membrane), to molecular O_2 in the interior of the phagocytic vacuole (i.e., the extracellular space). The initiation of electron transfer (oxidase activation) requires the recruitment of Rac as well as the cytosolic oxidase components p47^{phox}, p67^{phox}, and p40^{phox} to the cell membrane, and their association with flavocytochrome b_{558}. Recruitment of Rac and the other components may be independent of each other and it remains unclear what the precise relative roles of these two events are. The p67^{phox} molecule contains a proline-rich activation domain which binds directly to an activation sequence in the C-terminal of Nox2 to initiate the process of electron transfer; thus, p67^{phox} is also known as the Nox activator. In resting neutrophils, p40^{phox}, p47^{phox}, and p67^{phox} may exist in a cytosolic complex stabilized by SH3 domain interactions. Intramolecular autoinhibitory interactions maintain p47^{phox} in a closed conformation that is unfavorable for binding to the flavocytochrome. During neutrophil activation, p47^{phox} becomes heavily serine phosphorylated at up to 11 sites, which relieves the above autoinhibitory interactions and elicits interaction with phosphoinositides on the cell surface (5, 6). In addition to phosphorylation, intracellular generation of arachidonic acid (AA) (and possibly phosphatidic acid) via phospholipase A2 appears to be necessary for recruitment to the cell membrane (59, 60, 62, 199, 304). Binding of SH3 domains of p47^{phox} to a proline-rich domain of p22^{phox} then allows interaction of p67^{phox} with Nox2 and oxidase activation. Thus, p47^{phox} plays an essential role in the assembly of the oxidase complex. The protein kinase C (PKC) isoforms β, δ, and ζ are suggested to be the major kinases responsible for p47^{phox} phosphorylation but recent studies suggest that other

TABLE 1. EXPRESSION OF NOX ISOFORM mRNAS IN CARDIOVASCULAR CELLS AND TISSUE

	Cardiomyocytes	*Endothelial cells (EC)*	*Fibroblasts*	*Vascular smooth muscle cells (VSMC)*
Nox1		Isolated human coronary artery EC (313) Human umbilical vein EC (HUVEC) (8, 154) Rat aortic EC (8) Rat basilar artery EC (7)	Isolated human cardiac fibroblasts (313)	Isolated human coronary artery SMC (313) Human aortic SMC (330, 272, 111) Rat VSMC from mesenteric arteries (330) Rat aortic VSMC (191, 114, 318, 350) Rabbit pulmonary arterial SMC (353) Rabbit SMC from resistance arteries (353) Mouse aortic VSMC (124) A7r5 (rat aortic VSMC) (170, 356)
Nox2	Mouse left ventricle (29) Isolated mouse cardiomyocytes (29, 273) Isolated rat cardiomyocytes (362)	HUVEC (80, 111, 166, 289, 290, 343, 8, 154, 239) Isolated human coronary artery EC (313) Porcine pulmonary artery EC (147) Rat cardiac micro-vascular EC (24, 25) Rat aortic EC (8) Rat basilar artery EC (7) EA.Hy926 (transformed HUVEC) (111)	Isolated human cardiac fibroblasts (313) Adventitia of human coronary arteries (313) Adventitia of mouse aorta (348)	Isolated human coronary artery SMC (313) HVSMC from resistance arteries (330) Human aortic intimal SMC (167) Intimal cells of human coronary arteries (313) Rat aortic VSMC (191)
Nox4 `	Mouse left ventricle (38) Isolated mouse cardiomyocytes (273)	Isolated human coronary artery EC (313) HUVEC (8, 154) Rat aortic EC (8) Rat basilar artery EC (7)	Isolated human cardiac fibroblasts (313, 57) Adventitia of human coronary arteries (313) Isolated adult rat cardiac fibroblasts (55)	Intimal cells of human coronary arteries (313) Human aortic media (167) Media of human coronary arteries (313) A7r5 cells (356, 170) Isolated human coronary artery SMC (313) Human VSMC from resis-tance arteries (330) Human aortic SMC (330, 272) Rat VSMC from mesenteric arteries (330) Rat aortic VSMC (191) Medial smooth muscle within rat cartoid arteries (323) Mouse aortic VSMC (124)
Nox5		HUVEC (20)`	Human cardiac fibroblasts (58)	Human VSMC (20) Human aortic SMC (272)
Duox1				Human aortic media (167) Intimal SMC within human aortic athero-aclerotic lesions (167)

kinases such as Akt (PKC B), p38MAPK, and p21 activated kinase (PAK) can also be involved (46, 61, 72, 149, 178, 183, 256, 269). $p67^{phox}$ and $p22^{phox}$ also become phosphorylated during NADPH oxidase activation although the relevance of this remains unclear (31, 378). Likewise, the precise role of $p40^{phox}$, which has significant homology to $p47^{phox}$, in oxidase activation is poorly understood.

Rac binds to an N-terminal TPR domain in $p67^{phox}$ and this interaction may regulate electron transfer. However, recent evidence suggests that Rac-GTP also interacts directly with the flavocytochrome b558 to regulate electron transfer (74). Rac-GTP has also been reported to be capable of initiating signaling pathways leading to translocation of cytosolic oxidase subunits in COS-7 cells (278, 279, 295). Rac translocation requires geranylgeranyl modification of its C-terminal (175), and this process is regulated by membrane-bound guanine nucleotide exchange factors (GEFs) which catalyze conversion of Rac-GDP to Rac GTP. A newly identified GEF, P-Rex1, which is activated either by phosphatidylinositols or $G_{\beta\gamma}$ subunits (141, 354), appears particularly important (354), while other GEFs that may be involved include Tiam-1, Trio, and Vav-1 (244, 278). With regard to potential therapeutic manipulation of oxidase activity, it is relevant that the synthesis of geranylgeranyl groups is inhibited by HMG-CoA reductase inhibitors (statins); therefore, some of the pleiotropic effects of statins may be mediated through inhibition of Rac translocation.

B. Mechanisms underlying acute activation cardiovascular NADPH oxidases

The continuous low-level NADPH oxidase-derived ROS production in cardiovascular (and other nonphagocytic) cells, even in the absence of agonist activation, has no parallel in the neutrophil oxidase. In EC, studies from our laboratory and others found that a significant proportion of the Nox2-based NADPH oxidase exists as fully preassembled and functional ROS-generating complexes associated with the perinuclear intracellular cytoskeleton, even in unstimulated cells (Fig. 4) (24, 94, 202, 330). This observation may provide a potential explanation for the continuous low-level activity in these cells. Intriguingly, experiments with $p47^{phox}$ depletion and transfection in some of these studies have suggested that unphosphorylated $p47^{phox}$ may act to modestly inhibit basal oxidase activity in unstimulated EC or aorta (208). However, more recent studies have also suggested that the continuous activity seen in the absence of agonist stimulation may be Nox4 oxidase-based (8, 11, 229, 303). Knockdown of Nox4 reduced basal ROS production in cultured EC and VSMC (8, 90), while in transfected HEK cells, EC, and VSMC, Nox4 oxidase activity was unaffected by the cytosolic subunits $p67^{phox}$, $p47^{phox}$, NOXA1 or NOXO1—suggesting that it does not require binding to these oxidase components for its activation but may be constitutively active (11, 103, 229, 303). Some studies have also suggested that Rac1 may regulate basal oxidase activity based on the finding that statin withdrawal after chronic treatment in animals stimulates endothelial $O_2^{\bullet-}$ generation through Rac1–dependent activation of NADPH oxidase (339).

In addition to basal ROS production, NADPH oxidase activity in cardiovascular cells is acutely upregulated by a large number of stimuli (Fig. 5). In many cases, however, the Nox isoform that is responsible has not been definitively identified and it remains unclear whether activation is isoform-specific. There may also be significant variations in the responses to similar stimuli among different cell types, at least in part due to the heterogeneity in Nox isoform expression. While upstream signaling events leading to cardiovascular Nox2- and Nox1-based oxidase activation have been quite well studied for some agonists (54, 98, 337), the molecular events involved in oxidase activation at the level of the enzyme itself are relatively poorly characterized. Data on activation of Nox4-based oxidases are also extremely scanty. The precise location of ROS production (either basally or after agonist-induced activation) remains a matter of some debate (206, 363), largely because current methods for imaging ROS lack sufficient spatial resolution, but appears to be both intracellular and extracellular.

In general, the key features of Nox2 oxidase activation in cardiovascular cells are similar to the phagocytic enzyme insofar as the roles of $p47^{phox}$ phosphorylation and Rac1

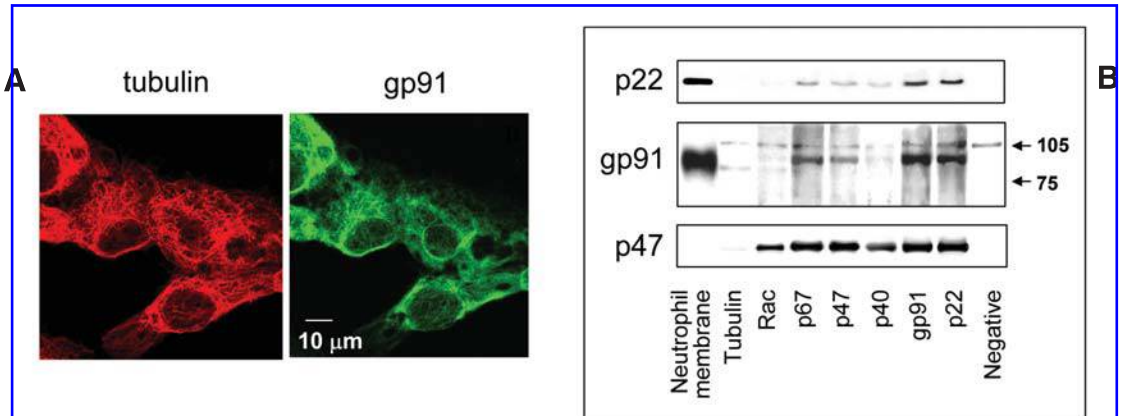

FIG. 4. NADPH oxidase localization and assembly in endothelial cells. (**A**) Confocal micrographs demonstrating cytoskeletal microtube distribution (*left*) and Nox2 (*right*) in porcine iliac arterial endothelial cells. Cells were co-labeled with a monoclonal anti-α-tubulin antibody and an anti-Nox2 polyclonal antibody. There is significant overlap between the tubulin and Nox2 distributions, particularly in the perinuclear region. (**B**). Co-immunoprecipitation of NADPH oxidase subunits in endothelial cells. NADPH oxidase subunits were immunoprecipitated using polyclonal antibodies as labeled below each lane. Subsequent immunodetection for coexistence of other subunits was performed with antibodies to $p22^{phox}$, Nox2, and $p47^{phox}$. $p22^{phox}$ was readily detected in the immunoprecipitates of $p67^{phox}$, $p47^{phox}$, $p40^{phox}$, and Nox2. Similarly, Nox2 was detected in the immunoprecipitates of $p67^{phox}$, $p47^{phox}$, $p40^{phox}$, $p22^{phox}$, and Rac1. The $p47^{phox}$ subunit was also co immunoprecipitated down with all the NADPH oxidase subunits. These studies confirm the association of oxidase subunits into complexes in unstimulated endothelial cells. Adapted from Ref. 202 with permission.

translocation are concerned. Thus, Rac translocation is implicated in oxidase activation and response to altered shear stress (334, 368), phorbol esters (344), vascular endothelial growth factor (VEGF) (335), tumor necrosis factor α (TNFα) (48, 65, 68, 107, 258), hypoxia-reoxygenation (173), ischemia–reperfusion (173), depolarization (310), and nutrient deprivation (219) (Fig. 5). Similarly, the phosphorylation of $p47^{phox}$ and its translocation and association with cytochrome b_{558} is involved in oxidase activation in response to angiotensin II (Ang II) (208), TNFα (207), VEGF (360), chronic oscillatory shear (153), and other stimuli. PKC-dependent phosphorylation was implicated in the responses to Ang II and TNFα (207, 208), whereas the response to hyperoxia of human pulmonary artery EC (52) or to VEGF in human umbilical vein endothelial cells (HUVEC) (360) appears to involve tyrosine phosphorylation of $p47^{phox}$. In contrast to Nox2, the roles of $p47^{phox}$ phosphorylation and translocation and Rac translocation in the activation of Nox1-based activity in cardiovascular cells remain to be definitively demonstrated but appear likely (e.g., in cultured VSMC).

Interestingly, recent data indicate that $p47^{phox}$ may have additional roles in nonphagocytic cells. It has been suggested that protein–protein interactions involving $p47^{phox}$ and other nonoxidase factors may play an important role in the spatial confinement of NADPH oxidase-derived ROS signals and thereby in local redox signaling (206, 363). A yeast two-hybrid screen of lung and EC libraries for interaction partners of $p47^{phox}$ by Xu *et al*. recovered several different proteins including the TNF receptor-associated factor 4 (TRAF4) (363). In HUVEC, Wu *et al*. (360) reported that VEGF-induced translocation of $p47^{phox}$ to membrane ruffles involved a direct interaction with WAVE1, an important regulator of cytoskeleton, which may act as a scaffold to recruit the NADPH oxidase to a complex involved with both cytoskeletal regulation and downstream JNK activation; the WAVE1-dependent complex also contained Rac1 and the kinase PAK1. Similarly, in human microvascular EC, we showed that the association of phosphorylated $p47^{phox}$ with TRAF4 was critical for TNFα-induced ROS-dependent activation of ERK1/2 (206) (Fig. 6). In WEHI 231 lymphomas, CD40-induced NADPH oxidase activation required TRAF3 (129). Analogous to these roles of $p47^{phox}$, protein–protein interactions involving Rac1 or Rac-GEFs may also be important in targeted redox signaling. Thus, in VSMC, AT_1 receptor-dependent Rac1 and NADPH oxidase activation and EGF-receptor transactivation required caveolin-1-dependent GEF phosphorylation and trafficking into lipid rafts (382). Interaction with cytoskeletal elements also appears to have an important regulatory role in NADPH

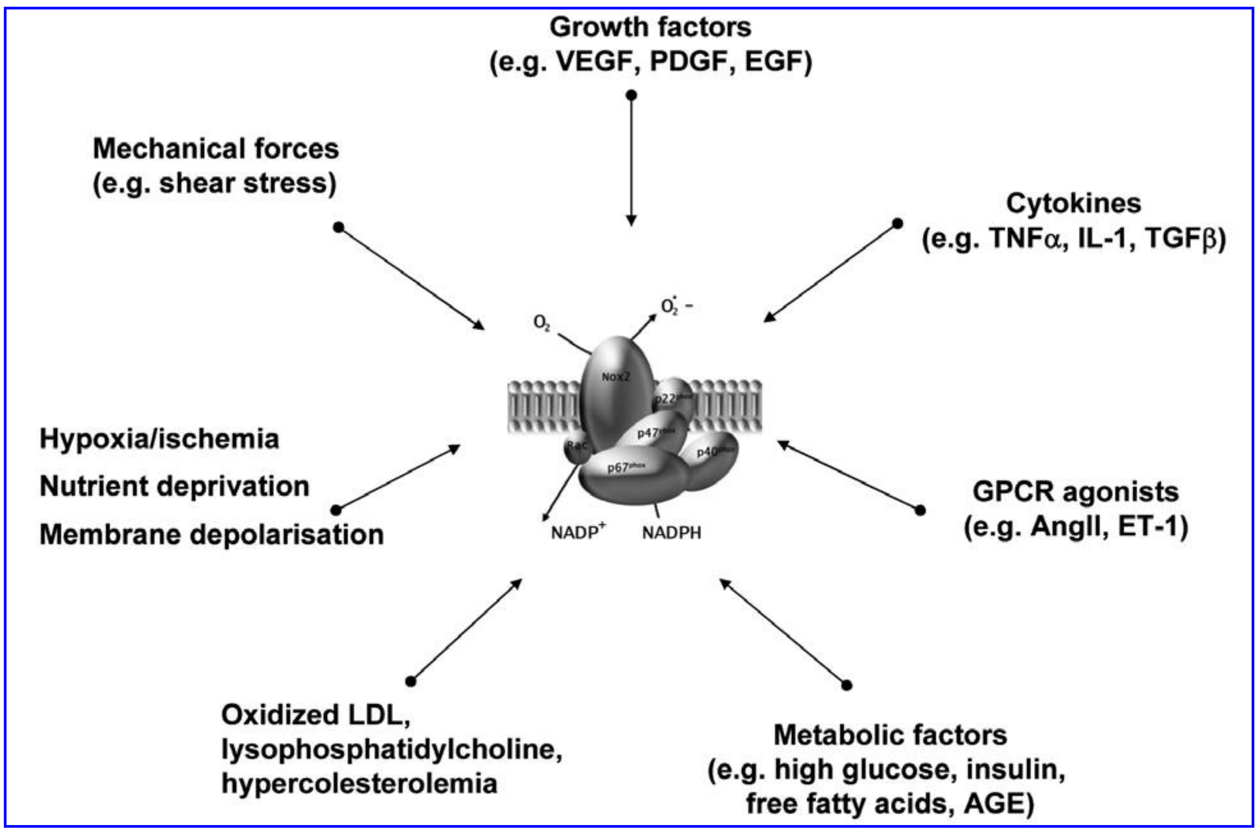

FIG. 5. Schematic diagram illustrating known activators of the Nox2 oxidase. A diverse range of signals activate the oxidase including G-protein coupled receptor (GPCR) agonists such as angiotensin II (Ang II) and endothelin-1 (ET-1), mechanical forces, ischemia-associated factors, metabolic factors, and growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and endothelial growth factor (EGF). AGE, advanced glycation end-products; LDL, low density lipoprotein.

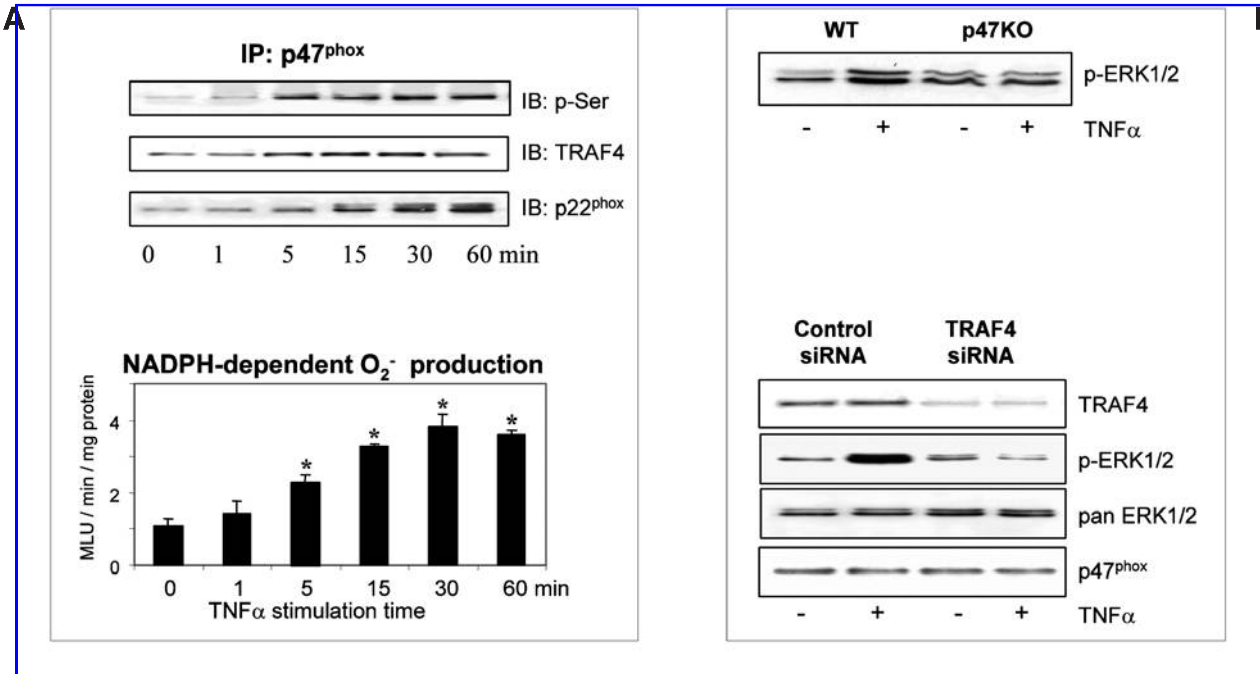

FIG. 6. Requirement of p47^{phox} phosphorylation and TRAF4 for acute TNF-α induced redox signaling in endothelial cells. (**A**) Time course of TNF-α-induced p47^{phox} phosphorylation, p47^{phox}–TRAF4 binding, p47^{phox}–p22^{phox} binding (*top panels, immunoblots*) and NADPH-dependent SOD-inhibitable O_2^- production (*bottom panel*) in human microvascular endothelial cells (HMEC-1). Immunoblots demonstrate that TNF-α-induced p47^{phox} phosphorylation and the association of p47^{phox} with TRAF4 was detectable after 5 min, with a peak at 15–30 min. Along with p47^{phox} phosphorylation, the amount of p22^{phox} which co-immunoprecipitated with p47^{phox} rapidly increased after TNF-α stimulation, being maximal at ~60 min. TNF-α also induced a significant increase in NADPH-dependent O_2^- production which peaked at ~30 min. (**B**) Role of p47^{phox} and TRAF4 in TNF-α-induced ERK activation. *Top panel*: Representative immunoblots showing ERK-1/2 phosphorylation in wild-type and p47$^{phox-/-}$ coronary microvascular endothelial cells. No ERK phosphorylation was detected in the absence of p47^{phox}. *Bottom panel*: Effect of siRNA-mediated knockdown of TRAF4 on acute TNF-α-induced ERK1/2 activation. TRAF4 protein expression was substantially reduced after siRNA treatment, and TNF-α induced-ERK1/2 phosphorylation was concomitantly inhibited. Adapted from Ref. 206 with permission.

oxidase activation and downstream redox signaling. The translocation of p47^{phox} to the membrane is reported to be assisted by interactions with the cytoskeleton mediated by moesin (374). In VSMC, Ang II-stimulated ROS production and the phosphorylation of p38MAP kinase and JNK were attenuated by treatment with cytochalasin B, which disrupts the cytoskeleton; p47^{phox} colocalized with the actin cytoskeleton in Ang II-stimulated cells in this study (331). In human EC exposed to human immunodeficiency virus type 1 Tat, p47^{phox} becomes phosphorylated and rapidly redistributed to membrane ruffles; this response is associated with stress fiber disassembly and peripheral retraction and is mediated by PAK (359). In EC exposed to arsenic, NADPH oxidase activation and ROS production were shown to involve Cdc42-mediated actin filament reorganization; either overexpression of a dominant negative Cdc42 mutant or pretreatment with an actin filament stabilizing reagent, jasplakinolide, abrogated arsenic-induced NADPH oxidase activation (282). Taken together, these data suggest that interactions of p47^{phox} and other oxidase components with cytoskeletal proteins and with other signaling molecules may play an important role in spatially confined redox signaling in response to specific agonists. This could take place either around specialized regions of the plasma membrane (such as caveolae) or in the vicinity of intracellular membranous compartments.

C. Activating stimuli for cardiovascular NADPH oxidases

Cardiovascular NADPH oxidase activity may be acutely upregulated by a wide variety of (patho)physiological stimuli which include (a) G-protein coupled receptor agonists such as Ang II and endothelin-1 (ET-1) (29, 80, 91, 187, 189, 210, 330, 332); (b) growth factors such as VEGF (54, 335), thrombin (135), platelet-derived growth factor (PDGF) (231), and EGF (113); (c) cytokines such as TNFα (107), interleukin 1 (IL-1), and transforming growth factor β (TGFβ) (113, 126); (d) "metabolic" factors such as elevated glucose (53, 152, 156), insulin (168), free fatty acids (156), and advanced glycation end products (AGE) (351, 377); (e) oxidized LDL, lysophosphatidylcholine, and hypercholesterolemia; (100, 134, 259, 261, 262); (f) mechanical forces such as oscillatory shear stress (144, 145); and (g) ischemia-related stimuli such as nutrient deprivation, membrane depolarization, flow cessation, hypoxia–reoxygenation, and ischemia (9, 218, 219) (Fig. 5). For detailed reviews of the signaling pathways upstream of

NADPH oxidase activation that may be involved, the reader is referred to several excellent recent publications (34, 118, 119, 333). Here, we discuss what is known about the Nox isoforms that are activated in response to the above stimuli.

Ang II was first reported to upregulate NADPH oxidase activity in cultured VSMC in a seminal study by Griendling and colleagues (117). It has subsequently been shown to have similar effects in EC (204, 208, 375), cardiomyocytes and fibroblasts (122, 254, 264). Ang II-induced acute activation of NADPH oxidase in VSMC appears to involve Nox1, since antisense Nox1 cDNA inhibited this response (191). In EC (348) and cardiac tissue (29), however, Ang II-induced oxidase activation is critically dependent upon Nox2. Ang II is reported to induce ROS production with a biphasic timecourse in VSMC, with initial very rapid NADPH oxidase activation occurring via PKC, whereas subsequent maintained activation involves EGF receptor transactivation and robust Rac activation (301).

The Nox isoform involved in *PDGF*-induced oxidase activation in VSMC (231) and fibroblasts is unclear but the requirement for cytosolic subunits suggests that it is probably Nox1 rather than Nox4 (3, 196, 292). In Caco-2 and HEK293 cells, *EGF* stimulates Nox1-dependent radical generation (315). Similarly in Caco-2 cells, the inflammatory mediator IFN-γ induced an increase in Nox1 mRNA levels (274, 369). In rat cardiomyocytes, we have recently shown that NADPH oxidase activation induced by glycated albumin is dependent on Nox2 (377).

Mechanical stimuli, in particular cyclic stretch, induce NADPH oxidase activation in several cardiovascular cell types, including human coronary artery VSMC and human aortic EC (144, 145). A role for $p47^{phox}$ has been demonstrated in stretch-induced ROS formation and MMP2 activation in cultured VSMC (124) and in high pressure-induced ERK activation (260). Mechanically-induced oxidase activation is likely to be pathophysiologically important in many conditions (e.g., hypertension), but the Nox isoform(s) involved remain unclear (162).

D. Transcriptional regulation of oxidase subunits

In addition to acute activation of NADPH oxidase in cardiovascular cells, enzyme activity is also modulated by the transcriptional upregulation of oxidase subunits which presumably increases the pool of enzyme complexes available for activation. Indeed, chronic increases in oxidase activity (over hours or days) either *in vitro* in response to specific stimuli or *in vivo* in a variety of pathological contexts (*see later sections*) correlate in many cases with an increase in mRNA expression level of one or more oxidase subunit (including the Noxs) (290). However, in the *in vivo* setting it has not always been possible to determine which cell type is mediating the increase. It remains unclear whether simultaneous increases in all oxidase subunits are required to allow an increase in activity. In the case of Nox-based oxidase activity in EC, isolated increases in Nox2 may be sufficient since Nox2 mRNA expression level is low compared to other oxidase subunits (290). In the case of Nox4 oxidase, changes in Nox4 expression level may be the major mechanism responsible for modulating activity; $p22^{phox}$ is also required for activity but other subunits do not appear to be required (8, 229).

Available evidence indicates that distinct signaling pathways and/or effectors may be involved in the regulation of expression of different Nox isoforms. In VSMC, Nox1 mRNA expression is upregulated by serum, Ang II, PGF2α, LDL, phorbol ester, and mechanical stretch (124, 170, 191, 318, 356), downregulated by atorvastatin (350), and unaffected by endothelin-1, lipopolysaccharide, interleukin-1β, thrombin, or the oxysteroid 7-ketocholesterol (90, 272, 356). By contrast, Nox4 mRNA is downregulated by thrombin and interleukin-1β (90) but upregulated by human urotensin II (77) and 7-ketocholesterol (272), whereas the effects of Ang II and serum are conflicting (356, 191). In EC, Nox2 expression is reportedly upregulated by Ang II, endothelin-1, oxidized LDL, and shear stress (80, 155, 289, 290), while statins, estrogens, BMP-4, and pulsatile flow all cause a decrease in expression (155, 289, 314, 343). Nox4 expression is also reportedly upregulated by Ang II and shear stress (155, 365) and to be downregulated by pulsatile flow, although in another report, shear stress acted to decrease Nox4 mRNA (155, 314). In contrast to the Nox isoforms, increased expression of regulatory oxidase subunits in response to several agonists often appears to occur in a coordinated fashion. For example, in VSMC, chronic exposure to Ang II upregulates the expression of $p22^{phox}$ as well as $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$ (65, 77, 112, 330). Likewise, in EC, Ang II upregulates $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$ (290). The expression of $p22^{phox}$ or regulatory subunits may also be specifically downregulated by various agents, such as dexamethasone (230), activation of PPAR-α or –γ (157, 158) or statins (157, 158).

Taken together, the above data clearly indicate that the regulation of individual Nox isoforms and oxidase subunits is quite different and potentially complex even within a single cell type. It is clearly therefore of importance to determine the molecular mechanisms which effect the agonist-induced changes in transcription of NADPH oxidase subunits, as these may inform therapeutic strategies to target expression. The identification of cis-acting regulatory elements within the gene loci which mediate the agonist-induced transcriptional changes, and identification of the trans-acting factors that bind to these elements would begin to elucidate the pathways involved. To date, the gene whose regulation has been best characterized at the molecular level is Nox2. The minimal Nox2 promoter region required for monocyte/macrophage expression was identified as a 450 bp region proximal to the transcription initiation site (309), which includes binding sites for both positive and negative regulators of transcription (86, 87, 161, 222, 223, 341). In terminally differentiated phagocytic cells, Nox2 expression is induced by the immune mediator interferon-γ (IFNγ), and this response involves the hematopoietic-lineage specific transcription factor PU.1. The latter binds to an element within the proximal Nox2 promoter and can form a complex with interferon regulatory factor-1, interferon consensus sequence binding protein and CREB binding protein (85, 87). In addition, eosinophil-specific regulation of Nox2 transcription was shown to be dependant upon activation by the direct binding of GATA-1, and competitive inhibition by the binding of GATA-2 to the same site (366). Point mutations within the Nox2 promoter have also been identified which act to specifically repress expression within neutrophils, but do not affect Nox2 transcription in the patients' eosinophils (352). However, the functional Nox2 promoter within cardiovascular cells has not as yet been characterized. The cis-acting elements that regulate expression of other Nox isoforms in any cell type are only just beginning to be studied. In the case of Nox1, we have recently identified the promoter sequences that drive expression in colon epithelial cells and shown that maximal expression is dependent upon binding of a GATA factor (35).

The regulation of the promoters of $p47^{phox}$ and $p67^{phox}$ has also thus far only been studied in myeloid cells. As was found to be the case for Nox2, both were dependent upon binding of PU.1 (171, 212). In the case of $p67^{phox}$, cooperation between PU.1, IRF-1, ICSBP, and CBP was also required for full myeloid expression, as with Nox2 (171). In addition, the protein tyrosine phosphatase, SHP1, was shown to decrease the interactions of these proteins with the promoter elements, and so downregulate expression of both $p67^{phox}$ and Nox2 (171). Functional binding sites for the ubiquitous transcription factors Sp1 and AP-1 were also characterized within the $p67^{phox}$ promoter (213), but their significance in cardiovascular cells is unknown. In the case of $p22^{phox}$, the identification of five polymorphisms present within the sequence of the $p22^{phox}$ promoter in spontaneously hypertensive rats (SHR) is of potential interest. In a transient transfection assay, these polymorphisms were shown to significantly increase promoter activity in rat VSMCs (372); however, the factors that potentially bind to these regions have not yet been characterized.

VIII. PHYSIOLOGICAL ROLES OF CARDIOVASCULAR NADPH OXIDASES

Whether NADPH oxidase-derived ROS have physiological (in addition to their well-recognized pathophysiological) roles in the cardiovascular system is an interesting question that is open to debate. A physiological role would provide at least a teleological explanation for the existence of these enzymes. As a minimum, the effects of NADPH oxidases on cell growth, migration, proliferation, activation, etc. which have been documented in pathological settings (*see later*) could clearly also serve important physiological functions during development or reparative processes. NADPH oxidase-derived ROS could also be relevant to the physiological regulation of vascular smooth muscle tone and in oxygen sensing.

A. Effect on vascular tone

In most vascular beds, the local production and activity of NO is pivotally involved in the endothelial regulation of vasomotor tone in health (266). NO-dependent regulation is rapidly sensitive to alterations in local stimuli (such as increased shear stress) and appropriate *local* vasodilator actions are central to the achievement of integrated increases and/or redistribution of blood flow among specific vascular beds. The local levels of $O_2^{\bullet-}$ (together with molecules such as hemoglobin and antioxidants such as the SODs, which all influence NO bioactivity) are potentially important in the spatial restriction of NO action, even in health. In this regard, it is of interest that increased flow is a potent stimulus for the release of $O_2^{\bullet-}$ (as well as NO) in vessels (193). The involvement of NADPH oxidases remains to be demonstrated but oxidase activity is known to be increased by shear stress (155).

In addition to indirect effects through inactivation of NO, $O_2^{\bullet-}$ may also exert direct effects on vascular tone following dismutation to H_2O_2. Indeed, recent studies suggest that H_2O_2 released from the endothelium may account for endothelium-derived hyperpolarizing factor (EDHF) vasodilator activity in murine and human mesenteric arteries and in human coronary arterioles, where it is involved in flow-induced dilatation (232, 233, 243, 247). Studies by Matoba *et al*. (233) have suggested that NO synthases are responsible for the EDHF-like activity attributed to H_2O_2. To date, the specific involvement of NADPH oxidases in this response has not been demonstrated. However, these findings were not supported by those of Ellis *et al*. (88) who reported that catalase had minimal effects on endothelium-dependent relaxations in both aorta and small mesenteric arteries. It should be noted that H_2O_2 may also have vasodilator actions that are independent of hyperpolarization. In one report, the myogenic constrictor response of arteriolar vascular smooth muscle to increases in transmural pressure was found to be NADPH oxidase-dependent as it was inhibited either by pharmacological inhibition of the oxidase or in vessels from $p47^{phox}$-deficient mice (257).

B. Role in oxygen sensing?

Maintenance of O_2 homeostasis is paramount for survival and consequently a number of different mechanisms have evolved to safeguard and mitigate deleterious reductions in O_2 tension. In mammals, hypoxia is acutely sensed by the glomus cells of the carotid bodies, which through afferent regulatory pathways influence appropriate central nervous system responses, for example, increases in alveolar ventilation (37, 117). The equivalent counterparts in the airways are the neuroepithelial bodies (NEB). At a local level, reflex hypoxic pulmonary vasoconstriction allows regulation and optimization of ventilation-perfusion matching whilst in systemic vascular beds such as the coronary circulation, hypoxic vasodilatation serves to maintain O_2 delivery (342). Chronic hypoxia also evokes many adaptive changes in gene expression in cardiovascular cells, for example, genes involved in angiogenesis, energy metabolism, cell proliferation, and vascular remodelling (37, 109).

The precise configurations of the O_2-sensing pathways that regulate the above processes in different cells and tissues remains a hotly debated subject despite considerable advances in understanding several components of these pathways, for example, the role of the ROS-sensitive transcription factor hypoxia-inducible factor-1 (HIF-1) in regulating O_2-dependent gene expression (see Refs. 109, 164, and 300, for detailed reviews). It is likely that the detailed configurations will differ among different cells and tissues. An involvement of ROS-generating proteins in the proximal part of the O_2-sensing pathways has been suggested in many cell types and a possible role of NADPH oxidases has been speculated upon (37). In keeping with a possible role of NADPH oxidase, the enzyme is suggested to generate ROS in a dose-dependent manner in response to variations in local O_2 tension (2).

Evidence from studies in Nox2-deficient mice suggested that the oxidase is integral to O_2 sensing in NEBs, through interactions with K^+ channels (95). However, Nox2 was not essential for O_2-sensing in the carotid bodies of these mice (131, 288), nor was it required for the hypoxic pulmonary vasoconstriction response (12). On the other hand, mice lacking p47^{phox} had potentiated respiratory responses to a hypoxic stimulus, leading to suggestions that other Nox homologues may be involved (293). Recently, Nox4 has been proposed to act as an oxygen sensor in conjunction with the potassium channel TASK-1 in transfected HEK293 cells (198). Furthermore, in a cell culture model using human lung adenocarcinoma A549 cells, an increase in Nox1 mRNA and protein and in ROS generation were observed in response to hypoxia (115). Cells stably transfected with Nox1 showed significant accumulation of HIF-1α, which increased further on exposure to hypoxia. HIF-1-dependent gene transcription was attenuated by either catalase or the NADPH oxidase inhibitor, DPI, suggesting a link between Nox1 and HIF-1 activation.

IX. NADPH OXIDASES IN ENDOTHELIAL CELL ACTIVATION AND INFLAMMATION

Inflammation describes the stereotyped response of vascularized tissues to injury and various stresses, and mainly involves vascular leak and leukocyte extravasation at the level of the microvasculature. In addition to being a major part of immune responses, components of this process are also fundamental in the initiation and perpetuation of diseases such as atherosclerosis. An early step in the process of inflammation is EC "activation," which involves the regulated expression of cell surface adhesion molecules and cytokines that enable the recruitment and adhesion of circulating leukocytes, and is accompanied by an increase in endothelial permeability, allowing the transmigration of inflammatory cells into the affected tissue. Expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), endothelial leukocyte adhesion molecule-1 (E-selectin), and P-selectin, is induced by several stimuli including pro-inflammatory cytokines (e.g., IL-1β, TNFα, and IFN-γ) (228), altered vascular wall shear stress (45), hypercholesterolemia, oxidized low density lipoprotein (ox-LDL), and ischemia–reperfusion (221).

Intracellular ROS production and redox signalling are implicated in the induction of EC adhesion molecule expression and associated changes and several studies suggest an important role for NADPH oxidases as sources of the ROS, for example, in the context of increased oscillatory shear stress, ischemia–reperfusion, activation of the renin–angiotensin system, or exposure to AGEs (150, 281, 324, 351). The NADPH oxidase-derived ROS may emanate from leukocytes and inflammatory cells (169) as well as EC themselves (234, 249). A contribution from other ROS sources such as xanthine oxidase is also reported (324). TNFα-induced NF-κB-dependent VCAM-1, E-selectin, and ICAM-1 gene expression in human aortic EC is inhibited by adenoviral overexpression of dominant negative Rac1 or SOD, consistent with an involvement of NADPH oxidase (47). In another study, the involvement of NADPH oxidase was clearly demonstrated in TNFα-induced increases in endothelial permeability, which involved a ROS-dependent, JNK-mediated phosphorylation of VE cadherin (258). Stokes *et al*. (317) clearly demonstrated that leukocyte–endothelial adhesion in response to a high cholesterol diet involved NADPH oxidase in that it was attenuated in p47$^{phox-/-}$ mice compared to wild type. Furthermore, with the use of bone marrow chimeras to dissect out the contributions of the vessel wall versus bone marrow-derived cells, these authors demonstrated an important role for NADPH oxidase in both cell types (Fig. 7). The same group also showed that P-selectin-dependent adhesion of platelets and leukocytes in the cerebral microcirculation in response to hypercholesterolemia was blunted in Nox2$^{-/-}$ mice (160).

X. VASCULAR CELL GROWTH AND APOPTOSIS

Inherent to the understanding of vascular disorders such as atherosclerosis, restenosis, and hypertensive vascular remodeling, is an appreciation of the processes involved in the proliferation and/or apoptosis of vascular cells (i.e., VSMC, EC, and fibroblasts). It is now clear that ROS may significantly modulate cellular growth, proliferation, and death. Low concentrations of H_2O_2 stimulate VSMC proliferation and hypertrophy (370), whereas high concentrations initiate growth arrest and cell death (69, 211). H_2O_2 may also have a role in cell survival, as exemplified by the finding that rat aortic VSMC exhibit reduced proliferation and an increased rate of apoptosis following adenoviral-mediated overexpression of catalase (36). Transfection of NIH 3T3 fibroblast cells with Nox1 induced an increase in $O_2^{\bullet-}$ and to an even greater extent H_2O_2 levels, and increased cell growth and tumorigenicity and upregulated a battery of genes critical to cell growth and neoplasia. Subsequent overexpression of catalase in these cells reduced ROS levels and partially normalized a range of cell growth parameters (13). Ang II increases VSMC growth and hypertrophy and this process is dependent on NADPH oxidase-derived ROS, probably mainly Nox1-derived (370). Thus, Ang II-induced VSMC hypertrophy is attenuated by pharmacological inhibitors of the oxidase, depletion of p22^{phox}, or catalase overexpression (Fig. 8) (117, 336, 370). Furthermore, hypertrophy of cultured VSMCs following stimulation by either thrombin or serum was found to be p47^{phox}-dependent but did not require Nox2 suggesting the involvement of the Nox1 isoform (22).

EC growth and survival are also influenced by NADPH oxidases. The proliferation of EC induced by VEGF is inhibited by three structurally unrelated NADPH oxidase inhibitors but not by xanthine oxidase or NOS inhibitors (1). Furthermore, Ushio-Fukai *et al.* (335) showed that VEGF-mediated proliferation is inhibited by dominant negative Rac1 or antisense Nox2 oligonucleotides. Other stimuli for EC proliferation, such as oxidized LDL (oxLDL) (289), Ang II (298), ET-1 (78), altered shear stress (241), and hypoxia (298), also appear to signal these effects via NADPH oxidase-derived ROS (298).

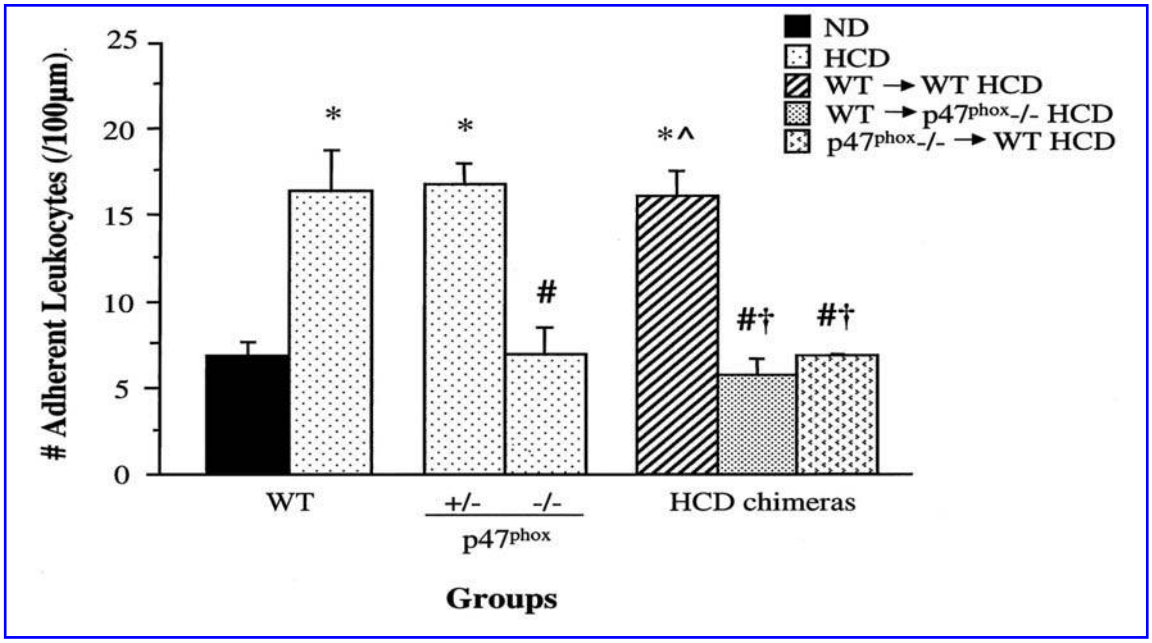

FIG. 7. Hypercholesterolemia-induced endothelial activation and leukocyte adhesion require NADPH oxidase both in the vessel wall and in circulating bone marrow-derived cells. Mean baseline leukocyte adhesion in postcapillary venules of cremaster muscle was quantified in wild-type (WT) and p47*phox*-deficient mice subjected to hypercholesterolemic diet (HCD) or normal diet (ND) for 2 weeks. Bone marrow chimeras were generated to produce animals lacking p47*phox* either in marrow cells alone or in all cells except marrow cells. HCD increased adhesion in WT and heterozygous p47*phox* mice (p47*phox*+/−) but not in homozygous p47*phox* mice (p47*phox*−/−). Leukocyte adhesion after HCD was reduced either in animals with p47*phox*-deficient marrow (and therefore, presumably leukocytes) (p47*phox*−/−→WT) or in animals with intact marrow but p47*phox* deficiency elsewhere (WT→p47*phox*−/−). $*p < 0.01$ vs. WT ND mice; $\#p < 0.05$ vs. WT HCD and p47*phox*+/- HCD mice; $\hat{}p < 0.005$ vs. p47*phox*−/− HCD mice; $\dagger p < 0.05$ vs WT→WT HCD mice. Reproduced from Ref. 317 with permission.

In sufficiently high doses, ROS predictably lead to irreversible cell damage and programmed cell death (apoptosis). In EC, TNFα-induced apoptosis was prevented by dominant negative Rac1 (70). Furthermore, EC apoptosis induced by other stimuli that activate NADPH oxidase, namely Ang II, oxidized LDL, and hyperglycemia, is attenuated by antioxidants (79, 99, 201). Finally, endothelial cell anoikis, a process in which cell detachment from the extracellular matrix induces cell death, is associated with rapid increases in intracellular ROS which appear to at least partly emanate from NADPH oxidase although mitochondria are also involved (200).

XI. EC MIGRATION, REGULATION OF EXTRACELLULAR MATRIX, AND ANGIOGENESIS

EC migration is important in inflammation, vascular injury, angiogenesis, and other disorders. Early studies showed that migration of cultured EC upon exposure to Ang II, ox-LDL, hypoxia, or VEGF necessitated NADPH oxidase-derived ROS (1, 289). Several different ROS-dependent processes probably contribute to this promigratory effect. The initial polarization of the cell towards the direction of intended migration involves significant reorganisation of the cytoskeleton and has been shown to require Rac1 (357) and ROS production (e.g., in EC monolayer wounding assays) (245). Actin filament reorganisation following exposure of EC to hypoxia–reoxygenation is also ROS-dependent (56). For cell migration to occur, the extracellular matrix within which cells are normally embedded needs to be remodeled; ROS are well known to regulate the activity of matrix metalloproteinases (MMP), the enzymes critical in matrix remodeling (284). Indeed, VSMCs subjected to cyclical mechanical stretch stimulate NADPH oxidase-derived ROS, leading to increases in MMP-2 expression (Fig. 9) (124). Moreover, vascular remodelling subsequent to chronic increases in arterial blood flow has recently been shown to involve p47phox-dependent (but not Nox2-dependent) ROS generation and MMP activation (42).

Angiogenesis involves a combination of EC and pericyte migration, proliferation, and appropriate spatial orientation to form new tubular conduits for the passage of blood. The process is important physiologically during embryological development and in wound repair, and is also relevant in the pathological settings of chronic ischemia, atherosclerosis, tumor vascularization, and diabetic retinopathy. Tissue hypoxia is one of the more potent stimuli for angiogenesis and rapidly induces proangiogenic growth factors such as VEGF (296, 305). The data discussed previously support an involvement of NADPH oxidase in angiogenesis and H_2O_2, when directly applied to cultured EC at a low concentration, does indeed stimulate tubular morphogenesis (367). Recently, Ushio-Fukai *et al.* (335) confirmed the role of NADPH oxidase in

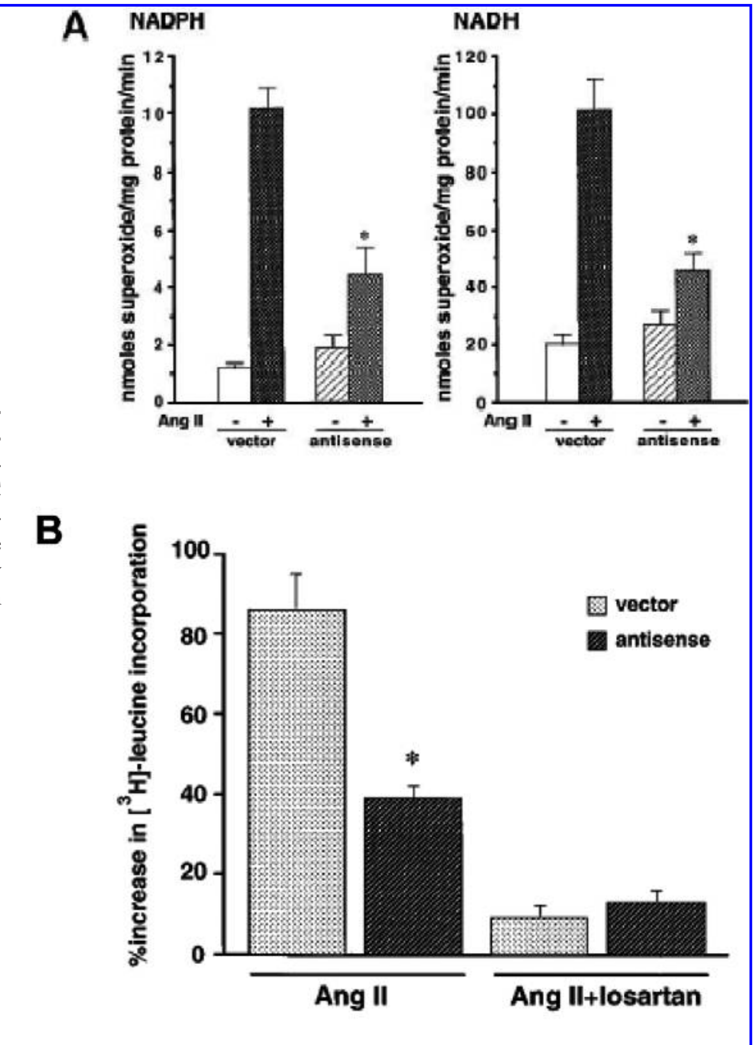

FIG. 8. Critical role of p22phox in angiotensin II-induced VSMC ROS production and hypertrophy. (**A**) Attenuation of angiotensin II (Ang II)-induced NADPH/NADH oxidase activity in VSMC transfected with antisense p22phox cDNA. (**B**) Inhibition of Ang II-induced hypertrophy by antisense p22phox. The Ang II response is also inhibited by the AT_1 receptor antagonist losartan. Adapted from Ref. 336 with permission.

angiogenesis. These authors reported that VEGF-induced angiogenesis involved a Nox2 oxidase since it was inhibited by transfection of antisense Nox2 oligonucleotides, DPI, or dominant negative Rac1 (Fig. 10). Furthermore, in an *in vivo* sponge implant assay, angiogenesis was significantly inhibited in $Nox2^{-/-}$ mice or in wild-type mice treated with antioxidants (335). The same group subsequently showed that ischemia-induced neovascularization in a hind-limb ligation model was significantly diminished in $Nox2^{-/-}$ mice (328).

XII. IMPAIRED ENDOTHELIUM-DEPENDENT VASODILATATION

Endothelial dysfunction is a broad term that describes an alteration in normal vascular homeostasis towards a state characterized by reduced endothelium-dependent vasodilatation and proinflammatory and prothrombotic tendencies (108). The most widely studied aspect of endothelial dysfunction is impaired endothelial-dependent vasodilatation which is commonly the result of a reduction in NO bioavailability. Importantly, the severity of endothelial dysfunction in conditions such as atherosclerosis, hypertension, chronic heart failure, and diabetes mellitus is a strong predictor of future cardiovascular morbidity and mortality (39, 297). The reduction in NO bioavailability arises through its scavenging by excess $O_2^{\bullet-}$ radicals (73), a decline in NO production due to reduced eNOS expression, a deficiency of eNOS substrate (L-arginine), or cofactors (BH_4), and/or NOS inhibition by endogenously generated antagonists such as asymmetrical dimethylarginine (ADMA) (240, 250, 347). Excess $O_2^{\bullet-}$ production (presumably extracellular) may emanate from multiple cell types including EC, VSMC, adventitial fibroblasts, and infiltrating inflammatory cells. An important role for NADPH oxidases in the genesis of endothelial dysfunction has now been reported by a large number of studies in experimental hypercholesterolemia, hypertension, diabetes, atherosclerosis, and heart failure (39, 142, 205), as well as in human arteries and veins from subjects with these conditions (Fig. 11) (127, 316). In addition to NADPH oxidases, other sources of $O_2^{\bullet-}$ relevant to endothelial dysfunction include xanthine oxidase and uncoupled eNOS. As discussed earlier, in many settings, NADPH oxidase-derived ROS may promote or augment $O_2^{\bullet-}$ production by these enzymes (Fig. 12) (188, 195, 237).

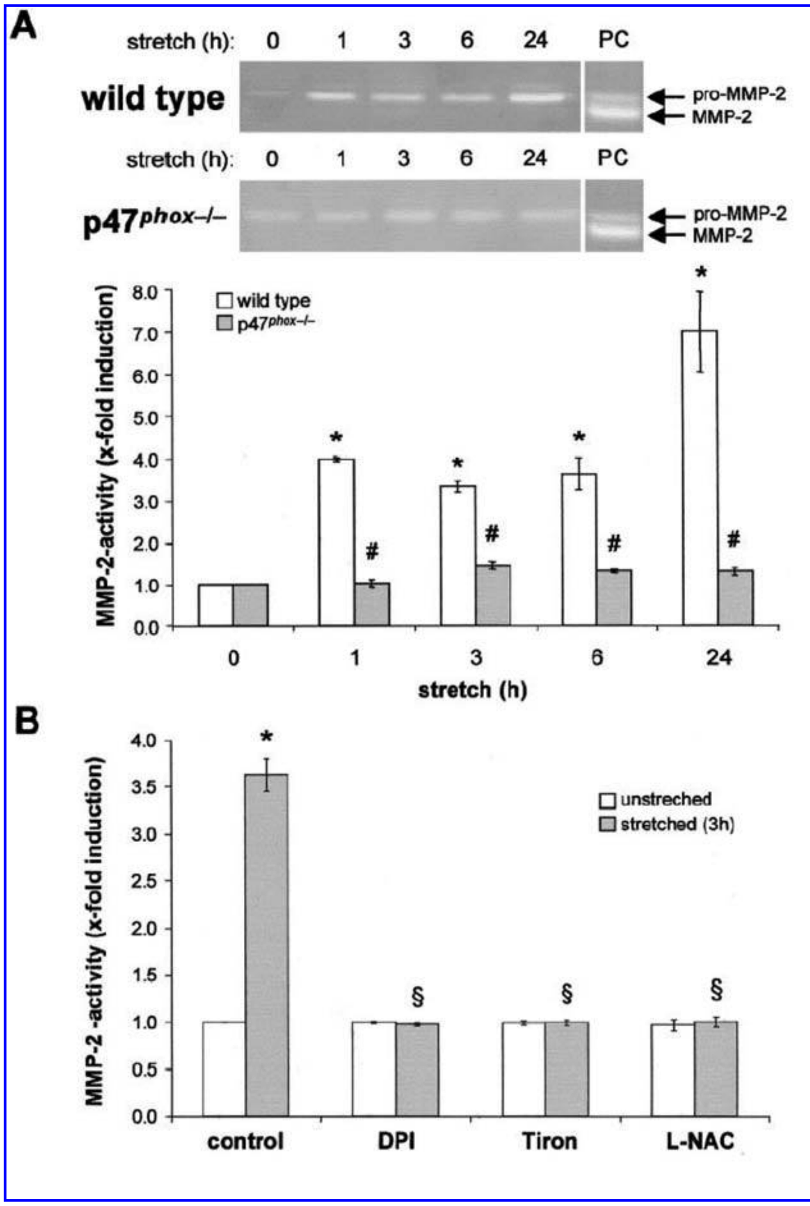

FIG. 9. Mechanical stretch enhances pro-MMP-2 release via p47*phox*. Cultured VSMCs from WT and p47$^{phox-/-}$ mice were subjected to mechanical stretch (0.5 Hz, 15% elongation) for the indicated time. Supernatants were tested for MMP-2 activity by gelatin zymography. (**A**) Time-dependent release of pro MMP-2 in WT and p47$^{phox-/-}$ VSMCs. PC indicates positive control. (**B**) Influence of DPI, Tiron, and L-NAC on stretch-induced pro-MMP-2 release in WT VSMCs after 3 h. *$p < 0.01$ for stretched vs. unstretched; #$p < 0.01$ for p47$^{phox-/-}$ vs. WT; §$p < 0.01$ for stretched with inhibitor vs, stretched without inhibitor. Reproduced from Ref. 124 with permission.

Increased vessel wall NADPH oxidase-derived $O_2^{\bullet-}$ is an important determinant of endothelial dysfunction in experimental Ang II-induced hypertension, renovascular hypertension, DOCA-salt hypertension, and genetic hypertension (188, 190, 194, 209, 210, 371, 379). Consistent with an important role for activation of the renin–angiotensin system in human hypertension, treatment of hypertensive subjects with an AT_1 receptor antagonist improved endothelial function assessed by forearm flow-mediated dilator response to hyperaemia, and reduced markers of inflammation and oxidant stress (177). In patients with renovascular hypertension, impaired endothelium-dependent vasodilatation was correlated with excessive oxidative stress and both improved after surgery to correct renal artery stenosis (138). On the other hand, an important driver for vascular NADPH oxidase activation and endothelial dysfunction in low renin hypertension (often studied experimentally using unilateral nephrectomy and administration of deoxycorticosterone acetate [DOCA] plus salt) appears to be endothelin-1 (210, 379).

Endothelial NADPH oxidase activation at least partly driven by Ang II appears to also be largely responsible for the endothelial dysfunction found in models of early atherosclerosis, such as heritable Watanabe hypercholesterolemic rabbits or cholesterol-fed normal rabbits (349). Similarly, diet-induced atherosclerosis and endothelial dysfunction in primates is associated with increased NADPH oxidase-derived $O_2^{\bullet-}$ (130). In human coronary arteries from patients with coronary artery disease, Spiekermann *et al*. (316) found that endothelial dysfunction was attributable to increased $O_2^{\bullet-}$ from both NADPH oxidase and xanthine oxidase.

In diabetes, most evidence suggests an involvement of both NADPH oxidase and uncoupled eNOS in the genesis of endothelial dysfunction, for example, in aorta from streptozotocin-treated rats (142) and mice (10) as well as in arteries

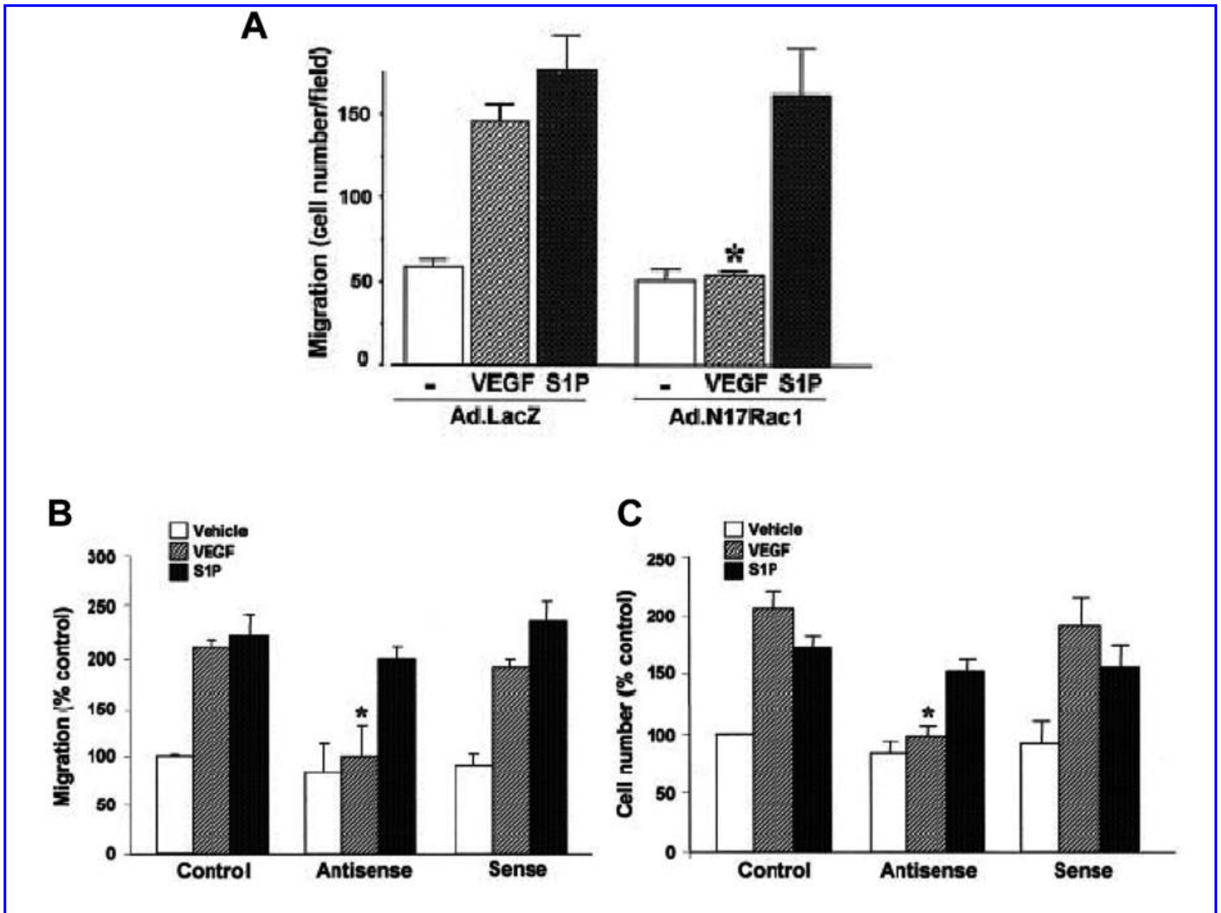

FIG. 10. Involvement of Nox2 and Rac in VEGF-induced endothelial cell migration and proliferation. (**A**) Transfection of HUVEC with Ad.N17Rac1 (dominant negative Rac1) significantly reduced cell migration when compared with control β-galactosidase transfected cells (Ad.LacZ). (**B**) Involvement of Nox2 in VEGF-induced cell migration and proliferation. HUVEC were transfected with reagent alone (control), Nox2 antisense or sense oligonucleotides. Cells were then stimulated with vehicle, VEGF or sphingosine 1-phosphate (S1P) and cell migration and proliferation assessed. Reduction of Nox2 abolished the increase in cell migration and proliferation in response to VEGF but not S1P. Adapted from Ref. 335 with permission.

from human diabetic patients undergoing coronary artery bypass surgery (127). Increased NADPH oxidase-derived $O_2^{\bullet -}$ may also contribute to endothelial dysfunction in heart failure, where the ensuing vascular dysfunction may contribute to increased loading of the heart and reduced exercise tolerance (186). In experimental heart failure induced by coronary ligation in rats, aortic endothelial dysfunction was attributable to increased $O_2^{\bullet -}$ production from NADPH oxidase (23). Similarly, our own group showed that NADPH oxidase-derived ROS contributed to impaired endothelium-dependent (NO-dependent) enhancement of left ventricular relaxation in experimental pressure overload cardiac hypertrophy and failure (225).

XIII. HYPERTENSION

Although the pathogenesis of hypertension is complex and multifactorial, a role for increased ROS generation has been suggested by many studies, especially in relation to Ang II-dependent hypertension (194). For example, vascular NADPH oxidase activity is increased in rats made hypertensive by chronic Ang II infusion (283), together with increases in the expression of Nox1, 2, and 4 (246) and p22*phox* mRNA (96). Similarly, Nox1 and Nox4 transcript levels were found to be higher in aortae of transgenic hypertensive rats overexpressing the Ren2 gene, compared to wild-type controls (356). Nox2 but not Nox4 mRNA levels were increased in artery ring segments of rabbits after aortic banding (268). In cerebral arteries of SHR, however, an increase in NADPH oxidase activity correlated with an upregulation in Nox4 but not Nox1 or Nox2 expression (267). It should be noted, however, that a causative relationship between increased oxidative stress and hypertension is much more contentious than that between oxidative stress and the endothelial dysfunction that often accompanies hypertension. In short-term Ang II-induced hypertension in mice, the infusion of a peptide inhibitor of NADPH oxidase attenuated the rise in blood pressure (286). Likewise, studies of p47*phox* knockout mice showed reduced levels of hypertension in response to chronic Ang II infusion (187). However, Touyz *et al*. (329) reported that the crossing of transgenic mice expressing human renin, which normally have an an-

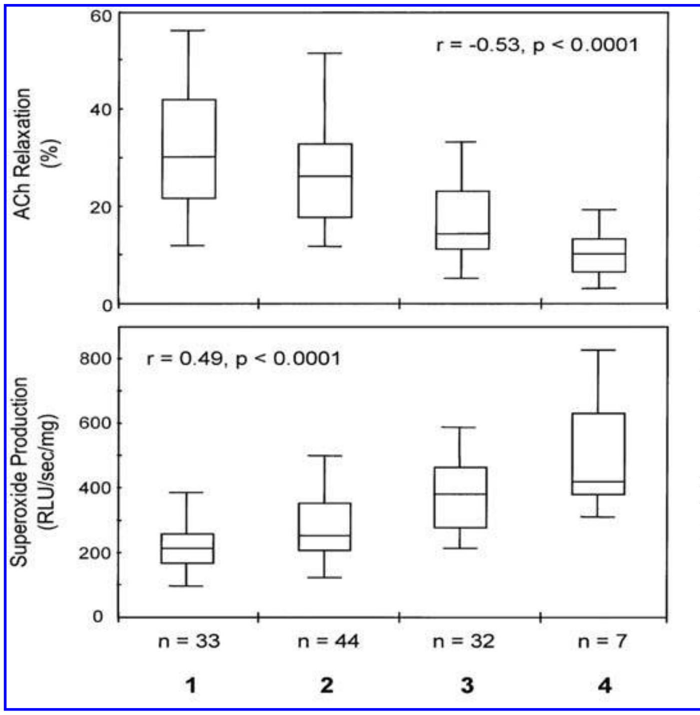

FIG. 11. Relationship between cardiovascular risk factors and maximal acetylcholine (Ach)-induced relaxation (*top panel*) or vascular NADPH –dependent superoxide production (*bottom panel*) in human saphenous veins. Risk factors were hypertension, diabetes mellitus, smoking, and hypercholesterolemia; 1 to 4 at the bottom indicates the number of risk factors. *Lines within boxes* represent median values; upper and lower limits of boxes are 75th and 25th percentiles, respectively; *upper and lower bars* of whiskers are 90th and 10th percentiles, respectively. Patients with greater numbers of risk factors had progressively higher superoxide production and lower Ach-induced relaxation. Reproduced from Ref. 128 with permission.

giotensin II-dependent hypertensive phenotype, with Nox2$^{-/-}$ mice did not prevent the development of hypertension. Alternative Nox isoforms may therefore be involved in short-term Ang II-driven hypertension. Dikalova *et al*. (75) recently reported that in a transgenic mouse with VSMC-specific overexpression of Nox1, Ang II-induced hypertension, and VSMC remodeling were significantly greater than in wild-type mice. In DOCA-salt hypertension in rats, where endothelin-1-dependent increases in NADPH oxidase activity seem to be important, a selective ET_A antagonist significantly reduced both ROS generation and hypertension (210).

The precise mechanism(s) involved in NADPH oxidase (ROS)-dependent hypertension remain to be established and could involve vascular or nonvascular pathways (such as altered regulation in the kidneys and brain). A large number of studies have implicated NADPH oxidase in vascular remodeling induced by hypertensive stimuli, for example, in response to Ang II infusion (340, 346, 373) (*discussed in earlier sections*). Furthermore, stretch-induced MMP-2 activation (and therefore potentially remodeling) in VSMCs was reported to be NADPH oxidase-dependent, being absent in p47$^{phox-/-}$ cells (124). However, several studies also show a dissociation between altered vascular $O_2^{\bullet-}$ and blood pressure (210, 311). Interestingly, recent studies suggest that cerebrovascular NADPH oxidase-derived ROS may contribute to the development of Ang II-induced hypertension (380).

XIV. ATHEROSCLEROSIS

A detailed discussion of the complex pathogenesis and pathophysiology of atherosclerosis is beyond the scope of this review but many aspects of this process are known to be redox-sensitive, for example, endothelial activation (*discussed earlier*), oxidative modification of lipids, the recruitment of immune and VSMC into atherosclerotic plaques, and VSMC proliferation (*discussed earlier*). Here, we focus specifically on evidence that implicates NADPH oxidase-derived ROS in one or more of these processes.

The accumulation of LDL at sites of atheromatous lesion predilection is crucial in the evolution of atherosclerosis. OxLDL is a potent stimulus for NADPH oxidase activation in EC (134) which contributes to the expression of adhesion molecules and recruitment of monocytes and other cells. Furthermore, it has been demonstrated that NADPH oxidase-derived ROS contribute to macrophage-mediated oxidation of LDL potentially leading to a vicious cycle (14). Once monocytes traverse the EC monolayer into the vessel wall, they transform into macrophages that avidly take up oxLDL to become foam cells. OxLDL also activates NADPH oxidase within macrophages which contributes to further ROS generation and amplification of the steps described thus far (134, 289). The subsequent process of VSMC migration (e.g., in response to growth factors such as PDGF) may also involve NADPH oxidase-derived ROS (321). Likewise, NADPH oxidase may contribute to VSMC proliferation within the atherosclerotic plaque (22).

Taken together, the above observations provide circumstantial support for the potential of NADPH oxidases to be involved in atherogenesis. Nevertheless, more direct evidence for a role of NADPH oxidases remains relatively limited. Barry-Lane *et al*. (22) crossed the apolipoprotein E knockout (apoE$^{-/-}$) mouse, which is predisposed to atherosclerotic lesions throughout the arterial tree but with a predilection for the aortic root, with p47$^{phox-/-}$ mice and found that lesion formation was significantly reduced in the descending aorta in p47^{phox}-deficient animals. However, a separate study using this double knockout mouse found comparable levels of ath-

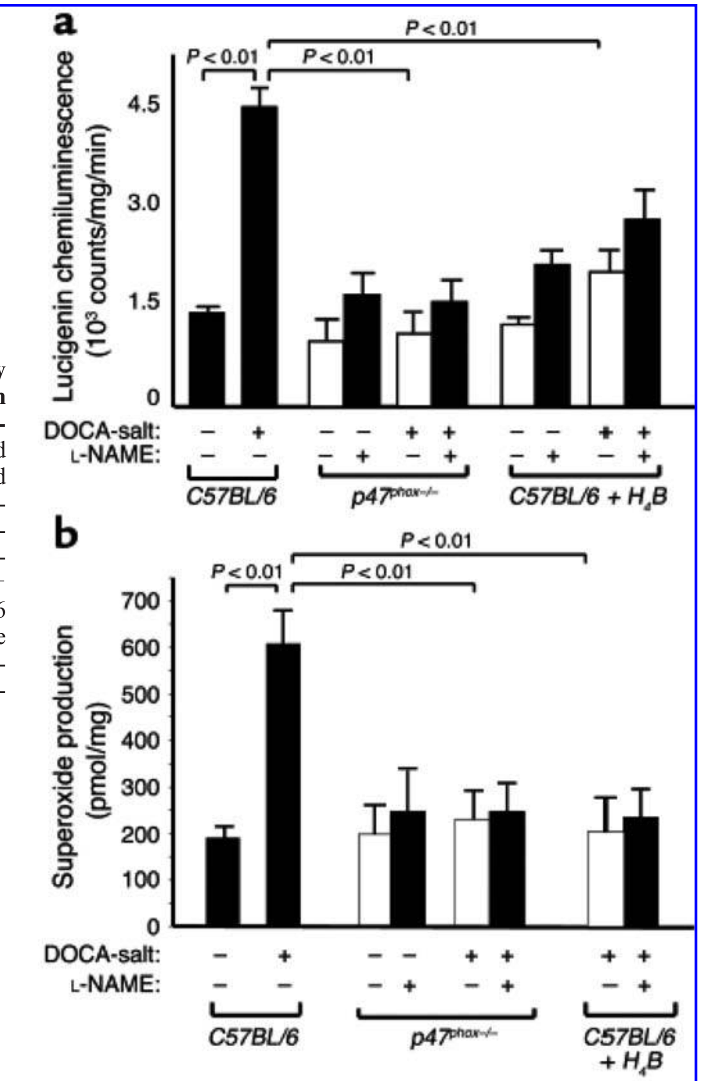

FIG. 12. Effect of NADPH oxidase deficiency (p47$^{phox-/-}$) and treatment with tetrahydrobiopterin (H_4B) on vascular $O\cdot_2^-$ production in hypertension induced by DOCA-salt. (**A**) $O\cdot_2^-$ production estimated by lucigenin-enhanced chemiluminescence in control and DOCA-salt hypertensive mice. (**B**) Vascular $O\cdot_2^-$ production measured by SOD-inhibitable cytochrome C reduction assay. DOCA-salt increased $O\cdot_2^-$ production in normal C57BL/6 mice but not p47$^{phox-/-}$ mice. $O\cdot_2^-$ production was also inhibited in hypertensive C57BL/6 mice treated with H_4B. The data demonstrate that in the absence of a functional NADPH oxidase, eNOS uncoupling does not occur in DOCA-salt hypertension. Reproduced from Ref. 188 with permission.

erosclerosis at the aortic root to wild-type animals, but importantly the descending aorta was not examined (151). Interestingly, apoE$^{-/-}$ mice are reported to have higher vascular expression of Nox1 relative to wild-type littermate controls (30). Nox1 mRNA was also found to be upregulated in both minimally and terminally diseased human coronary arteries (192), as was p22^{phox} expression in atherosclerotic coronary arteries (15, 313). In more advanced lesions, it appears that an infiltration of macrophages contributes significantly to increased NADPH oxidase expression and activity (15, 167, 313, 323). Nox1, Nox2, and p22^{phox} mRNAs were significantly increased within rat arteries during the early stages of restenosis after balloon injury (323). The latter could correspond to an upregulation in oxidase activity in VSMC and the adventitia respectively. By contrast, Nox4 expression in these experiments was not altered during the early stages of restenosis but increased in the neointima during the redifferentiation phase after cellular proliferation had ceased (323). The expression of p67^{phox} and p47^{phox} protein, assessed by immunocytochemistry, has also been suggested to increase in the adventitial fibroblasts of porcine coronary arteries after balloon-induced injury (302). In primates, Hathaway *et al.* (130) were able to correlate superoxide levels and expression of p22^{phox} and p47^{phox} subunits, with diet-induced atherosclerosis over a 4 year period and subsequent regression whilst on a normal diet over an 8 month period. These data have been further corroborated by human studies. Increased expression of the p22^{phox} subunit was found throughout the wall of human coronary atherosclerotic vessels (15). Finally, not only was Nox subunit expression found to be associated with the severity of atherosclerosis in human coronary arteries, but also greater superoxide was detected in the plaque shoulder, suggesting a possible role of NADPH oxidase in plaque instability (313).

XV. DIABETES MELLITUS

A substantial body of evidence implicates oxidative stress as an important pathogenic factor in diabetic cardiovascular complications, in both Type I and Type II diabetes mellitus. The drivers of this oxidative stress include hyperglycemia,

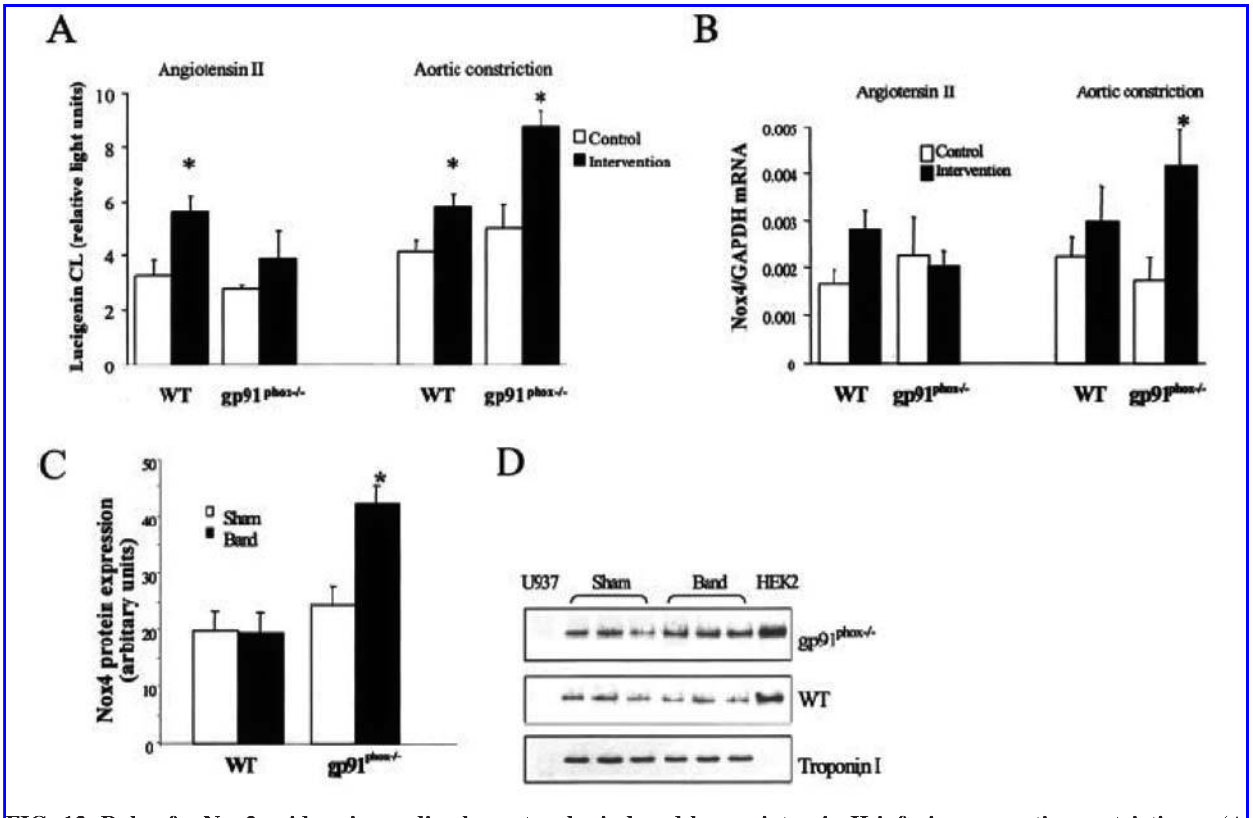

FIG. 13. Role of a Nox2 oxidase in cardiac hypertrophy induced by angiotensin II infusion or aortic constriction. (**A**) NADPH oxidase activity measured by lucigenin chemiluminescence (CL). (**B**) Nox4 mRNA expression, normalized to GAPDH levels (real-time RT-PCR). (**C**) Nox4 protein expression in LV homogenates from wild-type and gp91$^{phox-/-}$ mice after aortic constriction or sham surgery (mean data). (**D**) Representative immunoblot showing increased Nox4 protein expression (~65 kDa) in Nox2$^{-/-}$ animals following aortic constriction. HEK2 cell protein was a positive control for Nox4, U937 cell protein a negative control, and troponin I was a loading control. *$p < 0.05$ for treated vs. respective control group (*panels A–C*). Reproduced from Ref. 38 with permission.

hyperinsulinemia, and the elevated free fatty acids and lipids that are usually associated with diabetes. Hyperglycemia promotes the production of ROS and nitrogen species (RNS) in many cell types (142, 197, 280, 376) and when present chronically also promotes the formation of AGEs which themselves are capable of inducing ROS production (351, 358, 364, 367). Hyperglycemia-induced ROS production undoubtedly emanates from several different sources, including mitochondria and uncoupled NOSs, but NADPH oxidases are important source in many settings. An NADPH oxidase inhibitor, apocynin, as well as a PKC inhibitor inhibited vascular ROS generation in three different animal models of diabetes, namely streptozotocin-induced diabetes (a model of Type 1 diabetes), obese ob/ob mice, and Zucker fatty rats (both models of Type II diabetes) (312). In cultured aortic VSMC and EC, exposure to high glucose for 72 h also significantly increased ROS production, which was inhibited by either DPI or a PKC inhibitor (156). In isolated rat cardiomyocytes, incubation with high glucose for 24 h resulted in an enhanced free radical production and significant contractile dysfunction which was prevented by an AT_1 receptor antagonist, DPI or apocynin (280). In glomerular mesangial cells, however, high glucose-induced ROS production was effectively blocked by rotenone, an inhibitor of the mitochondrial electron transport chain complex I, as well as a PKC inhibitor, DPI or apocynin (197). In bovine aortic EC, Nishikawa *et al*. (255) demonstrated that hyperglycemia-induced ROS production was prevented by an inhibitor of electron transport chain complex II, an uncoupler of oxidative phosphorylation, uncoupling protein-1 or manganese superoxide dismutase but not by rotenone (255). In contrast, Hink *et al*. (142) identified both uncoupled NOS and NADPH oxidase as $O_2^{\bullet-}$ sources in aorta from rats subjected to streptozocin-induced diabetes, in association with a seven-fold increase in Nox2 mRNA(142). Consistent with an important role for Nox2 in mediating the effects of glycated proteins, macrophages retrieved from Nox2$^{-/-}$ mice displayed a complete inhibition of AGE-induced tissue factor activity (351). Similarly, the induction of ROS and VCAM-1 expression by AGE in HUVEC was significantly inhibited by both apocynin and DPI (22). Apocynin also significantly inhibited AGE-induced NF-κB translocation (22). Recently, we have shown that glycated proteins also induce Nox2 oxidase activation in isolated cardiomyocytes (377). The potential for such increased ROS production to promote abnormalities such as endothelial dysfunction or atherosclerosis was discussed in earlier sections.

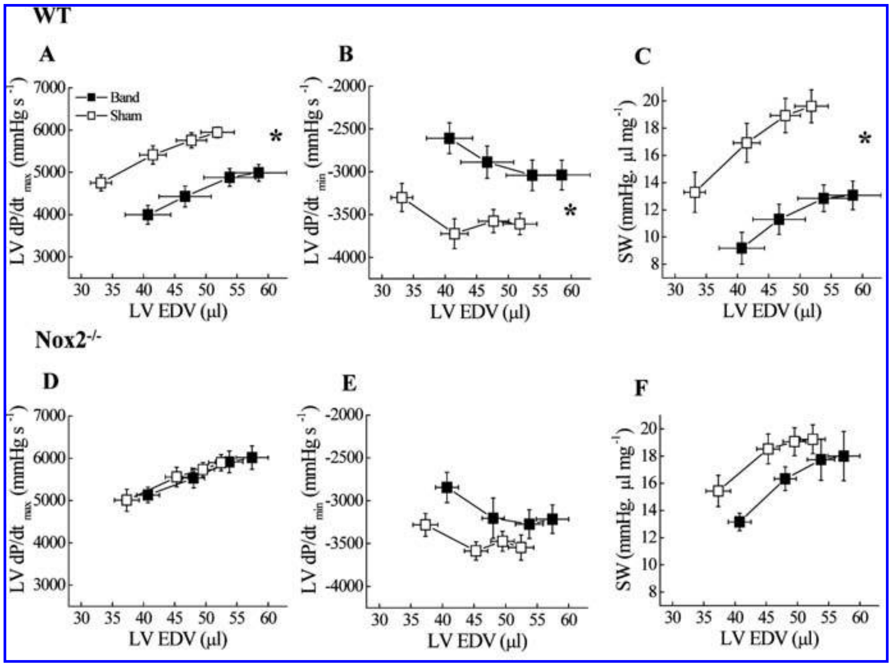

FIG. 14. LV function in isolated ejecting hearts from WT and Nox2-/- mice across a range of LV end-diastolic volumes (EDV). Changes in (**A, D**) LVdP/dtmax; (**B, E**) LVdP/ dtmin; (**C, F**) stroke work (SW). Data are means ± SEM from 8 animals; *$p < 0.05$ aortic banded (■) versus sham (□). Data demonstrate a marked protection against contractile dysfunction induced by aortic constriction in Nox2−/− animals. Reproduced from Ref. 120 with permission.

XVI. CARDIAC HYPERTROPHY

Chronic heart failure (CHF) occurs secondary to long-standing increases in heart workload, most commonly due to hypertension (pressure overload) or ischemic heart disease (322). The heart adapts to increased systemic pressure load through left ventricular hypertrophy (LVH), which involves alterations in cardiomyocyte, extracellular matrix, and coronary vessel structure and function. Persistent LVH usually progresses to contractile depression, cardiac dilatation, and CHF. Growing evidence supports an important role for oxidative stress and redox signaling in the pathophysiology of LVH. For example, hypertrophy of isolated cardiomyocytes induced by α-adrenergic agonists, Ang II, endothelin-1, TNFα, or cyclic stretch has been shown to involve increased ROS production (143, 251, 275). *In vivo*, the development of pressure overload LVH in mice or the transition from compensated to decompensated pressure overload LVH in guinea pigs are inhibited by antioxidants (63, 71). Patients with CHF also have evidence of increased oxidative stress which has been correlated with myocardial and endothelial dysfunction and overall severity of heart failure (89, 148, 227, 236).

While the sources of ROS production and the mechanisms by which ROS exert pathophysiological effects remain under investigation, a significant role for NADPH oxidases has been suggested. Of interest, many stimuli that activate NADPH oxidases (e.g., cyclic stretch, Ang II, α-adrenergic agonists, endothelin-1 and TNF-α (65, 66, 210, 336) are relevant to LVH and heart failure pathophysiology. In isolated rat cardiomyocytes (355, 362) hypertrophy induced by Ang II, endothelin-1, and norepinephrine may at least in part involve NADPH oxidases as evidenced by the use of oxidase inhibitors and the involvement of Rac1 (139, 277, 325). In experimental pressure overload LVH in guinea pigs, myocardial NADPH oxidase subunit expression and activity were increased in parallel with MAPK activation; oxidase expression was documented in both cardiomyocytes and EC in this study (203). Similar NADPH oxidase activation is observed in murine pressure overload LVH (38, 121, 235). Recently, it was confirmed that myocardium from end-stage human CHF patients demonstrated increased NADPH oxidase activity (137, 224).

More direct evidence for an involvement of NADPH oxidases in LVH comes from studies in Nox2$^{-/-}$ mice. In a model of short-term (7–14 days) subpressor Ang II infusion, both myocardial NADPH oxidase activation and the development of *in vivo* hypertrophy were significantly inhibited in Nox2$^{-/-}$ mice (Fig. 13) (29). In the setting of pressure overload induced by aortic banding, however, both morphological LVH and the associated rises in molecular markers such as ANF mRNA were similar in Nox2$^{-/-}$ and wild-type mice (38, 235). Interestingly, however, aortic banding significantly increased LV NADPH oxidase activity and *in situ* $O_2^{\bullet-}$ production not only in wild-type but also Nox2$^{-/-}$ mice, which was attributable to increased Nox4 expression in the banded Nox2$^{-/-}$ animals (38). Furthermore, chronic treatment of banded Nox2$^{-/-}$ mice with the antioxidant N-acetyl-cysteine significantly reduced the extent of LVH (38). These results suggest that, while Nox2 is pivotally involved in the development of Ang II-induced hypertrophy, LVH induced by pressure overload could be more dependent on Nox4. Nonetheless, further studies suggest that Nox2 plays an important role in the contractile dysfunction that accompanies pressure overload LVH even though it does not alter the extent of hypertrophy *per se*. Using pressure-volume analyses as well as echocardiography, we found that banded Nox2$^{-/-}$ mice were significantly protected against the LV systolic and diastolic dysfunction that occurred with banding in wild-type animals (Fig. 14) (120). Taken together, these data suggest distinct roles for Nox2 and Nox4 in different components of the overall hypertrophic response to pressure overload with the two isoforms exhibiting different activation as well as distinct downstream effects.

Potential redox-sensitive downstream signaling pathways that may be influenced by NADPH oxidase activation in the heart include RAS, the MAPKs (p38MAPK, ERK1/2, JNK), c-src, p90RSK, the PI3 kinase (PI3K)/Akt pathway, AP-1, NF-κB, HIF-1, and others (4, 77, 110, 136, 182, 191, 361). However, the involvement of NADPH oxidase in activating these pathways remains poorly characterized in cardiomyocytes. The small GTPase RAS has been suggested to be a redox-sensitive signaling switch in many cell types, and in NIH3T3 fibroblasts stably-transformed with a constitutively active isoform of p21Ras, H-Ras^{v12}, it was suggested to mediate NADPH oxidase activation (159). In keeping with such a mechanism, it was recently reported that α-adrenoceptor-induced hypertrophic signaling in rat cardiomyocytes involved a posttranslational oxidative modification of RAS, with downstream phosphorylation of MEK1/2, ERK1/2, and p90SRK (182, 361). Many studies have shown that activation of several members of the MAPK family is redox-sensitive (118, 136, 174, 182, 215, 361), but the possible role of NAPDH oxidase-dependent MAPK activation in the heart remains speculative.

XVII. CARDIAC REMODELING AND FIBROSIS

Interstitial fibrosis contributes significantly to the pathophysiology of cardiac dysfunction associated with LVH, myocardial ischemia, senescence, inflammatory processes, and diabetes. Under these conditions, interstitial fibroblasts transform into myofibroblasts that express α-smooth muscle actin, angiotensin converting enzyme, high densities of Ang II receptors, and various MMPs and tissue inhibitors of MMP (TIMPs) (322). Following significant myocardial infarction, the heart typically dilates and becomes more spherical over a period of weeks and months in a process known as adverse remodeling, which is associated with alterations in contractile function and the development of CHF. Like fibrosis, adverse cardiac remodeling involves profound alterations in the composition of the extracellular matrix. Both fibrosis and remodeling are therefore markedly influenced by the balance between collagen deposition and matrix degradation, the latter being modulated largely by the activity of MMPs and TIMPS (16).

Persuasive evidence implicates intracellular redox balance as a key regulator of fibrosis and remodeling. Oxidative stress is profibrotic in the liver, lungs, kidney, and vasculature (276), and ROS modulate fibroblast proliferation and their transformation into matrix-producing myofibroblasts (13, 159). Profibrotic stimuli such as Ang II (216, 320), aldosterone (320), and cyclic load (181) all stimulate intracellular ROS production as discussed earlier. In addition, many signaling pathways and transcription factors implicated in fibrogenesis are redox-sensitive (32, 181, 253). Notably, MMP expression and activation are exquisitely redox-sensitive (13, 93, 248, 308). In the context of remodeling post-MI, local activation of the renin–angiotensin system may be important in increasing ROS production (41, 319).

Emerging evidence supports a role for NADPH oxidase in interstitial cardiac fibrosis and remodeling. Although a profibrotic role of Ang II is well recognized (29, 132, 165, 287), the involvement of NADPH oxidase in this process has been unclear. We addressed this question in Nox2$^{-/-}$ mice infused with Ang II and found that Ang II-induced increases in interstitial cardiac fibrosis were completely abolished independent of the hypertrophic and pressor efforts of Ang II (29, 165). Furthermore, mRNA expression of procollagen 1 and III and connective tissue growth factor (CTGF) as well as MMP-2 activation were suppressed in Ang II-treated Nox2$^{-/-}$ mice compared to wild type (165). Aldosterone is also a potent profibrotic agent and has recently been reported to activate vascular p38MAPK and NADPH oxidase (40). In line with this, we found in an experimental model of aldosterone-driven interstitial cardiac fibrosis that this was inhibited in Nox2$^{-/-}$ mice (165). Sun *et al*. (320) also reported evidence of increased myocardial oxidative stress together with increased Nox2 expression in a similar model although a cause–effect relationship between these observations was not established. Nox2 also appears to be profibrotic in pressure-overload LVH since we found that Nox2-deficient mice subjected to aortic banding had reduced interstitial fibrosis compared to banded wild-type controls (120). Thus, these studies strongly support a specific role of Nox2 in the development of cardiac fibrosis (Fig.15). However, the role of different cell types in this response remains unclear. Both Nox2 (263) and Nox4 (44) are expressed in aortic adventitial fibroblasts of rabbit and mouse, but in human cardiac fibroblasts it was recently reported that the main isoforms expressed at mRNA level were Nox4 and Nox5, whereas Nox1 and Nox2 were barely detectable (58). In the latter study, TGFβ-1 potently upregulated Nox4 mRNA expression and oxidase activity which led to increased expression of the myofibroblast marker smooth muscle α-actin (58). However, the role of Nox4 in mediating *in vivo* fibrosis was not addressed. Taken together, these results could be consistent with a role for both Nox2 (in nonfibroblasts) and Nox4 (in fibroblasts) in *in vivo* fibrosis or they could indicate significant species-specific differences.

NADPH oxidase could have a similarly important role in adverse cardiac remodeling but this remains an area under continuing investigation. An increase in ROS production and oxidative stress is well recognized to occur post-MI (140, 176, 307), and antioxidant treatment (e.g., with dimethylthiourea or probucol) reportedly attenuates LV remodeling following MI by attenuating increases in collagen volume fraction and MMP activity (176, 306). An increased myocardial expression of the NADPH oxidase subunits, Nox2 and p22^{phox}, has been reported after MI both in animal models (97, 220) and human myocardium (180). In recent preliminary studies in Nox2$^{-/-}$ mice subjected to coronary ligation, we have found that cardiac remodeling is significantly reduced compared to wild type, supporting an important role for Nox2 in this process (unpublished data).

XVIII. MYOCARDIAL ISCHEMIA–REPERFUSION AND CARDIOPROTECTION

Oxidative stress is increased in cellular and experimental models of ischemia–reperfusion injury, with reperfusion thought to be the more potent stimulus for ROS production

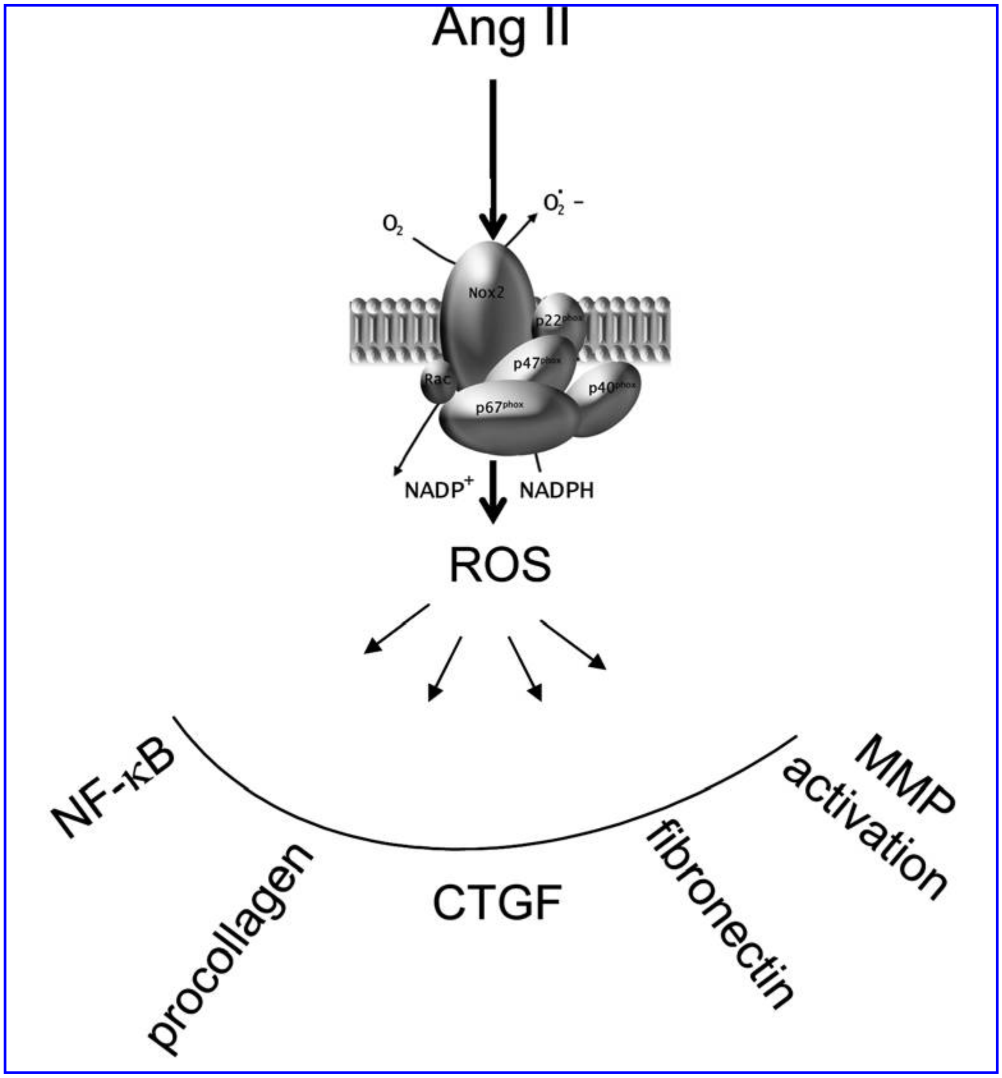

FIG. 15. Schematic illustrating the downstream profibrotic effects of NADPH oxidase-derived ROS following chronic Ang II infusion. CTGF, connective tissue growth factor; MMP, matrix metalloproteinase.

(26, 173). However, as yet, no convincing evidence for an involvement of NADPH oxidases in this process has been reported. Indeed, myocardial infarction following 30 min ischemia and 24 h reperfusion in p47$^{phox-/-}$ mice was not found to be significantly different from wild-type mice (146).

In contrast, a recent study suggests that NADPH oxidase-derived ROS may play a significant role in the signaling of early ischemic preconditioining (i.e., may have beneficial effects). Bell *et al*. (27) showed that ischemic preconditioning-induced reductions in infarct size were abolished in Nox2$^{-/-}$ mice although these animals could be preconditioned by an adenosine analogue, suggesting a significant role for Nox2 in the adaptive response to brief ischemia.

XIX. SEPSIS

The systemic sepsis syndrome (e.g., in response to gram negative bacterial infection) is characterized by hypotension, vascular hyporeactivity, intrinsic cardiac depression, and multiorgan dysfunction, and has a high mortality despite treatment (123, 270). Significant oxidative stress is a well-recognized feature of the syndrome (33, 51), at least in part the result of inflammatory cytokine-induced production of ROS (51, 92, 291). The increased ROS production may contribute to cardiac contractile depression and reversible injury (101, 226) and endotoxemia-induced dysfunction is significantly decreased in transgenic mice overexpressing either human extracellular glutathione peroxidase or the intracellular isoform (242). A few studies suggest that NADPH oxidase-derived ROS may contribute to the oxidative stress. DeLeo *et al*. (67) demonstrated that LPS rendered neutrophils more responsive to other stimuli as a result of increased translocation of Rac2, p47^{phox}, and p67^{phox} (i.e., “priming”). Sanlioglu *et al*. (294) also reported that LPS induced Rac1-dependent ROS production and TNFα secretion in macrophages. Ben Shaul *et al.* (28) found that NADPH oxidase activity increased in rat hearts subjected to LPS injection *in vivo*, while pretreatment with the oxidase inhibitor apocynin significantly reduced mortality. Similarly, LPS treatment of rats enhanced vascular expression of p22^{phox}, p67^{phox}, Nox2, and xanthine oxidase, and increased O_2^- and $ONOO^-$ formation (33). ROS formation was partially sensitive to both DPI and the xanthine oxidase inhibitor oxypurinol but scavenging of O_2^- did not restore endothelial dysfunction (33). More recently, a study in isolated cultured mouse neonatal cardiomyocytes showed that LPS-induced TNFα expression and myocardial depression involved a Nox2 oxidase (273).

XX. CONCLUSIONS

The knowledge that increased oxidative stress plays important roles in the pathophysiology of many cardiovascular disorders has naturally led to consideration of the potential therapeutic benefit of antioxidant agents. Although treatment with antioxidants or with SOD has been found to be effective in reducing markers of oxidative stress and improving functional parameters such as endothelium-dependent relaxation in many settings, the results of large antioxidant trials in patients at risk of cardiovascular morbidity and mortality have been singularly disappointing (163). However, recent advances in our understanding of the complexity of oxidative stress and redox signaling, as well as the concept that ROS production may be specifically regulated by distinct stimuli and pathways, leads to renewed enthusiasm regarding therapeutic possibilities. The NADPH oxidases provide perhaps the best example of an enzyme system that appears to be specifically designed for redox signaling. Furthermore, the complexity of their regulation may in itself provide the possibility of targeted therapeutic manipulation in cell-, tissue- and pathway-specific manners at appropriate points in the disease process. Instead of biologically inefficient and nonspecific antioxidants (e.g., vitamins) that have been disappointing, targeted drugs may be more promising. Indeed, several successful existing drugs are now appreciated to exert at least part of their effects in this manner (e.g., statins, ACE inhibitors, and AT_1 antagonists). Further understanding of the detailed mechanisms and roles of ROS sources such as the NADPH oxidase family in cardiovascular disorders should provide the basis for devising novel therapies for some of these conditions.

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ABBREVIATIONS

AA, arachidonic acid; AGE, advanced glycation end products; Ang II, angiotensin II; CGD, chronic granulomatous disease; CHF, chronic heart failure; CTGF, connective tissue growth factor (CTGF); DOCA, deoxycorticosterone acetate; DPI, diphenylene iodonium; EC, endothelial cells; EDHF, endothelium-derived hyperpolarizing factor; GEF, guanine nucleotide exchange factor; HIF-1, hypoxia-inducible factor-1; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IFNγ, interferon-γ; IL-1, interleukin 1; LPS, lipopolysaccharide; LVH, left ventricular hypertrophy; MMP, matrix metalloproteinases; NO, nitric oxide; NOS, nitric oxide synthase; Nox, NADPH oxidase; NoxO1, Nox organizer 1; NoxA1, Nox activator 1; oxLDL, oxidized low density lipoprotein; PAK, p21 activated kinase; PDGF, platelet derived growth factor; PKC, protein kinase C; PMA, phorbol myristate; ROS, reactive oxygen species; RNS, reactive nitrogen species; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase; TGFβ, transforming growth factor β; TIMPs, tissue inhibitors of MMP; TNFα, tumor necrosis factor α; TRAF4, TNF receptor-associated factor 4; VEGF, vascular endothelial growth factor; VCAM-1, vascular cell adhesion molecule-1; VSMC, vascular smooth muscle cells.

REFERENCES

1. Abid MR, Kachra Z, Spokes KC, and Aird WC. NADPH oxidase activity is required for endothelial cell proliferation and migration. *FEBS Lett* 486: 252–256, 2000.
2. Acker H. Mechanisms and meaning of cellular oxygen sensing in the organism. *Respir Physiol* 95: 1–10, 1994.
3. Adachi T, Togashi H, Suzuki A, Kasai S, Ito S, Sugahara K, and Kawata S. NADPH oxidase plays a crucial role in PDGF-induced proliferation of hepatic stellate cells. *Hepatology* 41: 1272–1281, 2005.
4. Adachi T, Pimentel DR, Heibeck T, Hou X, Lee YJ, Jiang B, Ido Y, and Cohen RA. S-glutathiolation of Ras mediates redox-sensitive signaling by angiotensin II in vascular smooth muscle cells. *J Biol Chem* 279: 29857–29862, 2004.
5. Ago T, Kuribayashi F, Hiroaki H, Takeya R, Ito T, Kohda D, and Sumimoto H. Phosphorylation of p47phox directs phox homology domain from SH3 domain toward phosphoinositides, leading to phagocyte NADPH oxidase activation. *Proc Natl Acad Sci USA* 100: 4474–4479, 2003.
6. Ago T, Nunoi H, Ito T, and Sumimoto H. Mechanism for phosphorylation-induced activation of the phagocyte NADPH oxidase protein p47(phox). Triple replacement of serines 303, 304, and 328 with aspartates disrupts the SH3 domain-mediated intramolecular interaction in p47 (phox), thereby activating the oxidase. *J Biol Chem* 274: 33644–33653, 1999.
7. Ago T, Kitazono T, Kuroda J, Kumai Y, Kamouchi M, Ooboshi H, Wakisaka M, Kawahara T, Rokutan K, Ibayashi S, and Iida M. NAD(P)H oxidases in rat basilar arterial endothelial cells. *Stroke* 36: 1040–1046, 2005.
8. Ago T, Kitazono T, Ooboshi H, Iyama T, Han YH, Takada J, Wakisaka M, Ibayashi S, Utsumi H, and Iida M. Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase. *Circulation* 109: 227–233, 2004.
9. Al-Mehdi AB, Zhao G, Dodia C, Tozawa K, Costa K, Muzykantov V, Ross C, Blecha F, Dinauer M, and Fisher AB. Endothelial NADPH oxidase as the source of oxidants in lungs exposed to ischemia or high K^+. *Circ Res* 83: 730–737, 1998.

10. Alp NJ, Mussa S, Khoo J, Cai S, Guzik T, Jefferson A, Goh N, Rockett KA, and Channon KM. Tetrahydrobiopterin-dependent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I overexpression. *J Clin Invest* 112: 725–735, 2003.
11. Ambasta RK, Kumar P, Griendling KK, Schmidt HHHW, Busse R, and Brandes RP. Direct Interaction of the novel Nox nroteins with p22^{phox} is required for the formation of a functionally active NADPH oxidase. *J Biol Chem* 279: 45935–45941, 2004.
12. Archer SL, Reeve HL, Michelakis E, Puttagunta L, Waite R, Nelson DP, Dinauer MC, and Weir EK. 0_2 sensing is preserved in mice lacking the gp91^{phox} subunit of NADPH oxidase. *Proc Natl Acad Sci USA* 96: 7944–7949, 1999.
13. Arnold RS, Shi J, Murad E, Whalen AM, Sun CQ, Polavarapu R, Parthasarathy S, Petros JA, and Lambeth JD. Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1. *PNAS* 98: 5550–5555, 2001.
14. Aviram M, Rosenblat M, Etzioni A, and Levy R. Activation of NADPH oxidase required for macrophage-mediated oxidation of low-density lipoprotein. *Metabolism* 45: 1069–1079, 1996.
15. Azumi H, Inoue N, Takeshita S, Rikitake Y, Kawashima S, Hayashi Y, Itoh H, and Yokoyama M. Expression of NADH/NADPH oxidase p22^{phox} in human coronary arteries. *Circulation* 100: 1494–1498, 1999.
16. Baker AH, Zaltsman AB, George SJ, and Newby AC. Divergent effects of tissue inhibitor of mettalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death *in vitro*. TIMP-3 promotes apoptosis. *J Clin Invest* 101: 1478–1487, 1998.
17. Banfi B, Clark RA, Steger K, and Krause KH. Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. *J Biol Chem* 278: 3510–3513, 2003.
18. Banfi B, Malgrange B, Knisz J, Steger K, Dubois-Dauphin M, and Krause KH. NOX3, a superoxide-generating NADPH oxidase of the inner ear. *J Biol Chem* 279: 46065–46072, 2004.
19. Banfi B, Maturana A, Jaconi S, Arnaudeau S, Laforge T, Sinha B, Ligeti E, Demaurex N, and Krause KH. A mammalian H^+ channel generated through alternative splicing of the NADPH oxidase homolog NOH-1. *Science* 287: 138–142, 2000.
20. Banfi B, Molnar G, Maturana A, Steger K, Hegedus B, Demaurex N, and Krause KH. A Ca^{2+}-activated NADPH oxidase in testis, spleen, and lymph nodes. *J Biol Chem* 276: 37594–37601, 2001.
21. Banfi B, Tirone F, Durussel I, Knisz J, Moskwa P, Molnar GZ, Krause KH, and Cox JA. Mechanism of Ca^{2+} activation of the NADPH oxidase 5 (NOX5). *J Biol Chem* 279: 18583–18591, 2004.
22. Barry-Lane PA, Patterson C, van der MM, Hu Z, Holland SM, Yeh ET, and Runge MS. p47^{phox} is required for atherosclerotic lesion progression in ApoE$^{(-/-)}$ mice. *J Clin Invest* 108: 1513–1522, 2001.
23. 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.
24. Bayraktutan U, Blayney L, and Shah AM. Molecular characterization and localization of the NAD(P)H oxidase components gp91-^{phox} and p22-^{phox} in endothelial cells. *Arterioscler Thromb Vasc Biol* 20: 1903–1911, 2000.
25. Bayraktutan U, Draper N, Lang D, and Shah AM. Expression of a functional neutrophil-type NADPH oxidase in cultured rat coronary microvascular endothelial cells. *Cardiovasc Res* 38: 256–262, 1998.
26. Becker LB, Vanden Hoek TL, Shao ZH, Li CQ, and Schumacker PT. Generation of superoxide in cardiomyocytes during ischemia before reperfusion. *Am J Physiol* 277: H2240–H2246, 1999.
27. Bell RM, Cave AC, Johar S, Hearse DJ, Shah AM, and Shattock MJ. Pivotal role of NOX-2-containing NADPH oxidase in early ischemic preconditioning. *FASEB J*. 19: 2037–2039, 2005.
28. Ben Shaul V, Lomnitski L, Nyska A, Zurovsky Y, Bergman M, and Grossman S. The effect of natural antioxidants, NAO and apocynin, on oxidative stress in the rat heart following LPS challenge. *Toxicology Lett* 123: 1–10, 2001.
29. Bendall JK, Cave AC, Heymes C, Gall N, and Shah AM. Pivotal role of a gp91^{phox}-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. *Circulation* 105: 293–296, 2002.
30. Bengtsson SH, Gulluyan LM, Dusting GJ, and Drummond GR. Novel isoforms of NADPH oxidase in vascular physiology and pathophysiology. *Clin Exper Pharmacol Physiol* 30: 849–854, 2003.
31. Bey EA, Xu B, Bhattacharjee A, Oldfield CM, Zhao X, Li Q, Subbulakshmi V, Feldman GM, Wientjes FB, and Cathcart MK. Protein kinase Cd is required for p47^{phox} phosphorylation and translocation in activated human monocytes. *J Immunol* 173: 5730–5738, 2004.
32. Bishop JE, Lindahl G. Regulation of cardiovascular collagen synthesis by mechanical load. *Cardiovasc Res* 42: 27–44, 1999.
33. Brandes RP, Koddenberg G, Gwinner W, Kim DY, Kruse HJ, Busse R, and Mugge A. Role of increased production of superoxide anions by NAD(P)H oxidase and xanthine oxidase in prolonged endotoxemia. *Hypertension* 33: 1243–1249, 1999.
34. Brandes RP, Kreuzer J. Vascular NADPH oxidases: molecular mechanisms of activation. *Cardiovasc Res* 65: 16–27, 2005.
35. Brewer AC, Sparks EC, and Shah AM. Transcriptional regulation of the NADPH oxidase isoform, Nox1, in colon epithelial cells:Role of GATA binding factor(s). *Free Rad Biol Med* 40: 260–274, 2006.
36. Brown MR, Miller FJ, Jr., Li WG, Ellingson AN, Mozena JD, Chatterjee P, Engelhardt JF, Zwacka RM, Oberley LW, Fang X, Spector AA, and Weintraub NL. Overexpression of human catalase inhibits proliferation and promotes apoptosis in vascular smooth muscle cells. *Circ Res* 85: 524–533, 1999.
37. Bunn HF, Poyton RO. Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* 76: 839–885, 1996.

38. Byrne JA, Grieve DJ, Bendall JK, Li JM, Gove C, Lambeth JD, Cave AC, and Shah AM. Contrasting roles of NADPH oxidase isoforms in pressure-overload versus angiotensin II-induced cardiac hypertrophy. *Circ Res* 93: 802–805, 2003.
39. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 87: 840–844, 2000.
40. Callera GE, Touyz RM, Tostes RC, Yogi A, He Y, Malkinson S, and Schiffrin EL. Aldosterone activates vascular p38MAP kinase and NADPH oxidase via c-Src. *Hypertension* 45: 773–779, 2005.
41. Campbell SE, Katwa LC. Angiotensin II stimulated expression of transforming growth factor-b_1 in cardiac fibroblasts and myofibroblasts. *J Mol Cell Cardiol* 29: 1947–1958, 1997.
42. Castier Y, Brandes RP, Leseche G, Tedgui A, and Lehoux S. $p47^{phox}$-dependent NADPH oxidase regulates flow-induced vascular remodeling. *Circ Res* 97: 533–540, 2005.
43. Chai Y-C, Ashraf SS, Rokutan K, Johnston RB, and Thomas JA. S-thiolation of individual human neutrophil proteins including actin by stimulation of the respiratory burst: Evidence against a role for glutathione disulfide. *Arch Biochem Biophys* 310: 273–281, 1994.
44. Chamseddine AH, Miller FJ, Jr. gp91phox contributes to NADPH oxidase activity in aortic fibroblasts but not smooth muscle cells. *Am J Physiol* 285: H2284–H2289, 2003.
45. Chappell DC, Varner SE, Nerem RM, Medford RM, and Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. *Circ Res* 82: 532–539, 1998.
46. Chen Q, Powell DW, Rane MJ, Singh S, Butt W, Klein JB, and McLeish KR. Akt phosphorylates $p47^{phox}$ and mediates respiratory burst activity in human neutrophils. *J Immunol* 170: 5302–5308, 2003.
47. Chen XL, Zhang Q, Zhao R, Ding X, Tummala PE, and Medford RM. Rac1 and superoxide are required for the expression of cell adhesion molecules induced by tumor necrosis factor-alpha in endothelial cells. *J Pharmacol Exp Ther* 305: 573–580, 2003.
48. Chen XL, Zhang Q, Zhao R, and Medford RM. Superoxide, H_2O_2, and iron are required for TNF-a-induced MCP-1 gene expression in endothelial cells: role of Rac1 and NADPH oxidase. *Am J Physiol Heart Circ Physiol* 286: H1001–H1007, 2004.
49. Cheng G, Cao Z, Xu X, Van Meir EG, and Lambeth JD. Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene* 269: 131–140, 2001.
50. Cheng G, Lambeth JD. NoxO1, regulation of lipid binding, localization, and activiation of Nox1 by the phox homology (PX) domain. *J Biol Chem* 279: 4737–4742, 2004.
51. Cheng XS, Shimokawa H, Momii H, Oyama J, Fukuyama N, Egashira K, Nakazawa H, and Takeshita A. Role of superoxide anion in the pathogenesis of cytokine-induced myocardial dysfunction in dogs in vivo. *Cardiovasc Res* 42: 651–659, 1999.
52. Chowdhury AK, Watkins T, Parinandi NL, Saatian B, Kleinberg ME, Usatyuk PV, and Natarajan V. Src-mediated tyrosine phosphorylation of $p47^{phox}$ in hyperoxia-induced activation of NADPH oxidase and generation of reactive oxygen species in lung endothelial cells. *J Biol Chem* 280: 20700–20711, 2005.
53. Christ M, Bauersachs J, Liebetrau C, Heck M, Gunther A, and Wehling M. Glucose increases endothelial-dependent superoxide formation in coronary arteries by NAD(P)H oxidase activation: attenuation by the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor atorvastatin. *Diabetes* 51: 2648–2652, 2002.
54. Colavitti R, Pani G, Bedogni B, Anzevino R, Borrello S, Waltenberger J, and Galeotti T. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. *J Biol Chem* 277: 3101–3108, 2002.
55. Colston JT, de la Rosa SD, Strader JR, Anderson MA, and Freeman GL. H_2O_2 activates Nox4 through PLA_2-dependent arachidonic acid production in adult cardiac fibroblasts. *FEBS Lett* 579: 2533–2540, 2005.
56. Crawford LE, Milliken EE, Irani K, Zweier JL, Becker LC, Johnson TM, Eissa NT, Crystal RG, Finkel T, and Goldschmidt-Clermont PJ. Superoxide-mediated actin response in post-hypoxic endothelial cells. *J Biol Chem* 271: 26863–26867, 1996.
57. Cucoranu I, Clempus RE, and Sorescu D. Nox4-based NAD(P)H oxidase mediates TGF-b1-induced differentiation of human cardiac fibroblasts into myofibroblasts. *Circulation* 110: III–157, 2005.
58. Cucoranu I, Clempus R, Dikalova A, Phelan PJ, Ariyan S, Dikalov S, and Sorescu D. NAD(P)H oxidase 4 mediates transforming growth factor-{beta}1-induced differentiation of cardiac fibroblasts into myofibroblasts. *Circ Res* 97:900–907, 2005.
59. Dana R, Leto TL, Malech HL, and Levy R. Essential requirement of cytosolic phospholipase A_2 for activation of the phagocyte NADPH oxidase. *J Biol Chem* 273: 441–445, 1998.
60. Dana R, Malech HL, and Levy R. The requirement for phospholipase A_2 for activation of the assembled NADPH oxidase in human neutrophils. *Biochem J* 297: 217–223, 1994.
61. Dang PM, Fontayne A, Hakim J, El BJ, and Perianin A. Protein kinase C zeta phosphorylates a subset of selective sites of the NADPH oxidase component p47phox and participates in formyl peptide-mediated neutrophil respiratory burst. *J Immunol* 166: 1206–1213, 2001.
62. Daniels I, Lindsay MA, Keany CI, Burden RP, Fletcher J, and Haynes AP. Role of arachidonic acid and its metabolites in the priming of NADPH oxidase in human polymorphonuclear leukocytes by peritoneal dialysis effluent. *Clin Diagn Lab Immunol* 5: 683–689, 1998.
63. Date M, Morita T, Yamashita N, Nishida K, Yamaguchi O, Higuchi Y, Hirotani S, Matsumura Y, Hori M, Tada M, and Otsu K. The antioxidant N-2-mercaptopropionyl glycine attenuates left ventricular hypertrophy in in vivo murine pressure-overload model. *J AM COLL CARDIOL* 39: 907–912, 2002.
64. De Deken X, Wang D, Many MC, Costagliola S, Libert F, Vassart G, Dumont JE, and Miot F. Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. *J Biol Chem* 275: 23227–23233, 2000.

65. De Keulenaer GW, Alexander RW, Ushio-Fukai M, Ishizaka N, and Griendling KK. Tumour necrosis factor alpha activates a p22phox-based NADH oxidase in vascular smooth muscle. *Biochem J* 329: 653–657, 1998.
66. De Keulenaer GW, Chappell DC, Ishizaka N, Nerem RM, Alexander RW, and Griendling KK. Oscillatory and steady laminar shear stress differentially affect human endothelial redox state: Role of a superoxide-producing NADH Oxidase. *Circ Res* 82: 1094–1101, 1998.
67. DeLeo FR, Renee J, McCormick S, Nakamura M, Apicella M, Weiss JP, and Nauseef WM. Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. *J Clin Invest* 101: 455–463, 1998.
68. Deshpande DA, Walseth TF, Panettieri RA, and Kannan MS. CD38/cyclic ADP-ribose-mediated Ca^{2+} signaling contributes to airway smooth muscle hyper-responsiveness. *FASEB J* 17: 452–454, 2003.
69. Deshpande NN, Sorescu D, Seshiah P, Ushio-Fukai M, Akers M, Yin Q, and Griendling KK. Mechanism of hydrogen peroxide-induced cell cycle arrest in vascular smooth muscle. *Antioxid Redox Signal* 4: 845–854, 2002.
70. Deshpande SS, Angkeow P, Huang J, Ozaki M, and Irani K. Rac1 inhibits TNF-a-induced endothelial cell apoptosis: dual regulation by reactive oxygen species. *FASEB J* 14: 1705–1714, 2000.
71. Dhalla AK, Hill MF, and Singal PK. Role of oxidative stress in transition of hypertrophy to heart failure. *J Am Coll Cardiol* 28: 506–514, 1996.
72. Didichenko SA, Tilton B, Hemmings BA, Ballmer-Hofer K, and Thelen M. Constitutive activation of protein kinase B and phosphorylation of $p47^{phox}$ by a membrane-targeted phosphoinositide 3-kinase. *Curr Biol* 6: 1271–1278, 1996.
73. Didion SP, Ryan MJ, Didion LA, Fegan PE, Sigmund CD, and Faraci FM. Increased superoxide and vascular dysfunction in CuZnSOD-deficient mice. *Circ Res* 91: 938–944, 2002.
74. Diebold BA, Bokoch GM. Molecular basis for Rac2 regulation of phagocyte NADPH oxidase. *Nat Immunol* 2: 211–215, 2001.
75. Dikalova A, Sutcliffe D, Dikalov S, Lassegue B, Weber D, Rocic P, Cheng G, Lambeth D, Owens G, and Griendling K. Nox1 overexpression in mice enhances Ang II-superoxide production and hypertension. *Circulation* 108: IV-45-2005.
76. Dinauer MC, Orkin SH. Chronic granulomatous disease. *Annu Rev Med* 43: 117–124, 1992.
77. Djordjevic T, BelAiba RS, Bonello S, Pfeilschifter J, Hess J, and Gorlach A. Human urotensin II Is a novel activator of NADPH oxidase in human pulmonary artery smooth muscle cells. *Arterioscler Thromb Vasc Biol* 25: 519–525, 2005.
78. Dong F, Zhang X, Wold LE, Ren Q, Zhang Z, and Ren J. Endothelin-1 enhances oxidative stress, cell proliferation and reduces apoptosis in human umbilical vein endothelial cells: role of ETB receptor, NADPH oxidase and caveolin-1. *Br J Pharmacol* 145: 323–333, 2005.
79. Du X, Stocklauser-Farber K, and Rosen P. Generation of reactive oxygen intermediates, activation of NF-kappaB, and induction of apoptosis in human endothelial cells by glucose: role of nitric oxide synthase? *Free Radic Biol Med* 27: 752–763, 1999.
80. Duerrschmidt N, Wippich N, Goettsch W, Broemme HJ, and Morawietz H. Endothelin-1 induces NAD(P)H oxidase in human endothelial cells. *Biochem Biophys Res Commun* 269: 713–717, 2000.
81. Dupuy C, Ohayon R, Valent A, Noel-Hudson MS, Deme D, and Virion A. Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. Cloning of the porcine and human cDNAs. *J Biol Chem* 274: 37265–37269, 1999.
82. Eaton P, Hearse DJ, and Shattock MJ. S-thiolation of the a_1-catalytic but not the b_1 rgulatory subunit of the Na/K ATPase during early cardiac reperfusion. *Circulation* 98: I–805, 1998.
83. Eaton P, Byers HL, Leeds N, Ward MA, and Shattock MJ. Detection, quantitation, purification, and identification of cardiac proteins S-thiolated during ischemia and reperfusion. *J Biol Chem* 277: 9806–9811, 2002.
84. Edens WA, Sharling L, Cheng G, Shapira R, Kinkade JM, Lee T, Edens HA, Tang X, Sullards C, Flaherty DB, Benian GM, and Lambeth JD. Tyrosine cross-linking of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit $gp91^{phox}$. *J Cell Biol* 154: 879–892, 2001.
85. Eklund EA, Jalava A, and Kakar R. PU.1, Interferon regulatory factor 1, and interferon consensus sequence-binding protein cooperate to increase $gp91^{phox}$ expression. *J Biol Chem* 273: 13957–13965, 1998.
86. Eklund EA, Jalava A, and Kakar R. Tyrosine phosphorylation of HoxA10 decreases DNA binding and transcriptional repression during interferon gamma -induced differentiation of myeloid leukemia cell lines. *J Biol Chem* 275: 20117–20126, 2000.
87. Eklund EA, Kakar R. Recruitment of CREB-binding protein by PU.1, IFN-regulatory factor-1, and the IFN consensus sequence-binding protein is necessary for IFN-{gamma}-induced p67phox and gp91phox expression. *J Immunol* 163: 6095–6105, 1999.
88. Ellis A, Pannirselvam M, Anderson TJ, and Triggle CR. Catalase has negligible inhibitory effects on endothelium-dependent relaxations in mouse isolated aorta and small mesenteric artery. *Br J Pharmcol* 140: 1193–1200, 2003.
89. Ellis GR, Anderson RA, Lang D, Blackman DJ, Morris RHK, Morris-Thurgood J, McDowell IFW, Jackson SK, Lewis MJ, and Frenneaux MP. Neutrophil superoxide anion-generating capacity, endothelial function and oxidative stress in chronic heart failure: effects of short- and long-term vitamin C therapy. *J Am Coll Cardiol* 36: 1474–1482, 2000.
90. Ellmark SHM, Dusting GJ, Ng Tang Fui M, Guzzo-Pernell N, and Drummond GR. The contribution of Nox4 to NADPH oxidase activity in mouse vascular smooth muscle. *Cardiovasc Res* 65: 495–504, 2005.
91. Fei J, Viedt C, Soto U, Elsing C, Jahn L, and Kreuzer J. Endothelin-1 and smooth muscle cells: Induction of Jun amino-terminal kinase through an oxygen radical-sensitive mechanism. *Arterioscler Thromb Vasc Biol* 20: 1244–1249, 2000.
92. Ferdinandy P, Danial H, Ambrus I, Rothery RA, and Schulz R. Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure. *Circ Res* 87: 241–247, 2000.

93. Ferrans VJ. New insights into the world of matrix metalloproteinases. *Circulation* 105: 405–407, 2002.
94. Frey RS, Rahman A, Kefer JC, Minshall RD, and Malik AB. PKCz regulates TNF-a-induced activation of NADPH oxidase in endothelial cells. *Circ Res* 90: 1012–1019, 2002.
95. Fu XW, Wang D, Nurse CA, Dinauer MC, and Cutz E. NADPH oxidase is an O_2 sensor in airway chemoreceptors: evidence from K^+ current modulation in wild-type and oxidase-deficient mice. *PNAS* 97: 4374–4379, 2000.
96. Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers Q, Taylor WR, Harrison DG, Wilcox JN, and Griendling KK. $p22^{phox}$ mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. *Circulation* 80: 45–51, 1997.
97. Fukui T, Yoshiyama M, Hanatani A, Omura T, Yoshikawa J, and Abe Y. Expression of $p22\text{-}^{phox}$ and $gp91\text{-}^{phox}$, essential components of NADPH oxidase, increases after myocardial infarction. *Biochem Biophys Res Commun* 281: 1200–1206, 2001.
98. Furst R, Brueckl C, Kuebler WM, Zahler S, Krotz F, Gorlach A, Vollmar AM, and Kiemer AK. Atrial natriuretic peptide induces mitogen-activated protein kinase phosphatase-1 in human endothelial cells via Rac1 and NAD(P)H oxidase/Nox2-activation. *Circ Res* 96: 43–53, 2005.
99. Galle J, Heinloth A, Wanner C, and Heermeier K. Dual effect of oxidized LDL on cell cycle in human endothelial cells through oxidative stress. *Kidney Int Suppl* 78: S120–S123, 2001.
100. Galle J, Schneider R, Heinloth A, Wanner C, Galle PR, Conzelmann E, Dimmeler S, and Heermeier K. Lp(a) and LDL induce apoptosis in human endothelial cells and in rabbit aorta: role of oxidative stress. *Kidney Int* 55: 1450–1461, 1999.
101. Gao WD, Liu Y, and Marban E. Selective effects of oxygen free radicals on excitation-contraction coupling in ventricular muscle: Implications for the mechanism of stunned myocardium. *Circulation* 94: 2597–2604, 1996.
102. Gaston BM, Carver J, Doctor A, and Palmer LA. S-nitrosylation signaling in cell biology. *Mol Interv* 3: 253–263, 2003.
103. Geiszt M, Kopp JB, Varnai P, and Leto TL. Identification of Renox, an NAD(P)H oxidase in kidney. *PNAS* 97: 8010–8014, 2000.
104. Geiszt M, Lekstrom K, and Leto TL. Analysis of mRNA transcripts from the NAD(P)H oxidase 1 (Nox1) gene: Evidence against production of the NADPH oxidase homolog-1 short (NOH-1S) transcript variant. *J Biol Chem* 279: 51661–51668, 2004.
105. Geiszt M, Lekstrom K, Witta J, and Leto TL. Proteins homologous to $p47^{phox}$ and $p67^{phox}$ support superoxide production by NAD(P)H oxidase 1 in colon epithelial cells. *J Biol Chem* 278: 20006–20012, 2003.
106. Georgiou G. How to flip the (redox) switch. *Cell* 111: 607–610, 2002.
107. Gertzberg N, Neumann P, Rizzo V, and Johnson A. NAD(P)H oxidase mediates the endothelial barrier dysfunction induced by TNF-alpha. *Am J Physiol Lung Cell Mol Physiol* 286: L37–L48, 2004.
108. Gimbrone MA, Jr. Vascular endothelium: an integrator of pathophysiologic stimuli in atherosclerosis. *Am J Cardiol* 75: 67B–70B, 1995.
109. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest* 115: 500–508, 2005.
110. Gorin Y, Ricono JM, Kim NH, Bhandari B, Choudhury GG, and Abboud HE. Nox4 mediates angiotensin II-induced activation of Akt/protein kinase B in mesangial cells. *Am J Physiol Renal Physiol* 285: F219–F229, 2003.
111. Gorlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, and Busse R. A $gp91^{phox}$ containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. *Circ Res* 87: 26–32, 2000.
112. Gorlach A, Brandes RP, Bassus S, Kronemann N, Kirchmaier C, Busse R, and Schini-Kerth VB. Oxidative stress and expression of p22phox are involved in the up-regulation of tissue factor in vascular smooth muscle cells in response to activated platelets. *FASEB J* 14: 1518–1528, 2000.
113. Gorlach A, Diebold I, Schini-Kerth VB, Berchner-Pfannschmidt U, Roth U, Brandes RP, Kietzmann T, and Busse R. Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: role of the $p22^{phox}$-containing NADPH oxidase. *Circ Res* 89: 47–54, 2001.
114. Gosgnach W, Messika-Zeitoun D, Gonzalez W, Philipe M, and Michel JB. Shear stress induces iNOS expression in cultured smooth muscle cells: role of oxidative stress. *Am J Physiol Cell Physiol* 279: C1880–C1888, 2000.
115. Goyal P, Weissman N, Grimminger F, Hegal C, Bader L, Rose F, Fink L, Ghofrani HA, Schermuly RT, Schmidt HHHW, Seeger W, and Hanze J. Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygne species. *Free Radic Biol Med* 36: 1279–1288, 2004.
116. Goyal P, Weissmann N, Rose F, Grimminger F, Schafers HJ, Seeger W, and Hanze J. Identification of novel Nox4 splice variants with impact on ROS levels in A549 cells. *Biochem Biophys Res Commun* 329: 32–39, 2005.
117. Griendling KK, Minieri CA, Ollerenshaw JD, and Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74: 1141–1148, 1994.
118. Griendling KK, Sorescu D, Lassegue B, and Ushio-Fukai M. Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. *Arterioscler Thromb Vasc Biol* 20: 2175–2183, 2000.
119. Griendling KK and Ushio-Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. *Regul Pept* 91: 21–27, 2000.
120. Grieve DJ, Byrne JA, Siva A, Layland J, Johar S, Cave AC, and Shah AM. Involvement of the NADPH oxidase isoform Nox2 in cardiac contractile dysfunction occurring in response to pressure-overload. *J Am Coll Cardiol* 47: 817–826, 2006.
121. Grieve DJ, Siva A, Byrne JA, Cave AC, and Shah AM. Divergent effects of a Nox2-containing NADPH oxidase on cardiac contractile function and hypertrophy after im-

position of chronic pressure overload. *Circulation* 110: III–133, 2004.

122. Grishko V, Pastukh V, Solodushko V, Gillespie M, Azuma J, and Schaffer S. Apoptotic cascade initiated by angiotensin II in neonatal cardiomyocytes: role of DNA damage. *Am J Physiol Heart Circ Physiol* 285: H2364–H2372, 2003.
123. Grocott-Mason RM, Shah AM. Cardiac dysfunction in sepsis: new theories and clinical implications. *Intensive Care Med* 24: 286–295, 1998.
124. Grote K, Flach I, Luchtefeld M, Akin E, Holland SM, Drexler H, and Schieffer B. Mechanical stretch enhances mRNA expression and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species. *Circ Res* 92: 80e–86e, 2003.
125. Gryglewski RJ, Palmer RM, and Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* 320: 454–456, 1986.
126. Gu Y, Xu YC, Wu RF, Nwariaku FE, Souza RF, Flores SC, and Terada LS. p47^{phox} participates in activation of RelA in endothelial cells. *J Biol Chem* 278: 17210–17217, 2003.
127. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, and Channon KM. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation* 105: 1656–1662, 2002.
128. Guzik TJ, West NEJ, Black E, McDonald D, Ratnatunga C, Pillai R, and Channon KM. Vascular superoxide production by NAD(P)H oxidase : association with endothelial dysfunction and clinical risk factors. *Circ Res* 86: E85–E90, 2000.
129. Ha YJ and Lee JR. Role of TNF receptor-associated factor 3 in the CD40 signaling by production of reactive oxygen species through association with p40phox, a cytosolic subunit of nicotinamide adenine dinucleotide phosphate oxidase. *J Immunol* 172: 231–239, 2004.
130. Hathaway CA, Heistad DD, Piegors DJ, and Miller FJ, Jr. Regression of atherosclerosis in monkeys reduces vascular superoxide levels. *Circ Res* 90: 277–283, 2002.
131. He L, Chen J, Dinger B, Sanders K, Sundar K, Hoidal J, and Fidone S. Characteristics of carotid body chemosensitivity in NADPH oxidase-deficient mice. *Am J Physiol Cell Physiol* 282: C27–C33, 2002.
132. He Z, Way KJ, Arikawa E, Chou E, Opland DM, Clermont A, Isshiki K, Ma RCW, Scott JA, Schoen FJ, Feener EP, and King GL. Differential regulation of angiotensin II-induced expression of connective tissue growth factor by protein kinase C isoforms in the myocardium. *J Biol Chem* 280: 15719–15726, 2005.
133. Heidari Y, Shah AM, and Gove C. NOX-2S is a new member of the NOX family of NADPH oxidases. *Gene* 335: 133–140, 2004.
134. Heinloth A, Heermeier K, Raff U, Wanner C, and Galle J. Stimulation of NADPH oxidase by oxidized low-density lipoprotein induces proliferation of human vascular endothelial cells. *J Am Soc Nephrol* 11: 1819–1825, 2000.
135. Herkert O, Djordjevic T, BelAiba RS, and Gorlach A. Insights into the redox control of blood coagulation: role of vascular NADPH oxidase-derived reactive oxygen species in the thrombogenic cycle. *Antioxid Redox Signal* 6: 765–776, 2004.
136. Herkert O, Diebold I, Brandes RP, Hess J, Busse R, and Gorlach A. NADPH oxidase mediates tissue factor-dependent surface procoagulant activity by thrombin in human vascular smooth muscle cells. *Circulation* 105: 2030–2036, 2002.
137. Heymes C, Bendall JK, Ratajczak P, Cave AC, Samuel JL, Hasenfuss G, and Shah AM. Increased myocardial NADPH oxidase activity in human heart failure. *J Am Coll Cardiol* 41: 2164–2171, 2003.
138. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, and Chayama K. Endothelial function and oxidative stress in renovascular hypertension. *N Engl J Med* 346: 1954–1962, 2002.
139. Higuchi Y, Otsu K, Nishida K, Hirotani S, Nakayama H, Yamaguchi O, Hikoso S, Kashiwase K, Takeda T, Watanabe T, Mano T, Matsumura Y, Ueno H, and Hori M. The small GTP-binding protein Rac1 induces cardiac myocyte hypertrophy through the activation of apoptosis signal-regulating kinase 1 and Nuclear Factor-kB. *J Biol Chem* 278: 20770–20777, 2003.
140. Hill MF, Singal PK. Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats. *Am J Pathol* 148: 291–300, 1996.
141. Hill K, Krugmann S, Andrews SR, Coadwell WJ, Finan P, Welch HCE, Hawkins PT, and Stephens LR. Regulation of P-Rex1 by phosphatidylinositol (3,4,5)-trisphosphate and Gbg subunits. *J Biol Chem* 280: 4166–4173, 2005.
142. Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl RAK, Warnholtz A, Meinertz T, Griendling K, Harrison DG, Forstermann U, and Munzel T. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 88: 14e–22, 2001.
143. Hirotani S, Otsu K, Nishida K, Higuchi Y, Morita T, Nakayama H, Yamaguchi O, Mano T, Matsumura Y, Ueno H, Tada M, and Hori M. Involvement of nuclear factor-kB and apoptosis signal-regulating kinase 1 in G-protein-coupled receptor agonist-induced cardiomyocyte hypertrophy. *Circulation* 105: 509–515, 2002.
144. Hishikawa K and Luscher TF. Pulsatile stretch stimulates superoxide production in human aortic endothelial cells. *Circulation* 96: 3610–3616, 1997.
145. Hishikawa K, Oemar BS, Yang Z, and Luscher TF. Pulsatile stretch stimulates superoxide production and activates nuclear factor-kB in human coronary smooth muscle. *Circ Res* 81: 797–803, 1997.
146. Hoffmeyer MR, Jones SP, Ross CR, Sharp B, Grisham MB, Laroux FS, Stalker TJ, Scalia R, and Lefer DJ. Myocardial ischemia/reperfusion injury in NADPH oxidase-deficient mice. *Circ Res* 87: 812–817, 2000.
147. Hohler B, Holzapfel B, and Kummer W. NADPH oxidase subunits and superoxide production in porcine pulmonary artery endothelial cells. *Histochem Cell Biol* 114: 29–37, 2000.
148. Hornig B, Arakawa N, Kohler C, and Drexler H. Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation* 97: 363–368, 1998.

149. Hoyal CR, Gutierrez A, Young BM, Catz SD, Lin JH, Tsichlis PN, and Babior BM. Modulation of p47phox activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase. *Proc Natl Acad Sci USA* 100: 5130–5135, 2003.
150. Hsiai TK, Cho SK, Wong PK, Ing M, Salazar A, Sevanian A, Navab M, Demer LL, and Ho CM. Monocyte recruitment to endothelial cells in response to oscillatory shear stress. *FASEB J* 17: 1648–1657, 2003.
151. Hsich E, Segal BH, Pagano PJ, Rey FE, Paigen B, Deleonardis J, Hoyt RF, Holland SM, and Finkel T. Vascular effects following homozygous disruption of p47phox. An essential component of NADPH oxidase. *Circulation* 101: 1234–1236, 2000.
152. Hua H, Munk S, Goldberg H, Fantus IG, and Whiteside CI. High glucose-suppressed endothelin-1 Ca^{2+} signaling via NADPH oxidase and diacylglycerol-sensitive protein kinase C isozymes in mesangial cells. *J Biol Chem* 278: 33951–33962, 2003.
153. Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, Dikalov S, Giddens DP, Griendling KK, Harrison DG, and Jo H. Oscillatory shear stress stimulates endothelial production of O_2^- from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion. *J Biol Chem* 278: 47291–47298, 2003.
154. Hwang J, Kleinhenz DJ, Lassegue B, Griendling KK, Dikalov S, and Hart CM. Peroxisome proliferator-activated receptor-{g} ligands regulate endothelial membrane superoxide production. *Am J Physiol Cell Physiol* 288: C899–C905, 2005.
155. Hwang J, Ing MH, Salazar A, Lassegue B, Griendling K, Navab M, Sevanian A, and Hsiai TK. Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation. *Circ Res* 93: 1225–1232, 2003.
156. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, and Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C–dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* 49: 1939–1945, 2000.
157. Inoue I, Goto Si, Matsunaga T, Nakajima T, Awata T, Hokari S, Komoda T, and Katayama S. The ligands/activators for peroxisome proliferator-activated receptor[nbsp][a] (PPARa) and PPARg increase Cu^{2+}, Zn^{2+}-superoxide dismutase and decrease p22phox message expressions in primary endothelial cells. *Metab Clin Exp* 50: 3–11, 2001.
158. Inoue I, Goto Si, Mizotani K, Awata T, Mastunaga T, Kawai Si, Nakajima T, Hokari S, Komoda T, and Katayama S. Lipophilic HMG-CoA reductase inhibitor has an anti-inflammatory effect: reduction of mRNA levels for interleukin-1[beta], interleukin-6, cyclooxygenase-2, and p22phox by regulation of peroxisome proliferator-activated receptor [a] (PPAR[a]) in primary endothelial cells. Life Sciences 67: 863–876, 2000.
159. Irani K, Xia Y, Zweier JL, Sollott SJ, Der CJ, Fearon ER, Sundaresan M, Finkel T, and Goldschmidt-Clermont PJ. Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. *Science* 275: 1649–1652, 1997.
160. Ishikawa M, Stokes KY, Zhang JH, Nanda A, and Granger DN. Cerebral microvascular responses to hypercholesterolemia: roles of NADPH oxidase and P-selectin. *Circ Res* 94: 239–244, 2004.
161. Jacobsen BM, Skalnik DG. YY1 Binds five cis-Elements and trans-activates the myeloid cell-restricted gp91phox promoter. *J Biol Chem* 274: 29984–29993, 1999.
162. Janssens V, Goris J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem J* 353: 417–439, 2001.
163. Jialal I, Devaraj S. Antioxidants and atherosclerosis: Don't throw out the baby with the bath water. *Circulation* 107: 926–928, 2003.
164. Jiang BH, Semenza GL, Bauer C, and Marti HH. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O_2 tension. *Am J Physiol* 271: C1172–C1180, 1996.
165. Johar S, Cave AC, Grieve DJ, and Shah AM. A critical role for a gp91phox-containing NADPH oxidase in interstitial cardiac fibrosis. *Circulation* 110: III–188, 2004.
166. Jones SA, O'Donnell VB, Wood JD, Broughton JP, Hughes EJ, and Jones OT. Expression of phagocyte NADPH oxidase components in human endothelial cells. *Am J Physiol* 271: H1626–H1634, 1996.
167. Kalinina N, Agrotis A, Tararak E, Antropova Y, Kanellakis P, Ilyinskaya O, Quinn MT, Smirnov V, and Bobik A. Cytochrome b_{558}-dependent NAD(P)H Oxidase-Phox units in smooth muscle and macrophages of atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 22: 2037–2043, 2002.
168. Kashiwagi A, Shinozaki K, Nishio Y, Maegawa H, Maeno Y, Kanazawa A, Kojima H, Haneda M, Hidaka H, Yasuda H, and Kikkawa R. Endothelium-specific activation of NAD(P)H oxidase in aortas of exogenously hyperinsulinemic rats. *Am J Physiol* 277: E976–E983, 1999.
169. Kass DA. Ventricular arterial stiffening: integrating the pathophysiology. *Hypertension* 46: 185–193, 2005.
170. Katsuyama M, Fan C, and Yabe-Nishimura C. NADPH oxidase is involved in prostaglandin F2a -induced hypertrophy of vascular smooth muscle cells. induction of NOX1 by PGF2a. *J Biol Chem* 277: 13438–13442, 2002.
171. Kautz B, Kakar R, David E, and Eklund EA. SHP1 protein-tyrosine phosphatase inhibits gp91phox and p67phox expression by inhibiting interaction of PU.1, IRF1, interferon consensus sequence-binding protein, and CREB-binding protein with homologous cis elements in the CYBB and NCF2 genes. *J Biol Chem* 276: 37868–37878, 2001.
172. Keaney JF, Jr., Larson MG, Vasan RS, Wilson PWF, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, and Benjamin EJ. Obesity and systemic oxidative stress: Clinical correlates of oxidative stress in The Framingham Study. *Arterioscler Thromb Vasc Biol* 23: 434–439, 2003.
173. Kim K-S, Takeda K, Sethi R, Pracyk JB, Tanaka K, Zhou YF, Yu Z-X, Ferrans VJ, Bruder JT, Kovesdi I, Irani K, Goldschmidt-Clermont P, and Finkel T. Protection from reoxygenation injury by inhibition of Rac1. *J Clin Invest* 101: 1821–1826, 1998.
174. Kimura S, Zhang GX, Nishiyama A, Shokoji T, Yao L, Fan YY, Rahman M, and Abe Y. Mitochondria-derived re-

active oxygen species and vascular MAP kinases: comparison of angiotensin II and diazoxide. *Hypertension* 45: 438–444, 2005.
175. Kinsella BT, Erdman RA, and Maltese WA. Carboxyl-terminal isoprenylation of ras-related GTP-binding proteins encoded by rac1, rac2, and ralA. *J Biol Chem* 266: 9786–9794, 1991.
176. Kinugawa S, Tsutsui H, Hayashidani S, Ide T, Suematsu N, Satoh S, Utsumi H, and Takeshita A. Treatment with dimethylthiourea prevents left ventricular remodeling and failure after experimental myocardial infarction in mice. Role of oxidative stress. *Circ Res* 87: 392–398, 2000.
177. Koh KK, Ahn JY, Han SH, Kim DS, Jin DK, Kim HS, Shin MS, Ahn TH, Choi IS, and Shin EK. Pleiotropic effects of angiotensin II receptor blocker in hypertensive patients. *J Am Coll Cardiol* 42: 905–910, 2003.
178. Korchak HM, Kilpatrick LE. Roles for beta II-protein kinase C and RACK1 in positive and negative signaling for superoxide anion generation in differentiated HL60 cells. *J Biol Chem* 276: 8910–8917, 2001.
179. Kovacic HN, Irani K, and Goldschmidt-Clermont PJ. Redox regulation of human Rac1 stability by the proteasome in human aortic endothelial cells. *J Biol Chem* 276: 45856–45861, 2001.
180. Krijnen PAJ, Meischl C, Hack CE, Meijer CJLM, Visser CA, Roos D, and Niessen HWM. Increased Nox2 expression in human cardiomyocytes after acute myocardial infarction. *J Clin Pathol* 56: 194–199, 2003.
181. Kunsch C and Medford RM. Oxidative stress as a regulator of gene expression in the vasculature. *Circ Res* 85: 753–766, 1999.
182. Kuster GM, Pimentel DR, Adachi T, Ido Y, Brenner DA, Cohen RA, Liao R, Siwik DA, and Colucci WS. a-Adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on ras. *Circulation* 111: 1192–1198, 2005.
183. Lal AS, Parker PJ, and Segal AW. Characterization and partial purification of a novel neutrophil membrane-associated kinase capable of phosphorylating the respiratory burst component p47phox. *Biochem J* 338: 359–366, 1999.
184. Lambeth JD, Cheng G, Arnold RS, and Edens WA. Novel homologs of gp91*phox*. *TIBS* 25: 459–461, 2000.
185. Lambeth JD. Nox enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4: 181–189, 2004.
186. Landmesser U, Hornig B, and Drexler H. Endothelial function: a critical determinant in atherosclerosis? *Circulation* 109: II27–II33, 2004.
187. Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, Holland SM, and Harrison DG. Role of p47^{phox} in vascular oxidative stress and hypertension caused by angiotensin II. *Hypertension* 40: 511–515, 2002.
188. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, and Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 111: 1201–1209, 2003.
189. Laplante MA, Wu R, Moreau P, and de CJ. Endothelin mediates superoxide production in angiotensin II-induced hypertension in rats. *Free Radic Biol Med* 38: 589–596, 2005.
190. Lassegue B, Griendling KK. Reactive oxygen species in hypertension; An update. *Am J Hypertens* 17: 852–860, 2004.
191. Lassegue B, Sorescu D, Szocs K, Yin Q, Akers M, Zhang Y, Grant SL, Lambeth JD, and Griendling KK. Novel gp91^{phox} homologues in vascular smooth muscle cells: Nox1 mediates angiotensin II-Induced superoxide formation and redox-sensitive signaling pathways. *Circ Res* 88: 888–894, 2001.
192. Lassegue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. *Am J Physiol Regul Integr Comp Physiol* 285: R277–R297, 2003.
193. Laurindo FR, Pedro MA, Barbeiro HV, Pileggi F, Carvalho MH, Augusto O, and da Luz PL. Vascular free radical release. Ex vivo and in vivo evidence for a flow-dependent endothelial mechanism. *Circ Res* 74: 700–709, 1994.
194. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, and Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* 95: 588–593, 1997.
195. Laursen JB, Somers M, Kurz S, McCann L, Warnholtz A, Freeman BA, Tarpey M, Fukai T, and Harrison DG. Endothelial regulation of vasomotion in ApoE-deficient mice. Implications for interactions between peroxynitrite and tetrahydrobiopterin. *Circulation* 103: 1282–1288, 2001.
196. Lavigne MC, Malech HL, Holland SM, and Leto TL. Genetic demonstration of p47phox-dependent superoxide anion production in murine vascular smooth muscle cells. *Circulation* 104: 79–84, 2001.
197. Lee HB, Yu MR, Yang Y, Jiang Z, and Ha H. Reactive oxygen species-regulated signaling pathways in diabetic nephropathy. *J Am Soc Nephrol* 14: S241–S245, 2003.
198. Lee YM, Kim BJ, Chun YS, So I, Choi H, Kim MS, and Park JW. NOX4 as an oxygen sensor to regulate TASK-1 activity. *Cell Signal* 18: 499–507, 2006.
199. Levy R, Lowenthal A, and Dana R. Cytosolic phospholipase A_2 is required for the activation of the NADPH oxidase associated H+ channel in phagocyte-like cells. *Adv Exp Med Biol* 479: 125–135, 2000.
200. Li AE, Ito H, Rovira II, Kim KS, Takeda K, Yu ZY, Ferrans VJ, and Finkel T. A role for reactive oxygen species in endothelial cell anoikis. *Circ Res* 85: 304–310, 1999.
201. Li D, Yang B, Philips MI, and Mehta JL. Proapoptotic effects of ANG II in human coronary artery endothelial cells: role of AT1 receptor and PKC activation. *Am J Physiol* 276: H786–H792, 1999.
202. Li J-M, Shah AM. Intracellular localization and preassembly of the NAPH oxidase complex in cultured endothelial cells. *J Biol Chem* 277: 19952–19960, 2002.
203. Li J-M, Gall NP, Grieve DJ, Chen M, and Shah AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. *Hypertension* 40: 477–484, 2002.
204. Li JM, Mullen AM, and Shah AM. Phenotypic properties and characteristics of superoxide production by mouse coronary microvascular endothelial cells. *J Mol Cell Cardiol* 33: 1119–1131, 2001.
205. Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiol-

ogy. *Am J Physiol Regul Integr Comp Physiol* 287: R1014–R1030, 2004.

206. Li JM, Fan LM, Christie MR, and Shah AM. Acute Tumor Necrosis Factor a signaling via NADPH oxidase in microvascular endothelial cells: Role of p47phox phosphorylation and binding to TRAF4. *Mol Cell Biol* 25: 2320–2330, 2005.
207. Li JM, Mullen AM, Yun S, Wientjes F, Brouns GY, Thrasher AJ, and Shah AM. Essential role of the NADPH oxidase subunit p47phox in endothelial cell superoxide production in response to phorbol ester and tumor necrosis factor-a. *Circ Res* 90;143–150 2002.
208. Li JM, Shah AM. Mechanism of endothelial cell NADPH oxidase activation by angiotensin II. Role of the p47phox subunit. *J Biol Chem* 278: 12094–12100, 2003.
209. Li JM, Wheatcroft S, Fan LM, Kearney MT, and Shah AM. Opposing roles of p47phox in basal versus angiotensin II-stimulated alterations in vascular O_2^- production, vascular tone, and mitogen-activated protein kinase activation. *Circulation* 109: 1307–1313, 2004.
210. Li L, Fink GD, Watts SW, Northcott CA, Galligan JJ, Pagano PJ, and Chen AF. Endothelin-1 increases vascular superoxide via endothelinA-NADPH oxidase pathway in low-renin hypertension. *Circulation* 107: 1053–1058, 2003.
211. Li PF, Dietz R, and von HR. Reactive oxygen species induce apoptosis of vascular smooth muscle cell. *FEBS Lett* 404: 249–252, 1997.
212. Li SL, Schlegel W, Valente AJ, and Clark RA. Critical flanking sequences of PU.1 binding sites in myeloid-specific promoters. *J Biol Chem* 274: 32453–32460, 1999.
213. Li SL, Valente AJ, Wang L, Gamez MJ, and Clark RA. Transcriptional regulation of the p67phox gene. Role of AP-1 in concert with myeloid-specific transcription factors. *J Biol Chem* 276: 39368–39378, 2001.
214. Li WG, Miller FJ, Jr., Zhang HJ, Spitz DR, Oberley LW, and Weintraub NL. H_2O_2-induced O_2^- production by a non-phagocytic NAD(P)H oxidase causes oxidant injury. *J Biol Chem* 276: 29251–29256, 2001.
215. Li X, Lee JW, Graves LM, and Earp HS. Angiotensin II stimulates ERK via two pathways in epithelial cells: protein kinase C suppresses a G-protein coupled receptor-EGF receptor transactivation pathway. *EMBO J* 17: 2574–2583, 1998.
216. Liu J, Yang F, Yang X-P, Jankowski M, and Pagano PJ. NAD(P)H oxidase mediates angiotensin II-induced vascular macrophage infiltration and medial hypertrophy. *Arterioscler Thromb Vasc Biol* 23: 776–782, 2003.
217. Liu J, Ormsby A, Oja-Tebbe N, and Pagano PJ. Gene transfer of NAD(P)H oxidase inhibitor to the vascular adventitia attenuates medial smooth muscle hypertrophy. *Circ Res* 95: 587–594, 2004.
218. Liu JQ, Zelko IN, and Folz RJ. Reoxygenation-induced Constriction in Murine Coronary Arteries: The role of endothelial NADPH oxidase (gp91phox) and intracellular superoxide. *J Biol Chem* 279: 24493–24497, 2004.
219. Lopes NH, Vasudevan SS, Gregg D, Selvakumar B, Pagano PJ, Kovacic H, and Goldschmidt-Clermont PJ. Rac-dependent monocyte chemoattractant protein-1 production is induced by nutrient deprivation. *Circ Res* 91: 798–805, 2002.
220. Lu L, Quinn MT, and Sun Y. Oxidative stress in the infarcted heart: role of de novo angiotensin II production. *Biochem Biophysic Res Commun* 325: 943–951, 2004.
221. Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. *Am J Physiol Cell Physiol* 280: C719–C741, 2001.
222. Luo W, Skalnik DG. CCAAT Displacement protein competes with multiple transcriptional activators for binding to four sites in the proximal gp91phox promoter. *J Biol Chem* 271: 18203–18210, 1996.
223. Luo W, Skalnik DG. Interferon regulatory factor-2 directs transcription from the gp91phox promoter. *J Biol Chem* 271: 23445–23451, 1996.
224. Maack C, Kartes T, Kilter H, Schafers HJ, Nickenig G, Bohm M, and Laufs U. Oxygen free radical release in human failing myocardium is associated with increased activity of Rac1-GTPase and represents a target for statin treatment. *Circulation* 108: 1567–1574, 2003.
225. MacCarthy PA, Grieve DJ, Li JM, Dunster C, Kelly FJ, and Shah AM. Impaired endothelial regulation of ventricular relaxation in cardiac hypertrophy: role of reactive oxygen species and NADPH oxidase. *Circulation* 104: 2967–2974, 2001.
226. MacFarlane NG, Miller DJ. Depression of peak force without altering calcium sensitivity by the superoxide anion in chemically skinned cardiac muscle of rat. *Circ Res* 70: 1217–1224, 1992.
227. Mallat Z, Philip I, Lebret M, Chatel D, Maclouf J, and Tedgui A. Elevated levels of 8-iso-prostaglandin F2a in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilatation and progression to heart failure. *Circulation* 97: 1536–1539, 1998.
228. Mantovani A, Bussolino F, and Dejana E. Cytokine regulation of endothelial cell function. *FASEB J* 6: 2591–2599, 1992.
229. Martyn KD, Frederick LM, von Loehneysen K, Dinauer MC, and Knaus UG. Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. *Cell Signal* 18: 69–82, 2006.
230. Marumo T, Schini-Kerth VB, Brandes RP, and Busse R. Glucocorticoids inhibit superoxide anion production and p22 phox mRNA expression in human aortic smooth muscle cells. *Hypertension* 32: 1083–1088, 1998.
231. Marumo T, Schini-Kerth VB, Fisslthaler B, and Busse R. Platelet-derived growth factor-stimulated superoxide anion production modulates activation of transcription factor NF-kB and expression of monocyte chemoattractant protein 1 in human aortic smooth muscle cells. *Circulation* 96: 2361–2367, 1997.
232. Matoba T, Shimokawa H, Kubota H, Morikawa K, Fujiki T, Kunihiro I, Mukai Y, Hirakawa Y, and Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. *Biochem Biophys Res Commun* 290: 909–913, 2002.
233. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, and Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J Clin Invest* 106: 1521–1530, 2000.

234. Matsubara T, Ziff M. Increased superoxide anion release from human endothelial cells in response to cytokines. *J Immunol* 137: 3295–3298, 1986.
235. Maytin M, Siwik DA, Ito M, Xiao L, Sawyer DB, Liao R, and Colucci WS. Pressure overload-induced myocardial hypertrophy in mice does not require gp91^{phox}. *Circulation* 109: 1168–1171, 2004.
236. McMurray J, Chopra M, Abdullah I, Smith WE, and Dargie HJ. Evidence of oxidative stress in chronic heart failure in humans. *Eur Heart J* 14: 1493–1498, 1993.
237. McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H, and Harrison DG. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol* 285: H2290–H2297, 2003.
238. Meneshian A, Bulkley GB. The physiology of endothelial xanthine oxidase: from urate catabolism to reperfusion injury to inflammatory signal transduction. *Microcirculation* 9: 161–175, 2002.
239. Meyer JW, Holland JA, Ziegler LM, Chang MM, Beebe G, and Schmitt ME. Identification of a functional leukocyte-type NADPH oxidase in human endothelial cells: a potential atherogenic source of reactive oxygen species. *Endothelium* 7: 11–22, 1999.
240. Miller FJ, Jr., Gutterman DD, Rios CD, Heistad DD, and Davidson BL. Superoxide production in vascular smooth muscle contributes to oxidative stress and impaired relaxation in atherosclerosis. *Circ Res* 82: 1298–1305, 1998.
241. Milovanova T, Manevich Y, Haddad A, Chatterjee S, Moore JS, and Fisher AB. Endothelial cell proliferation associated with abrupt reduction in shear stress is dependent on reactive oxygen species. *Antioxid Redox Signal* 6: 245–258, 2004.
242. Mirochnitchenko O, Prokopenko O, Palnitkar U, Kister I, Powell WS, and Inouye M. Endotoxemia in transgenic mice overexpressing human glutathione peroxidases. *Circ Res* 87: 289–295, 2000.
243. Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, and Gutterman DD. Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. *Circ Res* 92: e31–e40, 2003.
244. Mizrahi A, Molshanski-Mor S, Weinbaum C, Zheng Y, Hirshberg M, and Pick E. Activation of the phagocyte NADPH oxidase by Rac Guanine nucleotide exchange factors in conjunction with ATP and nucleoside diphosphate kinase. *J Biol Chem* 280: 3802–3811, 2005.
245. Moldovan L, Moldovan NI, Sohn RH, Parikh SA, and Goldschmidt–Clermont PJ. Redox changes of cultured endothelial cells and actin dynamics. *Circ Res* 86: 549–557, 2000.
246. Mollnau H, Wendt M, Szocs K, Lassegue B, Schulz E, Oelze M, Li H, Bodenschatz M, August M, Kleschyov AL, Tsilimingas N, Walter U, Forstermann U, Meinertz T, Griendling K, and Munzel T. Effects of angiotensin II Infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. *Circ Res* 90: 58e–65, 2002.
247. Morikawa K, Shimokawa H, Matoba T, Kubota H, Akaike T, Talukder MA, Hatanaka M, Fujiki T, Maeda H, Takahashi S, and Takeshita A. Pivotal role of Cu, Zn-superoxide dismutase in endothelium-dependent hyperpolarization. *J Clin Invest* 112: 1871–1879, 2003.
248. Morin I, Li WQ, Su S, Ahmad M, and Zafarullah M. Induction of stromelysin gene expression by tumor necrosis factor a is inhibited by dexamethasone, salicylate, and N-acetylcysteine in synovial fibroblasts. *J Pharmacol Exper Therap* 289: 1634–1640, 1999.
249. Munzel T, Daiber A, Ullrich V, and Mulsch A. Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. *Arterioscler Thromb Vasc Biol* 25: 1551–1557, 2005.
250. Munzel T, Sayegh H, Freeman BA, Tarpey MM, and Harrison DG. Evidence for enhanced vascular superoxide anion production in nitrate tolerance. A novel mechanism underlying tolerance and cross-tolerance. *J Clin Invest* 95: 187–194, 1995.
251. Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, and Namba M. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-a and angiotensin II. *Circulation* 98: 794–799, 1998.
252. Nakashima I, Takeda K, Kawamoto Y, Okuno Y, Kato M, and Suzuki H. Redox control of catalytic activities of membrane-associated protein tyrosine kinases. *Arch Biochem Biophys* 434: 3–10, 2005.
253. Nicoletti A, Michael J-B. Cardiac fibrosis and inflammation: interaction with hemodynamic and hormonal factors. *Cardiovasc Res* 41: 532–543, 2004.
254. Nishida M, Tanabe S, Maruyama Y, Mangmool S, Urayama K, Nagamatsu Y, Takagahara S, Turner JH, Kozasa T, Kobayashi H, Sato Y, Kawanishi T, Inoue R, Nagao T, and Kurose H. Gα12/13- and reactive oxygen species-dependent activation of c-Jun NH_2-terminal Kinase and p38 mitogen-activated protein kinase by angiotensin receptor stimulation in rat neonatal cardiomyocytes. *J Biol Chem* 280: 18434–18441, 2005.
255. Nishikawa T, Edelstein D, Du XL, Yamagishi SI, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, and Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404: 787–790, 2000.
256. Nixon JB, McPhail LC. Protein kinase C (PKC) isoforms translocate to Triton-insoluble fractions in stimulated human neutrophils: correlation of conventional PKC with activation of NADPH oxidase. *J Immunol* 163: 4574–4582, 1999.
257. Nowicki PT, Flavahan S, Hassanain H, Mitra S, Holland S, Goldschmidt-Clermont PJ, and Flavahan NA. Redox signaling of the arteriolar myogenic response. *Circ Res* 89: 114–116, 2001.
258. Nwariaku FE, Liu Z, Zhu X, Nahari D, Ingle C, Wu RF, Gu Y, Sarosi G, and Terada LS. NADPH oxidase mediates vascular endothelial cadherin phosphorylation and endothelial dysfunction. *Blood* 104: 3214–3220, 2004.
259. O'Donnell RW, Johnson DK, Ziegler LM, DiMattina AJ, Stone RI, and Holland JA. Endothelial NADPH oxidase: mechanism of activation by low-density lipoprotein. *Endothelium* 10: 291–297, 2003.

260. Oeckler RA, Kaminski PM, and Wolin MS. Stretch enhances contraction of bovine coronary arteries via an NAD(P)H oxidase-mediated activation of the extracellular signal-regulated kinase mitogen-activated protein kinase cascade. *Circ Res* 92: 23–31, 2003.
261. Ohara Y, Peterson TE, and Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 91: 2546–2551, 1993.
262. Ohara Y, Peterson TE, Zheng B, Kuo JF, and Harrison DG. Lysophosphatidylcholine increases vascular superoxide anion production via protein kinase C activation. *Arterioscler Thromb Vasc Biol* 14: 1007–1013, 1994.
263. Pagano PJ, Clark JK, Cifuentes-Pagano ME, Clark SM, Callis GM, and Quinn MT. Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. *Proc Natl Acad Sci USA* 94: 14483–14488, 1997.
264. Pagano PJ, Chanock SJ, Siwik DA, Colucci WS, and Clark JK. Angiotensin II induces $p67^{phox}$ mRNA expression and NADPH oxidase superoxide generation in rabbit aortic adventitial fibroblasts. *Hypertension* 32: 331–337, 1998.
265. Paget MSB and Buttner MJ. Thiol-based regulatory switches. *Annl Rev Genet* 37: 91–121, 2003.
266. Palmer RM, Ashton DS, and Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 333: 664–666, 1988.
267. Paravicini TM, Chrissobolis S, Drummond GR, and Sobey CG. Increased NADPH-oxidase activity and Nox4 expression during chronic hypertension is associated with enhanced cerebral vasodilatation to NADPH in vivo. *Stroke* 35: 584–589, 2004.
268. Paravicini TM, Gulluyan LM, Dusting GJ, and Drummond GR. Increased NADPH oxidase activity, $gp91^{phox}$ expression, and endothelium-dependent vasorelaxation during neointima formation in rabbits. *Circ Res* 91: 54–61, 2002.
269. Park HS, Lee SM, Lee JH, Kim YS, Bae YS, and Park JW. Phosphorylation of the leucocyte NADPH oxidase subunit $p47^{phox}$ by casein kinase 2: conformation-dependent phosphorylation and modulation of oxidase activity. *Biochem J* 358: 783–790, 2001.
270. Parrillo JE. Pathogenic mechanisms of septic shock. *N Engl J Med* 328: 1471–1477, 1993.
271. Patterson C, Ruef J, Madamanchi NR, Barry-Lane P, Hu Z, Horaist C, Ballinger CA, Brasier AR, Bode C, and Runge MS. Stimulation of a vascular smooth muscle cell NAD(P)H oxidase by thrombin; evidence that $p47^{phox}$ may participate in forming this oxidase in vitro and in vivo. *J Biol Chem* 274: 19814–19822, 1999.
272. Pedruzzi E, Guichard C, Ollivier V, Driss F, Fay M, Prunet C, Marie JC, Pouzet C, Samadi M, Elbim C, O'Dowd Y, Bens M, Vandewalle A, Gougerot-Pocidalo MA, Lizard G, and Ogier-Denis E. NAD(P)H oxidase Nox-4 mediates 7-ketocholesterol-induced endoplasmic reticulum stress and apoptosis in human aortic smooth muscle cells. *Mol Cell Biol* 24: 10703–10717, 2004.
273. Peng T, Lu X, and Feng Q. Pivotal Role of $gp91^{phox}$-containing NADH oxidase in lipopolysaccharide-induced tumor necrosis factor-a. expression and myocardial depression. *Circulation* 111: 1637–1644, 2005.
274. Perner A, Andresen L, Pedersen G, and Rask-Madsen J. Superoxide production and expression of NAD(P)H oxidases by transformed and primary human colonic epithelial cells. *Gut* 52: 231–236, 2003.
275. Pimentel DR, Amin JK, Xiao L, Miller T, Viereck J, Oliver-Krasinski J, Baliga R, Wang J, Siwik DA, Singh K, Pagano P, Colucci WS, and Sawyer DB. Reactive oxygen species mediate amplitude-dependent hypertrophic and apoptotic responses to mechanical stretch in cardiac myocytes. *Circ Res* 89: 453–460, 2001.
276. Poli G, Parola M. Oxidative damage and fibrogenesis. *Free Radic Biol Med* 22: 287–305, 1997.
277. Pracyk JB, Tanaka K, Hegland DD, Kim KS, Sethi R, Rovira II, Blazina DR, Lee L, Bruder JT, Kovesdi I, Goldshmidt-Clermont PJ, Irani K, and Finkel T. A requirement for the Rac1 GTPase in the signal transduction pathway leading to cardiac myocyte hypertrophy. *J Clin Invest* 102: 929–937, 1998.
278. Price MO, Atkinson SJ, Knaus UG, and Dinauer MC. Rac activation induces NADPH oxidase activity in transgenic COS^{phox} cells, and the level of superoxide production is exchange factor-dependent. *J Biol Chem* 277: 19220–19228, 2002.
279. Price MO, McPhail LC, Lambeth JD, Han CH, Knaus UG, and Dinauer MC. Creation of a genetic system for analysis of the phagocyte respiratory burst: high-level reconstitution of the NADPH oxidase in a nonhematopoietic system. *Blood* 99: 2653–2661, 2002.
280. Privratsky JR, Wold LE, Sowers JR, Quinn MT, and Ren J. AT1 Blockade prevents glucose-induced cardiac dysfunction in ventricular myocytes: Role of the AT_1 receptor and NADPH oxidase. *Hypertension* 42: 206–212, 2003.
281. Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Arnal JF, and Michel JB. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kB activation induced by intracellular oxidative stress. *Arterioscler Thromb Vasc Biol* 20: 645–651, 2000.
282. Qian Y, Liu KJ, Chen Y, Flynn DC, Castranova V, and Shi X. Cdc42 regulates arsenic-induced NADPH oxidase activation and cell migration through actin filament reorganization. *J Biol Chem* 280: 3875–3884, 2005.
283. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, and Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 97: 1916–1923, 1996.
284. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, and Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. *J Clin Invest* 98: 2572–2579, 1996.
285. Ray R, Shah A.M. NADPH oxidase and endothelial cell function. *Clin Sci (Lond)* 109: 217–226, 2005.
286. Rey FE, Cifuentes ME, Kiarash A, Quinn MT, and Pagano PJ. Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O_2^- and systolic blood pressure in mice. *Circ Res* 89: 408–414, 2001.

287. Robert V, Heymes C, Silvestre JS, Sabri A, Swynghedauw B, and Delcayre C. Angiotensin AT_1 receptor subtype as a cardiac target of aldosterone : Role in aldosterone-salt-induced fibrosis. *Hypertension* 33: 981–986, 1999.
288. Roy A, Rozanov C, Mokashi A, Daudu P, Al-Mehdi AB, Shams H, and Lahiri S. Mice lacking in $gp91^{phox}$ subunit of NAD(P)H oxidase showed glomus cell $[Ca^{2+}]_i$ and respiratory responses to hypoxia. *Brain Res* 872: 188–193, 2000.
289. Rueckschloss U, Galle J, Holtz J, Zerkowski HR, and Morawietz H. Induction of NAD(P)H oxidase by oxidized low-density lipoprotein in human endothelial cells: antioxidative potential of hydroxymethylglutaryl coenzyme A reductase inhibitor therapy. *Circulation* 104: 1767–1772, 2001.
290. Rueckschloss U, Quinn MT, Holtz J, and Morawietz H. Dose-dependent Rregulation of NAD(P)H oxidase expression by Angiotensin II in human endothelial cells: protective effect of angiotensin II type 1 receptor blockade in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 22: 1845–1851, 2002.
291. Sakaguchi S, Furusawa S, Yokota K, Sasaki K, Takayanagi M, and Takayanagi Y. The enhancing effect of tumour necrosis factor-alpha on oxidative stress in endotoxemia. *Pharmacol Toxicol* 79: 259–265, 1996.
292. Sambo P, Baroni SS, Luchetti M, Paroncini P, Dusi S, Orlandini G, and Gabrielli A. Oxidative stress in scleroderma: maintenance of scleroderma fibroblast phenotype by the constitutive up-regulation of reactive oxygen species generation through the NADPH oxidase complex pathway. *Arthritis Rheum* 44: 2653–2664, 2001.
293. Sanders KA, Sundar KM, He L, Dinger B, Fidone S, and Hoidal JR. Role of components of the phagocytic NADPH oxidase in oxygen sensing. *J Appl Physiol* 93: 1357–1364, 2002.
294. Sanlioglu S, Williams CM, Samavati L, Butler NS, Wang G, McCray PB, Jr., Ritchie TC, Hunninghake GW, Zandi E, and Engelhardt JF. Lipopolysaccharide induces Rac1-dependent reactive oxygen species formation and coordinates tumor necrosis factor-a secretion through IKK regulation of NF-kB. *J Biol Chem* 276: 30188–30198, 2001.
295. Sarfstein R, Gorzalczany Y, Mizrahi A, Berdichevsky Y, Molshanski-Mor S, Weinbaum C, Hirshberg M, Dagher MC, and Pick E. Dual role of Rac in the assembly of NADPH oxidase, tethering to the membrane and activation of $p67^{phox}$: a study based on mutagenesis of $p67^{phox}$-Rac1 chimeras. *J Biol Chem* 279: 16007–16016, 2004.
296. 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.
297. Schachinger V, Britten MB, and Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 101: 1899–1906, 2000.
298. Schafer M, Schafer C, Ewald N, Piper HM, and Noll T. Role of redox signaling in the autonomous proliferative response of endothelial cells to hypoxia. *Circ Res* 92: 1010–1015, 2003.
299. Schwedhelm E, Bartling A, Lenzen H, Tsikas D, Maas R, Brummer J, Gutzki FM, Berger J, Frolich JC, and Boger RH. Urinary 8-iso-prostaglandin F_2a as a risk marker in patients with coronary heart disease: A matched case-control study. *Circulation* 109: 843–848, 2004.
300. Semenza GL. HIF-1 and mechanisms of hypoxia sensing. *Curr Opin Cell Biol* 13: 167–171, 2001.
301. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, and Griendling KK. Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res* 91: 406–413, 2002.
302. Shi Y, Niculescu R, Wang D, Patel S, Davenpeck KL, and Zalewski A. Increased NAD(P)H oxidase and reactive oxygen species in coronary arteries after balloon injury. *Arterioscler Thromb Vasc Biol* 21: 739–745, 2001.
303. Shiose A, Kuroda J, Tsuruya K, Hirai M, Hirakata H, Naito S, Hattori M, Sakaki Y, and Sumimoto H. A novel superoxide-producing NAD(P)H oxidase in kidney. *J Biol Chem* 276: 1417–1423, 2001.
304. Shmelzer Z, Haddad N, Admon E, Pessach I, Leto TL, Eitan-Hazan Z, Hershfinkel M, and Levy R. Unique targeting of cytosolic phospholipase A_2 to plasma membranes mediated by the NADPH oxidase in phagocytes. *J Cell Biol* 162: 683–692, 2003.
305. Shweiki D, Itin A, Soffer D, and Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 359: 843–845, 1992.
306. Sia YT, Lapointe N, Parker TG, Tsoporis JN, Deschepper CF, Calderone A, Pourdjabbar A, Jasmin JF, Sarrazin JF, Liu P, Adam A, Butany J, and Rouleau JL. Beneficial effects of long-term use of the antioxidant probucol in heart failure in the rat. *Circulation* 105: 2549–2555, 2002.
307. Singh RB, Niaz MA, Rastogi SS, and Rastogi S. Usefulness of antioxidant vitamins in suspected acute myocardial infarction (the Indian experiment of infarct survival-3). *Am J Cardiol* 77: 232–236, 1996.
308. Siwik DA, Pagano PJ, and Colucci WS. Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts. *Am J Physiol Cell Physiol* 280: C53–C60, 2001.
309. Skalnik DG, Dorfman DM, Perkins AS, Jenkins NA, Copeland NG, and Orkin SH. Targeting of transgene expression to monocyte/macrophages by the gp91-phox promoter and consequent histiocytic malignancies. *PNAS* 88: 8505–8509, 1991.
310. Sohn H-Y, Keller M, Gloe T, Morawietz H, Rueckschloss U, and Pohl U. The small G-protein Rac mediates depolarization-induced superoxide formation in human endothelial cells. *J Biol Chem* 275: 18745–18750, 2000.
311. Somers MJ, Mavromatis K, Galis ZS, and Harrison DG. Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate-salt. *Circulation* 101: 1722–1728, 2000.
312. Sonta T, Inoguchi T, Tsubouchi H, Sekiguchi N, Kobayashi K, Matsumoto S, Utsumi H, and Nawata H. Evidence for contribution of vascular NAD(P)H oxidase to increased oxidative stress in animal models of diabetes and obesity. *Free Radic Bio Med* 37: 115–123, 2004.

313. Sorescu D, Weiss D, Lassegue B, Clempus RE, Szocs K, Sorescu G, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor R, and Griendling KK. Superoxide production and expression of Nox family proteins in human atherosclerosis. *Circulation* 105: 1429–1435, 2002.
314. Sorescu GP, Song H, Tressel SL, Hwang J, Dikalov S, Smith DA, Boyd NL, Platt MO, Lassegue B, Griendling KK, and Jo H. Bone morphogenic protein 4 produced in endothelial cells by oscillatory shear stress induces monocyte adhesion by stimulating reactive oxygen species production from a Nox1-based NADPH oxidase. *Circ Res* 95: 773–779, 2004.
315. Souza HP, Laurindo FRM, Ziegelstein RC, Berlowitz CO, and Zweier JL. Vascular NAD(P)H oxidase is disctinct from the phagocytic enzyme and modulates vascular reactivity control. *Am J Physiol* H658–H667, 2001.
316. Spiekermann S, Landmesser U, Dikalov S, Bredt M, Gamez G, Tatge H, Reepschlager N, Hornig B, Drexler H, and Harrison DG. Electron spin resonance Characterization of vascular xanthine and NAD(P)H oxidase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. *Circulation* 107: 1383–1389, 2003.
317. Stokes KY, Clanton EC, Russell JM, Ross CR, and Granger DN. NAD(P)H oxidase-derived superoxide mediates hypercholesterolemia-induced leukocyte-endothelial cell adhesion. *Circ Res* 88: 499–505, 2001.
318. Suh Y-A, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, and Lambeth JD. Cell transformation by the superoxide-generating oxidase Mox1. *Nature* 401: 79–82, 1999.
319. Sun Y, Weber KT. Infarct scar: a dynamic tissue. *Cardiovasc Res* 46: 250–256, 2000.
320. Sun Y, Zhang J, LL, Chen SS, Quinn MT, and Weber KT. Aldosterone-induced inflammation in the rat heart: role of oxidative stress. *Am J Pathol* 161: 1773–1781, 2002.
321. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, and Finkel T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* 270: 296–299, 1995.
322. Swynghedauw B. Molecular mechanisms of myocardial remodeling. *Physiol Rev* 79: 215–262, 1999.
323. Szocs K, Lassegue B, Sorescu D, Hilenski LL, Valppu L, Couse TL, Wilcox JN, Quinn MT, Lambeth JD, and Griendling KK. Upregulation of Nox-based NAD(P)H oxidases in restenosis after carotid injury. *Arterioscler Thromb Vasc Biol* 22: 21–27, 2002.
324. Takano M, Meneshian A, Sheikh E, Yamakawa Y, Wilkins KB, Hopkins EA, and Bulkley GB. Rapid upregulation of endothelial P-selectin expression via reactive oxygen species generation. *Am J Physiol Heart Circ Physiol* 283: H2054–H2061, 2002.
325. Takemoto M, Node K, Nakagami H, Liao Y, Grimm M, Takemoto Y, Kitakaze M, and Liao JK. Statins as antioxidant therapy for preventing cardiac myocyte hypertrophy. *J Clin Invest* 108: 1429–1437, 2001.
326. Takeya R, Ueno N, Kami K, Taura M, Kohjima M, Izaki T, Nunoi H, and Sumimoto H. Novel human homologues of $p47^{phox}$ and $p67^{phox}$ participate in activation of superoxide-producing NADPH oxidases. *J Biol Chem* 278: 25234–25246, 2003.
327. Thrasher AJ, Keep NH, Wientjes F, and Segal AW. Chronic granulomatomatous disease. *Biochim Biophys Acta* 1227: 1–24, 1994.
328. Tojo T, Ushio-Fukai M, Yamaoka-Tojo M, Ikeda S, Patrushev N, and Alexander RW. Role of $gp91^{phox}$ (Nox2)-Containing NAD(P)H oxidase in angiogenesis in response to hindlimb ischemia. *Circulation* 111: 2347–2355, 2005.
329. Touyz RM, Mercure C, He Y, Javeshghani D, Yao G, Callera GE, Yogi A, Lochard N, and Reudelhuber TL. Angiotensin II-dependent chronic hypertension and cardiac hypertrophy are unaffected by gp91phox-containing NADPH oxidase. *Hypertension* 45: 530–537, 2005.
330. Touyz RM, Chen X, Tabet F, Yao G, He G, Quinn MT, Pagano PJ, and Schiffrin EL. Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H Oxidase in smooth muscle cells from human resistance arteries: regulation by angiotensin II. *Circ Res* 90: 1205–1213, 2002.
331. Touyz RM, Yao G, and Schiffrin EL. Role of the actin cytoskeleton in angiotensin II signaling in human vascular smooth muscle cells. *Can J Physiol Pharmacol* 83: 91–97, 2005.
332. Touyz RM, Yao G, Viel E, Amiri F, and Schiffrin EL. Angiotensin II and endothelin-1 regulate MAP kinases through different redox-dependent mechanisms in human vascular smooth muscle cells. *J Hypertens* 22: 1141–1149, 2004.
333. Touyz RM, Tabet F, and Schiffrin EL. Redox-dependent signalling by angiotensin II and vascular remodelling in hypertension. *Clin Exper Pharmacol Physiol* 30: 860–866, 2003.
334. Tzima E, del Pozo MA, Kiosses WB, Mohamed SA, Li S, Chien S, and Schwartz MA. Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression. *EMBO J* 21: 6791–6800, 2002.
335. Ushio-Fukai M, Tang Y, Fukai T, Dikalov SI, Ma Y, Fujimoto M, Quinn MT, Pagano PJ, Johnson C, and Alexander RW. Novel role of $gp91^{phox}$-containing NAD(P)H oxidase in vascular endothelial growth factor-induced signaling and angiogenesis. *Circ Res* 91: 1160–1167, 2002.
336. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, and Griendling KK. $p22^{phox}$ is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotenisn II-induced hypertrophy in vascular smooth muscle cells. *J Biol Chem* 271: 23317–23321, 1996.
337. Ushio-Fukai M, Alexander RW, Akers M, Yin Q, Fujio Y, Walsh K, and Griendling KK. Reactive oxygen species mediate the activation of Akt/protein kinase B by angiotensin II in vascular smooth muscle cells. *J Biol Chem* 274: 22699–22704, 1999.
338. Van Buul JD, Fernandez-Borja M, Anthony EC, and Hordijk PL. Expression and localization of NOX2 and NOX4 in primary human endothelial cells. *Antioxid Redox Signal* 7: 308–317, 2005.
339. Vecchione C, Brandes RP. Withdrawal of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors elicits oxidative stress and induces endothelial dysfunction in mice. *Circ Res* 91: 173–179, 2002.

340. Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, and Schiffrin EL. Spironolactone improves angiotensin-induced vascular changes and oxidative stress. *Hypertension* 40: 504–510, 2002.
341. Voo KS, Skalnik DG. Elf-1 and PU.1 induce expression of gp91phox via a promoter element mutated in a subset of chronic granulomatous disease patients. *Blood* 93: 3512–3520, 1999.
342. Wadsworth RM. Vasoconstrictor and vasodilator effects of hypoxia. *Trends Pharmacol Sci* 15: 47–53, 1994.
343. Wagner AH, Schroeter MR, and Hecker M. 17b-estrodiol inhibition of NADPH oxidase expression in human endothelial cells. *FASEB J* 15: 2121–2130, 2001.
344. Wagner AH, Kohler T, Ruckschloss U, Just I, and Hecker M. Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. *Arterioscler Thromb Vasc Biol* 20: 61–69, 2000.
345. Wang H, Mao Y, Chen AY, Zhou N, LaVoie EJ, and Liu LF. Stimulation of Topoisomerase II-mediated DNA damage via a mechanism involving protein thiolation. *Biochemistry* 40: 3316–3323, 2001.
346. Wang HD, Hope S, Du Y, Quinn MT, Cayette A, Pagano PJ, and Cohen RA. Paracrine role of adventitial superoxide anion in mediating spontaneous tone of the isolated rat aorta in angiotensin II-induced hypertension. *Hypertension* 33: 1225–1232, 1999.
347. Wang HD, Pagano PJ, Du Y, Cayatte AJ, Quinn MT, Brecher P, and Cohen RA. Superoxide anion from the adventitia of the rat thoracic aorta inactivates nitric oxide. *Circ Res* 82: 810–818, 1998.
348. Wang HD, Xu S, Johns DG, Du Y, Quinn MT, Cayatte AJ, and Cohen RA. Role of NADPH oxidase in the vascular hypertrophic and oxidative stress response to angiotensin II in mice. *Circ Res* 88: 947–953, 2001.
349. Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, Heitzer T, Stasch JP, Griendling KK, Harrison DG, Bohm M, Meinertz T, and Munzel T. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis. Evidence for invovlement of the renin-angiotensin system. *Circulation* 99: 2027–2033, 1999.
350. Wassmann S, Laufs U, Muller K, Konkol C, Ahlbory K, Baumer AT, Linz W, Bohm M, and Nickenig G. Cellular antioxidant effects of atorvastatin in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 22: 300–305, 2002.
351. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, and Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab* 280: E685–E694, 2001.
352. Weening RS, De Boer M, Kuijpers TW, Neefjes VME, Hack WWM, and Roos D. Point mutations in the promoter region of the CYBB gene leading to mild chronic granulomatous disease. *Clin Exper Immunol* 122: 410–417, 2000.
353. Weissmann N, Tadic A, Hanze J, Rose F, Winterhalder S, Nollen M, Schermuly RT, Ghofrani HA, Seeger W, and Grimminger F. Hypoxic vasoconstriction in intact lungs: a role for NADPH oxidase-derived H_2O_2? *Am J Physiol Lung Cell Mol Physiol* 279: L683–L690, 2000.
354. Welch HC, Coadwell WJ, Ellson CD, Ferguson GJ, Andrews SR, Erdjument-Bromage H, Tempst P, Hawkins PT, and Stephens LR. P-Rex1, a PtdIns(3,4,5)P_3- and Gbg-regulated guanine-nucleotide exchange factor for Rac. *Cell* 108: 809–821, 2002.
355. Wenzel S, Taimor G, Piper HM, and Schluter KD. Redox-sensitive intermediates mediate angiotensin II-induced p38 MAP kinase activation, AP-1 binding activity, and TGF-b expression in adult ventricular cardiomyocytes. *FASEB J* 15: 2291–2293, 2001.
356. Wingler K, Wunsch S, Kreutz R, Rothermund L, Paul M, and Schmidt HH. Upregulation of the vascular NAD(P)H-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system in vitro and in vivo. *Free Radic Biol Med* 31: 1456–1464, 2001.
357. Wojciak-Stothard B, Ridley AJ. Shear stress-induced endothelial cell polarization is mediated by Rho and Rac but not Cdc42 or PI 3-kinases. *J Cell Biol* 161: 429–439, 2003.
358. Wong RKM, Pettit AI, Quinn PA, Jennings SC, Davies JE, and Ng LL. Advanced Glycation end products stimulate an enhanced neutrophil respiratory burst mediated through the activation of cytosolic phospholipase A_2 and generation of arachidonic acid. *Circulation* 108: 1858–1864, 2003.
359. Wu RF, Gu Y, Xu YC, Mitola S, Bussolino F, and Terada LS. Human immunodeficiency virus type 1 Tat regulates endothelial cell actin cytoskeletal dynamics through PAK1 activation and oxidant production. *J Virol* 78: 779–789, 2004.
360. Wu RF, Gu Y, Xu YC, Nwariaku FE, and Terada LS. Vascular endothelial growth factor causes translocation of p47phox to membrane ruffles through WAVE1. *J Biol Chem* 278: 36830–36840, 2003.
361. Xiao L, Pimental DR, Amin JK, Singh K, Sawyer DB, and Colucci WS. MEK1/2-ERK1/2 mediates a_1-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes. *J Mol Cell Cardiol* 33: 779–787, 2001.
362. Xiao L, Pimentel DR, Wang J, Singh K, Colucci WS, and Sawyer DB. Role of reactive oxygen species and NAD(P)H oxidase in alpha 1-adrenoceptor signaling in adult rat cardiac myocytes. *Am J Physiol Cell Physiol* 282: C926–C934, 2002.
363. Xu YC, Wu RF, Gu Y, Yang YS, Yang MC, Nwariaku FE, and Terada LS. Involvement of TRAF4 in oxidative activation of c-Jun N-terminal kinase. *J Biol Chem* 277: 28051–28057, 2002.
364. Yamagishi SI, Inagaki Y, Okamoto T, Amano S, Koga K, and Takeuchi M. Advanced glycation end products inhibit de novo protein synthesis and induce TGF-b overexpression in proximal tubular cells. *Kidney Int* 63: 464–473, 2003.
365. Yamagishi SI, Nakamura K, Ueda S, Kato S, and Imaizumi T. Pigment epithelium-derived factor (PEDF) blocks angiotensin II signaling in endothelial cells via suppression of NADPH oxidase: a novel anti-oxidative mechanism of PEDF. *Cell and Tissue Research* 320: 437–445, 2005.
366. Yang D, Suzuki S, Hao LJ, Fujii Y, Yamauchi A, Yamamoto M, Nakamura M, and Kumatori A. Eosinophil-specific regulation of gp91phox gene expression by tran-

scription factors GATA-1 and GATA-2. *J Biol Chem* 275: 9425–9432, 2000.
367. Yasuda M, Ohzeki Y, Shimizu S, Naito S, Ohtsuru A, Yamamoto T, and Kuroiwa Y. Stimulation of in vitro angiogenesis by hydrogen peroxide and the relation with ETS-1 in endothelial cells. *Life Sci* 64: 249–258, 1999.
368. Yeh LH, Park YJ, Hansalia RJ, Ahmed IS, Deshpande SS, Goldschmidt-Clermont PJ, Irani K, and Alevriadou BR. Shear-induced tyrosine phosphorylation in endothelial cells requires Rac1-dependent production of ROS. *Am J Physiol* 276: C838–C847, 1999.
369. Yoshida L, Nishida S, Shimoyama T, Kawahara T, Rokutan K, and Tsunawaki S. Expression of a $p67^{phox}$ homolog in Caco-2 cells giving O^{-}_{2}-reconstituting ability to cytochrome b_{558} together with recombinant $p47^{phox}$. *Biochem Biophys Res Commun* 296: 1322–1328, 2002.
370. Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, Taylor WR, and Griendling KK. Role of NADH/NADPH oxidase-derived H_2O_2 in angiotensin II-induced vascular hypertrophy. *Hypertension* 32: 488–495, 1998.
371. Zalba G, San Jose G, Moreno MU, Fortuno MA, Fortuno A, Beaumont FJ, and Diez J. Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. *Hypertension* 38: 1395–1399, 2001.
372. Zalba G, Jose GS, Beaumont FJ, Fortuno MA, Fortuno A, and Diez J. Polymorphisms and promoter overactivity of the p22phox gene in vascular smooth muscle cells from spontaneously hypertensive rats. *Circ Res* 88: 217–222, 2001.
373. Zalewski A, Shi Y. Vascular Myofibroblasts: Lessons from coronary repair and remodeling. *Arterioscler Thromb Vasc Biol* 17: 417–422, 1997.
374. Zhan Y, Virbasius JV, Song X, Pomerleau DP, and Zhou GW. The p40phox and p47phox PX domains of NADPH oxidase target cell membranes via direct and indirect recruitment by phosphoinositides. *J Biol Chem* 277: 4512–4518, 2002.
375. Zhang H, Schmeisser A, Garlichs.C.D., Plotze K, Damme U, Mugge A, and Daniel WG. Angiotensin II induced superoxide anion generation in human vascular endothelial cells: role of membrane-bound NADH/NADPH-oxidase. *Cardiovasc Res* 44: 215–222, 1999.
376. Zhang L, Zalewski A, Liu Y, Mazurek T, Cowan S, Martin JL, Hofmann SM, Vlassara H, and Shi Y. Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. *Circulation* 108: 472–478, 2003.
377. Zhang M, Kho AL, Anilkumar N, Chibber R, Pagano PJ, Shah AM, and Cave AC. Glycated proteins stimulate reactive oxygen species production in cardiac myocytes: involvement of a Nox2(gp91phox) containing NADPH oxidase. *Circulation* 113: 1235–1243, 2006.
378. Zhao X, Xu B, Bhattacharjee A, Oldfield CM, Wientjes FB, Feldman GM, and Cathcart MK. Protein kinase C d regulates p67phox phosphorylation in human monocytes. *J Leukoc Biol* 77: 414–420, 2005.
379. Zheng JS, Yang XQ, Lookingland KJ, Fink GD, Hesslinger C, Kapatos G, Kovesdi I, and Chen AF. Gene transfer of human guanosine 5'-triphosphate cyclohydrolase I restores vascular tetrahydrobiopterin level and endothelial function in low renin hypertension. *Circulation* 108: 1238–1245, 2003.
380. Zimmerman MC, Dunlay RP, Lazartigues E, Zhang Y, Sharma RV, Engelhardt JF, and Davisson RL. Requirement for Rac1-dependent NADPH oxidase in the cardiovascular and dipsogenic actions of angiotensin II in the brain. *Circ Res* 95: 532–539, 2004.
381. Zorov DB, Filburn CR, Klotz LO, Zweier JL, and Sollott SJ. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J Exp Med* 192: 1001–1014, 2000.
382. Zuo L, Ushio-Fukai M, Ikeda S, Hilenski L, Patrushev N, and Alexander RW. Caveolin-1 is essential for activation of Rac1 and NAD(P)H oxidase after angiotensin II type 1 receptor stimulation in vascular smooth muscle cells: role in redox signaling and vascular hypertrophy. *Arterioscler Thromb Vasc Biol* 25: 1824–1830, 2005.

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3. Asma Kassab, Agnieszka Piwowar. 2012. Cell oxidant stress delivery and cell dysfunction onset in type 2 diabetes. *Biochimie* **94**:9, 1837-1848. [CrossRef]
4. Boris B. Boyanovsky, William Bailey, Lauren Dixon, Preetha Shridas, Nancy R. Webb. 2012. Group V Secretory Phospholipase A2 Enhances the Progression of Angiotensin II–Induced Abdominal Aortic Aneurysms but Confers Protection against Angiotensin II–Induced Cardiac Fibrosis in ApoE-Deficient Mice. *The American Journal of Pathology* **181**:3, 1088-1098. [CrossRef]
5. Dhruv K. Singh. 2012. Diabetic nephropathy: associated risk factors in renal deterioration. *International Journal of Diabetes in Developing Countries* . [CrossRef]
6. Min Zhang , Alessia Perino , Alessandra Ghigo , Emilio Hirsch , Ajay M. Shah . NADPH Oxidases in Heart Failure: Poachers or Gamekeepers?. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Joonghoon Park, Eok Park, Bong-Hyun Ahn, Hyoung Jin Kim, Ji-hoon Park, Sun Young Koo, Hyo-Shin Kwak, Heui Sul Park, Dong Wook Kim, Myoungsub Song, Hyeon Joo Yim, Dong Ook Seo, Soon Ha Kim. 2012. NecroX-7 prevents oxidative stress-induced cardiomyopathy by inhibition of NADPH oxidase activity in rats. *Toxicology and Applied Pharmacology* **263**:1, 1-6. [CrossRef]
8. Mahesh Thirunavukkarasu, Ram Sudheer Adluri, Bela Juhasz, Samson Mathews Samuel, Lijun Zhan, Anupinder Kaur, Gautam Maulik, Juan A Sanchez, Janet Hager, Nilanjana Maulik. 2012. Novel role of NADPH oxidase in ischemic myocardium: a study with Nox2 knockout mice. *Functional & Integrative Genomics* **12**:3, 501-514. [CrossRef]
9. Roberto Cangemi, Andrea Celestini, Maria Ben, Pasquale Pignatelli, Roberto Carnevale, Marco Proietti, Cinzia Myriam Calabrese, Stefania Basili, Francesco Violi. 2012. Role of platelets in NOX2 activation mediated by TNF# in heart failure. *Internal and Emergency Medicine* . [CrossRef]
10. Smitha Malireddy , Sainath R. Kotha , Jordan D. Secor , Travis O. Gurney , Jamie L. Abbott , Gautam Maulik , Krishna R. Maddipati , Narasimham L. Parinandi . 2012. Phytochemical Antioxidants Modulate Mammalian Cellular Epigenome: Implications in Health and Disease. *Antioxidants & Redox Signaling* **17**:2, 327-339. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
11. Julie Turgeon, Paola Haddad, Sylvie Dussault, Jessika Groleau, Fritz Maingrette, Gemma Perez, Alain Rivard. 2012. Protection against vascular aging in Nox2-deficient mice: Impact on endothelial progenitor cells and reparative neovascularization. *Atherosclerosis* **223**:1, 122-129. [CrossRef]
12. Nynke E. Hahn, Christof Meischl, Tsukasa Kawahara, René J.P. Musters, Viola M.J. Verhoef, Jolanda van der Velden, Alexander B.A. Vonk, Walter J. Paulus, Albert C. van Rossum, Hans W.M. Niessen, Paul A.J. Krijnen. 2012. NOX5 Expression Is Increased in Intramyocardial Blood Vessels and Cardiomyocytes after Acute Myocardial Infarction in Humans. *The American Journal of Pathology* **180**:6, 2222-2229. [CrossRef]
13. Li Zhang, Yijiang Zhou, Jianhua Zhu, Qingbo Xu. 2012. An updated view on stem cell differentiation into smooth muscle cells. *Vascular Pharmacology* **56**:5-6, 280-287. [CrossRef]
14. Subir Kumar Maulik, Santosh Kumar. 2012. Oxidative stress and cardiac hypertrophy: a review. *Toxicology Mechanisms and Methods* 1-8. [CrossRef]
15. Vaithinathan Selvaraju, Mandip Joshi, Sumanth Suresh, Juan A. Sanchez, Nilanjana Maulik, Gautam Maulik. 2012. Diabetes, oxidative stress, molecular mechanism, and cardiovascular disease – an overview. *Toxicology Mechanisms and Methods* 1-6. [CrossRef]
16. Lorenzo Loffredo, Roberto Carnevale, Roberto Cangemi, Francesco Angelico, Teresa Augelletti, Serena Di Santo, Cinzia M. Calabrese, Luigi Della Volpe, Pasquale Pignatelli, Ludovica Perri, Stefania Basili, Francesco Violi. 2012. NOX2 up-regulation is associated with artery dysfunction in patients with peripheral artery disease. *International Journal of Cardiology* . [CrossRef]

17. G. Douglas, J. K. Bendall, M. J. Crabtree, A. L. Tatham, E. E. Carter, A. B. Hale, K. M. Channon. 2012. Endothelial-specific Nox2 overexpression increases vascular superoxide and macrophage recruitment in ApoE-/- mice. *Cardiovascular Research* . [CrossRef]

18. H Teoh, A Quan, A K Creighton, K W Annie Bang, K K Singh, P C Shukla, N Gupta, Y Pan, F Lovren, H Leong-Poi, M Al-Omran, S Verma. 2012. BRCA1 gene therapy reduces systemic inflammatory response and multiple organ failure and improves survival in experimental sepsis. *Gene Therapy* . [CrossRef]

19. Evangelos P. Daskalopoulos, Ben J.A. Janssen, W. Matthijs Blankesteijn. 2012. Myofibroblasts in the Infarct Area: Concepts and Challenges. *Microscopy and Microanalysis* 1-15. [CrossRef]

20. Bingqing Deng, Shuanglun Xie, Jingfeng Wang, Zhengyuan Xia, Ruqiong Nie. 2012. Inhibition of Protein Kinase C #₂ Prevents Tumor Necrosis Factor-#-Induced Apoptosis and Oxidative Stress in Endothelial Cells: The Role of NADPH Oxidase Subunits. *Journal of Vascular Research* **49**:2, 144-159. [CrossRef]

21. James B. Strait, Edward G. Lakatta. 2012. Aging-Associated Cardiovascular Changes and Their Relationship to Heart Failure. *Heart Failure Clinics* **8**:1, 143-164. [CrossRef]

22. Arpeeta Sharma, Pascal N. Bernatchez, Judy B. de Haan. 2012. Targeting Endothelial Dysfunction in Vascular Complications Associated with Diabetes. *International Journal of Vascular Medicine* **2012**, 1-12. [CrossRef]

23. Gabriella Leonarduzzi, Paola Gamba, Simona Gargiulo, Fiorella Biasi, Giuseppe Poli. 2012. Inflammation-related gene expression by lipid oxidation-derived products in the progression of atherosclerosis. *Free Radical Biology and Medicine* **52**:1, 19-34. [CrossRef]

24. James B. Strait, Edward G. LakattaCardiac Aging 639-659. [CrossRef]

25. Maria Del Ben, Mario Fabiani, Lorenzo Loffredo, Licia Polimeni, Roberto Carnevale, Francesco Baratta, Marco Brunori, Fabiana Albanese, Teresa Augelletti, Francesco Violi, Francesco Angelico. 2012. Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea and the effect of continuous positive airway pressure treatment. *BMC Pulmonary Medicine* **12**:1, 36. [CrossRef]

26. Benjamin S. Avner, Krystyna M. Shioura, Sarah B. Scruggs, Milana Grachoff, David L. Geenen, Donald L. Helseth, Mariam Farjah, Paul H. Goldspink, R. John Solaro. 2011. Myocardial infarction in mice alters sarcomeric function via post-translational protein modification. *Molecular and Cellular Biochemistry* . [CrossRef]

27. Gregory J. Gatto, Zhaohui Ao, Michael G. Kearse, Mei Zhou, Cyndi R. Morales, Erin Daniels, Benjamin T. Bradley, Matthew T. Goserud, Krista B. Goodman, Stephen A. Douglas, Mark R. Harpel, Douglas G. Johns. 2011. NADPH oxidase-dependent and -independent mechanisms of reported inhibitors of reactive oxygen generation. *Journal of Enzyme Inhibition and Medicinal Chemistry* 1-10. [CrossRef]

28. 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]

29. Herve Y. Sroussi, Yu Lu, Dana. Villines, Ying Sun. 2011. The down regulation of neutrophil oxidative metabolism by S100A8 and S100A9: Implication of the protease-activated receptor-2. *Molecular Immunology* . [CrossRef]

30. Lorenzo Loffredo. 2011. Chronic granulomatous disease. *Internal and Emergency Medicine* **6**:S1, 125-128. [CrossRef]

31. Svetlana V. Kostyuk, Aleksei V. Ermakov, Anna Yu. Alekseeva, Tatiana D. Smirnova, Kristina V. Glebova, Liudmila V. Efremova, Ancha Baranova, Natalya N. Veiko. 2011. Role Of Extracellular Dna #xidative Modification In Radiation Induced Bystander Effects In Human Endotheliocytes. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* . [CrossRef]

32. Shan Gao, Kuichang Yuan, Amin Shah, Jong Suk Kim, Woo Hyun Park, Suhn Hee Kim. 2011. Suppression of high pacing-induced ANP secretion by antioxidants in isolated rat atria. *Peptides* . [CrossRef]

33. Michael Y. Song , Ayako Makino , Jason X.-J. Yuan . 2011. Role of Reactive Oxygen Species and Redox in Regulating the Function of Transient Receptor Potential Channels. *Antioxidants & Redox Signaling* **15**:6, 1549-1565. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

34. Yanan Liu, Shaoqing Lei, Xia Gao, Xiaowen Mao, Tingting Wang, Gordon Wong, Paul Vanhoutte, Michael Irwin, Zhengyuan Xia. 2011. PKC-beta Inhibition with Ruboxistaurin Reduces Oxidative Stress And Attenuates Left Ventricular Hypertrophy and Dysfunction In Rats With Streptozotosin-Induced Diabetes. *Clinical Science* . [CrossRef]

35. Eric J. Belin de Chantemele, David W. Stepp. 2011. Influence of obesity and metabolic dysfunction on the endothelial control in the coronary circulation. *Journal of Molecular and Cellular Cardiology* . [CrossRef]

36. Pawel Niemiec, Tomasz Nowak, Anna Balcerzyk, Jolanta Krauze, Iwona Zak. 2011. The CYBA gene A640G polymorphism influences predispositions to coronary artery disease through interactions with cigarette smoking and hypercholesterolemia. *Biomarkers* **16**:5, 405-412. [CrossRef]
37. Paola Haddad, Sylvie Dussault, Jessika Groleau, Julie Turgeon, Fritz Maingrette, Alain Rivard. 2011. Nox2-derived reactive oxygen species contribute to hypercholesterolemia-induced inhibition of neovascularization: Effects on endothelial progenitor cells and mature endothelial cells. *Atherosclerosis* **217**:2, 340-349. [CrossRef]
38. Mariuca Vasa-Nicotera, Hailan Chen, Paola Tucci, Ai Li Yang, Gaelle Saintigny, Rossella Menghini, Christian Mahè, Massimiliano Agostini, Richard A. Knight, Gerry Melino, Massimo Federici. 2011. miR-146a is modulated in human endothelial cell with aging. *Atherosclerosis* **217**:2, 326-330. [CrossRef]
39. 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]
40. Paari Dominic Swaminathan, Anil Purohit, Siddarth Soni, Niels Voigt, Madhu V. Singh, Alexey V. Glukhov, Zhan Gao, B. Julie He, Elizabeth D. Luczak, Mei-ling A. Joiner, William Kutschke, Jinying Yang, J. Kevin Donahue, Robert M. Weiss, Isabella M. Grumbach, Masahiro Ogawa, Peng-Sheng Chen, Igor Efimov, Dobromir Dobrev, Peter J. Mohler, Thomas J. Hund, Mark E. Anderson. 2011. Oxidized CaMKII causes cardiac sinus node dysfunction in mice. *Journal of Clinical Investigation* . [CrossRef]
41. Tatjana Stankovi#, Vidosava #or#evi#, Borislav Kamenov, Hristina Stamenkovi#, Vladan #osi#, Radovan Mili#evi#, Vjeroslava Slavi#. 2011. Antioxidative Enzyme Activities and Lipid Peroxidation in Children with Inflammatory Endothelial Injury. *Journal of Medical Biochemistry* **30**:3, 250-254. [CrossRef]
42. Gabriel Loor, Jyothisri Kondapalli, Hirotaro Iwase, Navdeep S. Chandel, Gregory B. Waypa, Robert D. Guzy, Terry L. Vanden Hoek, Paul T. Schumacker. 2011. Mitochondrial oxidant stress triggers cell death in simulated ischemia–reperfusion. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1813**:7, 1382-1394. [CrossRef]
43. Hui-min Yan, Jing Zhao, De-zhong Ma, Hua Wang, Jia Wang, Zhi-hao Wang, Li Li, Yun Zhang, Wei Zhang, Ming Zhong. 2011. The effect of pitavastatin calcium on endothelial dysfunction induced by hypercholesterolemia. *Expert Opinion on Pharmacotherapy* **12**:10, 1463-1471. [CrossRef]
44. Shan Zhang, Beidong Chen, Wei Wu, Li Bao, Ruomei Qi. 2011. Ginkgolide B Reduces Inflammatory Protein Expression in Oxidized Low-density Lipoprotein-stimulated Human Vascular Endothelial Cells. *Journal of Cardiovascular Pharmacology* **57**:6, 721-727. [CrossRef]
45. Andrea L. Sheehan, Samuel Carrell, Bryon Johnson, Bojana Stanic, Botond Banfi, Francis J. Miller. 2011. Role for Nox1 NADPH oxidase in atherosclerosis. *Atherosclerosis* **216**:2, 321-326. [CrossRef]
46. Imad Al Ghouleh, Nicholas K.H. Khoo, Ulla G. Knaus, Kathy K. Griendling, Rhian M. Touyz, Victor J. Thannickal, Aaron Barchowsky, William M. Nauseef, Eric E. Kelley, Phillip M. Bauer, Victor Darley-Usmar, Sruti Shiva, Eugenia Cifuentes-Pagano, Bruce A. Freeman, Mark T. Gladwin, Patrick J. Pagano. 2011. Oxidases and peroxidases in cardiovascular and lung disease: New concepts in reactive oxygen species signaling. *Free Radical Biology and Medicine* . [CrossRef]
47. Alexander Sirker, Min Zhang, Ajay M. Shah. 2011. NADPH oxidases in cardiovascular disease: insights from in vivo models and clinical studies. *Basic Research in Cardiology* . [CrossRef]
48. Ana M. Briones, Fatiha Tabet, Glaucia E. Callera, Augusto C. Montezano, Alvaro Yogi, Ying He, Mark T. Quinn, Mercedes Salaices, Rhian M. Touyz. 2011. Differential regulation of Nox1, Nox2 and Nox4 in vascular smooth muscle cells from WKY and SHR. *Journal of the American Society of Hypertension* **5**:3, 137-153. [CrossRef]
49. Jeffrey L Barnes, Yves Gorin. 2011. Myofibroblast differentiation during fibrosis: role of NAD(P)H oxidases. *Kidney International* **79**:9, 944-956. [CrossRef]
50. Wenbo Zhang, Hua Liu, Modesto Rojas, Robert W Caldwell, Ruth B Caldwell. 2011. Anti-inflammatory therapy for diabetic retinopathy. *Immunotherapy* **3**:5, 609-628. [CrossRef]
51. Haixiang Wu, Chunhui Jiang, Dekang Gan, Yujie Liao, Hui Ren, Zhongcui Sun, Meng Zhang, Gezhi Xu. 2011. Different effects of low- and high-dose insulin on ROS production and VEGF expression in bovine retinal microvascular endothelial cells in the presence of high glucose. *Graefe's Archive for Clinical and Experimental Ophthalmology* . [CrossRef]
52. Gábor Csányi, Eugenia Cifuentes-Pagano, Imad Al Ghouleh, Daniel J. Ranayhossaini, Loreto Egaña, Lucia R. Lopes, Heather M. Jackson, Eric E. Kelley, Patrick J. Pagano. 2011. Nox2 B-loop peptide, Nox2ds, specifically inhibits the NADPH oxidase Nox2. *Free Radical Biology and Medicine* . [CrossRef]
53. Celio X.C. Santos, Narayana Anilkumar, Min Zhang, Alison C. Brewer, Ajay M. Shah. 2011. Redox signaling in cardiac myocytes. *Free Radical Biology and Medicine* **50**:7, 777-793. [CrossRef]

54. Mei-Hua Bao, Wen Dai, Yuan-Jian Li, Chang-Ping Hu. 2011. Rutaecarpine prevents hypoxia–reoxygenation-induced myocardial cell apoptosis via inhibition of NADPH oxidases. *Canadian Journal of Physiology and Pharmacology* **89**:3, 177-186. [CrossRef]
55. Toru Okazaki, Hajime Otani, Takayuki Shimazu, Kei Yoshioka, Masanori Fujita, Tayo Katano, Seiji Ito, Toshiji Iwasaka. 2011. Reversal of inducible nitric oxide synthase uncoupling unmasks tolerance to ischemia/reperfusion injury in the diabetic rat heart. *Journal of Molecular and Cellular Cardiology* **50**:3, 534-544. [CrossRef]
56. Dhruv K. Singh, Peter Winocour, Ken Farrington. 2011. Oxidative stress in early diabetic nephropathy: fueling the fire. *Nature Reviews Endocrinology* **7**:3, 176-184. [CrossRef]
57. 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]
58. Yisang Yoon , Chad A. Galloway , Bong Sook Jhun , Tianzheng Yu . 2011. Mitochondrial Dynamics in Diabetes. *Antioxidants & Redox Signaling* **14**:3, 439-457. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
59. Tianzheng Yu , Bong Sook Jhun , Yisang Yoon . 2011. High-Glucose Stimulation Increases Reactive Oxygen Species Production Through the Calcium and Mitogen-Activated Protein Kinase-Mediated Activation of Mitochondrial Fission. *Antioxidants & Redox Signaling* **14**:3, 425-437. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
60. Xinghua Cheng , Richard C.M. Siow , Giovanni E. Mann . 2011. Impaired Redox Signaling and Antioxidant Gene Expression in Endothelial Cells in Diabetes: A Role for Mitochondria and the Nuclear Factor-E2-Related Factor 2-Kelch-Like ECH-Associated Protein 1 Defense Pathway. *Antioxidants & Redox Signaling* **14**:3, 469-487. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
61. Claudia Piccoli, Giovanni Quarato, Annamaria D'Aprile, Eustacchio Montemurno, Rosella Scrima, Maria Ripoli, Monica Gomaraschi, Pietro Cirillo, Domenico Boffoli, Laura Calabresi, Loreto Gesualdo, Nazzareno Capitanio. 2011. Native LDL-induced oxidative stress in human proximal tubular cells: multiple players involved. *Journal of Cellular and Molecular Medicine* **15**:2, 375-395. [CrossRef]
62. 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]
63. Wei-Wen Kuo, Wei-Jan Wang, Cheng-Wen Lin, Peiying Pai, Tung-Yuan Lai, Chen-Yen Tsai. 2011. NADPH oxidase-derived superoxide anion-induced apoptosis is mediated via the JNK-dependent activation of NF-#B in cardiomyocytes exposed to high glucose. *Journal of Cellular Physiology* n/a-n/a. [CrossRef]
64. Yu Liu, He Huang, Wenfang Xia, Yanhong Tang, Mingjie Yuan, Qizhu Tang, Congxin Huang. 2011. Inhibition of NADPH oxidase up-regulates connexin 43 and ameliorates electrical remodeling in rabbits with heart failure. *Biomedicine & Aging Pathology* **1**:1, 33-38. [CrossRef]
65. Rhian M Touyz, Ana M Briones. 2011. Reactive oxygen species and vascular biology: implications in human hypertension. *Hypertension Research* **34**:1, 5-14. [CrossRef]
66. Adam Nabeebaccus, Min Zhang, Ajay M. Shah. 2011. NADPH oxidases and cardiac remodelling. *Heart Failure Reviews* **16**:1, 5-12. [CrossRef]
67. K Wingler, JJR Hermans, P Schiffers, AL Moens, M Paul, HHHW Schmidt. 2011. NOX 1, 2, 4, 5: Counting out oxidative stress. *British Journal of Pharmacology* no-no. [CrossRef]
68. Monisha Dhiman, Nisha Jain Garg. 2011. NADPH oxidase inhibition ameliorates Trypanosoma cruzi-induced myocarditis during Chagas disease. *The Journal of Pathology* n/a-n/a. [CrossRef]
69. Srikanth Pendyala, Viswanathan Natarajan. 2010. Redox regulation of Nox proteins#. *Respiratory Physiology & Neurobiology* **174**:3, 265-271. [CrossRef]
70. Richard C.M. Siow, Giovanni E. Mann. 2010. Dietary isoflavones and vascular protection: Activation of cellular antioxidant defenses by SERMs or hormesis?. *Molecular Aspects of Medicine* **31**:6, 468-477. [CrossRef]
71. Gabriel Loor, Jyothisri Kondapalli, Jacqueline M. Schriewer, Navdeep S. Chandel, Terry L. Vanden Hoek, Paul T. Schumacker. 2010. Menadione triggers cell death through ROS-dependent mechanisms involving PARP activation without requiring apoptosis. *Free Radical Biology and Medicine* **49**:12, 1925-1936. [CrossRef]
72. Meika Foster , Samir Samman . 2010. Zinc and Redox Signaling: Perturbations Associated with Cardiovascular Disease and Diabetes Mellitus. *Antioxidants & Redox Signaling* **13**:10, 1549-1573. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

73. Gabriella Leonarduzzi, Barbara Sottero, Giuseppe Poli. 2010. Targeting tissue oxidative damage by means of cell signaling modulators: The antioxidant concept revisited. *Pharmacology & Therapeutics* **128**:2, 336-374. [CrossRef]

74. Douglas B. Kell. 2010. Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Archives of Toxicology* **84**:11, 825-889. [CrossRef]

75. S Wind, K Beuerlein, T Eucker, H Müller, P Scheurer, ME Armitage, H Ho, HHHW Schmidt, K Wingler. 2010. Comparative pharmacology of chemically distinct NADPH oxidase inhibitors. *British Journal of Pharmacology* **161**:4, 885-898. [CrossRef]

76. Robert Schwartz, Yu Lu, Dana Villines, Herve Y. Sroussi. 2010. Effect of human immunodeficiency virus infection on S100A8/A9 inhibition of peripheral neutrophils oxidative metabolism. *Biomedicine & Pharmacotherapy* **64**:8, 572-575. [CrossRef]

77. Xian-Ju Huang, Xu Wang, Awais Ihsan, Qin Liu, Xi-Juan Xue, Shi-Jia Su, Chun-Hui Yang, Wen Zhou, Zong-Hui Yuan. 2010. Interactions of NADPH oxidase, renin–angiotensin–aldosterone system and reactive oxygen species in mequindox-mediated aldosterone secretion in Wistar rats. *Toxicology Letters* **198**:2, 112-118. [CrossRef]

78. Yu Liu, He Huang, Wenfang Xia, Yanhong Tang, Mingjie Yuan, Qizhu Tang, Congxin Huang. 2010. Inhibition of NADPH oxidase up-regulates connexin 43 and ameliorates electrical remodeling in rabbits with heart failure. *Biomedicine & Pharmacotherapy* . [CrossRef]

79. J. Kuroda, T. Ago, S. Matsushima, P. Zhai, M. D. Schneider, J. Sadoshima. 2010. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *Proceedings of the National Academy of Sciences* **107**:35, 15565-15570. [CrossRef]

80. Nedyalka V. Georgieva, Krasimir Stoyanchev, Nadia Bozakova, Ivanka Jotova. 2010. Combined Effects of Muscular Dystrophy, Ecological Stress, and Selenium on Blood Antioxidant Status in Broiler Chickens. *Biological Trace Element Research* . [CrossRef]

81. C. Bentley, N. Hathaway, J. Widdows, F. Bejta, C. De Pascale, M. Avella, C.P.D. Wheeler-Jones, K.M. Botham, C. Lawson. 2010. Influence of chylomicron remnants on human monocyte activation in vitro. *Nutrition, Metabolism and Cardiovascular Diseases* . [CrossRef]

82. Kuei-Chuan Lee, Ying-Ying Yang, Ying-Wen Wang, Fa-Yauh Lee, Che-Chuan Loong, Ming-Chih Hou, Han-Chieh Lin, Shou-Dong Lee. 2010. Increased Plasma Malondialdehyde in Patients with Viral Cirrhosis and Its Relationships to Plasma Nitric Oxide, Endotoxin, and Portal Pressure. *Digestive Diseases and Sciences* **55**:7, 2077-2085. [CrossRef]

83. Pasquale Pignatelli, Gaetano Tanzilli, Roberto Carnevale, Serena Di Santo, Lorenzo Loffredo, Andrea Celestini, Marco Proietti, Priscilla Tovaglia, Enrico Mangieri, Stefania Basili, Francesco Violi. 2010. Ascorbic Acid Infusion Blunts CD40L Upregulation in Patients Undergoing Coronary Stent. *Cardiovascular Therapeutics* no-no. [CrossRef]

84. Ana L. Luna, Leonor C. Acosta-Saavedra, Lizbeth Lopez-Carrillo, Patricia Conde, Eunice Vera, Andrea De Vizcaya-Ruiz, Mariana Bastida, Mariano E. Cebrian, Emma S. Calderon-Aranda. 2010. Arsenic alters monocyte superoxide anion and nitric oxide production in environmentally exposed children. *Toxicology and Applied Pharmacology* **245**:2, 244-251. [CrossRef]

85. Antje R. Weseler, Aalt Bast. 2010. Oxidative Stress and Vascular Function: Implications for Pharmacologic Treatments. *Current Hypertension Reports* **12**:3, 154-161. [CrossRef]

86. Guy Vassort, Belma Turan. 2010. Protective Role of Antioxidants in Diabetes-Induced Cardiac Dysfunction. *Cardiovascular Toxicology* **10**:2, 73-86. [CrossRef]

87. Kiyoko Uno, Stephen J Nicholls. 2010. Biomarkers of inflammation and oxidative stress in atherosclerosis. *Biomarkers in Medicine* **4**:3, 361-373. [CrossRef]

88. Chandan K. Sen Tiny New Genes Called MicroRNAs Regulate Blood Vessel Formation 353-358. [Abstract] [Full Text PDF] [Full Text PDF with Links]

89. Mingyi Wang, Jing Zhang, Simon J. Walker, Rafal Dworakowski, Edward G. Lakatta, Ajay M. Shah. 2010. Involvement of NADPH oxidase in age-associated cardiac remodeling. *Journal of Molecular and Cellular Cardiology* **48**:4, 765-772. [CrossRef]

90. Helen Imrie, Afroze Abbas, Mark Kearney. 2010. Insulin resistance, lipotoxicity and endothelial dysfunction. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1801**:3, 320-326. [CrossRef]

91. Chai Hui, Wo Like, Fu Yan, Xie Tian, Wang Qiuyan, Huang Lifeng. 2010. S-Allyl-L-Cysteine Sulfoxide Inhibits Tumor Necrosis Factor-Alpha Induced Monocyte Adhesion and Intercellular Cell Adhesion Molecule-1 Expression in Human Umbilical Vein Endothelial Cells. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology* **293**:3, 421-430. [CrossRef]

92. Zhirajr Mokini, M. Loredana Marcovecchio, Francesco Chiarelli. 2010. Molecular pathology of oxidative stress in diabetic angiopathy: Role of mitochondrial and cellular pathways. *Diabetes Research and Clinical Practice* **87**:3, 313-321. [CrossRef]
93. M. G. Vinokurov, M. M. Yurinskaya. 2010. Regulation of the apoptosis of neutrophils under the action of lipopolysaccharides. *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology* **4**:1, 13-21. [CrossRef]
94. Ashraf Taye, Adel H. Saad, Arun HS Kumar, Henning Morawietz. 2010. Effect of apocynin on NADPH oxidase-mediated oxidative stress-LOX-1-eNOS pathway in human endothelial cells exposed to high glucose. *European Journal of Pharmacology* **627**:1-3, 42-48. [CrossRef]
95. So-Yeon Kim, Jin-Gu Lee, Woo-Sung Cho, Kyong-Hyun Cho, Jun Sakong, Jae-Ryong Kim, Byung-Rho Chin, Suk-Hwan Baek. 2010. Role of NADPH oxidase-2 in lipopolysaccharide-induced matrix metalloproteinase expression and cell migration. *Immunology and Cell Biology* **88**:2, 197-204. [CrossRef]
96. Mark F. McCarty, Jorge Barroso-Aranda, Francisco Contreras. 2010. Potential complementarity of high-flavanol cocoa powder and spirulina for health protection. *Medical Hypotheses* **74**:2, 370-373. [CrossRef]
97. Weiwei Yin, Hanjoong Jo, Eberhard O. Voit. 2010. Systems Analysis of the Role of Bone Morphogenic Protein 4 in Endothelial Inflammation. *Annals of Biomedical Engineering* **38**:2, 291-307. [CrossRef]
98. Lang Wang, Li-Hua Zhu, Hong Jiang, Qi-Zhu Tang, Ling Yan, Dong Wang, Chen Liu, Zhou-Yan Bian, Hongliang Li. 2010. Grape seed proanthocyanidins attenuate vascular smooth muscle cell proliferation via blocking phosphatidylinositol 3-kinase-dependent signaling pathways. *Journal of Cellular Physiology* n/a-n/a. [CrossRef]
99. Joseph R. Burgoyne, Philip EatonA Rapid Approach for the Detection, Quantification, and Discovery of Novel Sulfenic Acid or S-Nitrosothiol Modified Proteins Using a Biotin-Switch Method **473**, 281-303. [CrossRef]
100. Giorgio Lenaz, Paola StrocchiReactive Oxygen Species in the Induction of Toxicity . [CrossRef]
101. Gábor Csányi, W. Robert Taylor, Patrick J. Pagano. 2009. NOX and inflammation in the vascular adventitia. *Free Radical Biology and Medicine* **47**:9, 1254-1266. [CrossRef]
102. David I. Brown, Kathy K. Griendling. 2009. Nox proteins in signal transduction. *Free Radical Biology and Medicine* **47**:9, 1239-1253. [CrossRef]
103. JiangYong Gu, Gu Yuan, YongHong Zhu, XiaoJie Xu. 2009. Computational pharmacological studies on cardiovascular disease by Qishen Yiqi Diwan. *Science in China Series B: Chemistry* **52**:11, 1871-1878. [CrossRef]
104. Célio X.C. Santos , Leonardo Y. Tanaka , João Wosniak , Jr. , Francisco R.M. Laurindo . 2009. Mechanisms and Implications of Reactive Oxygen Species Generation During the Unfolded Protein Response: Roles of Endoplasmic Reticulum Oxidoreductases, Mitochondrial Electron Transport, and NADPH Oxidase. *Antioxidants & Redox Signaling* **11**:10, 2409-2427. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
105. Barry H. Trachtenberg, Joshua M. Hare. 2009. Biomarkers of Oxidative Stress in Heart Failure. *Heart Failure Clinics* **5**:4, 561-577. [CrossRef]
106. Ge Gao , Samuel C. Dudley , Jr. . 2009. Redox Regulation, NF-#B, and Atrial Fibrillation. *Antioxidants & Redox Signaling* **11**:9, 2265-2277. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
107. Ian M. Fearon, Stephen P. Faux. 2009. Oxidative stress and cardiovascular disease: Novel tools give (free) radical insight. *Journal of Molecular and Cellular Cardiology* **47**:3, 372-381. [CrossRef]
108. Vyacheslav M. Shkryl, Adriano S. Martins, Nina D. Ullrich, Martha C. Nowycky, Ernst Niggli, Natalia Shirokova. 2009. Reciprocal amplification of ROS and Ca2+ signals in stressed mdx dystrophic skeletal muscle fibers. *Pflügers Archiv - European Journal of Physiology* **458**:5, 915-928. [CrossRef]
109. Lorenzo Loffredo, Francesco Violi. 2009. The Role of Nicotinamide Adenine Dinucleotide Phosphate Oxidase in the Pathogenesis of Hypertension. *High Blood Pressure & Cardiovascular Prevention* **16**:3, 87-92. [CrossRef]
110. Yin Hua Zhang, Lewis Dingle, Rachel Hall, Barbara Casadei. 2009. The role of nitric oxide and reactive oxygen species in the positive inotropic response to mechanical stretch in the mammalian myocardium. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1787**:7, 811-817. [CrossRef]
111. Ashwin Akki, Min Zhang, Colin Murdoch, Alison Brewer, Ajay M. Shah. 2009. NADPH oxidase signaling and cardiac myocyte function. *Journal of Molecular and Cellular Cardiology* **47**:1, 15-22. [CrossRef]
112. Il-Young Paik, Chan-Ho Jin, Hwa-Eun Jin, Young-Il Kim, Su-Youn Cho, Hee-Tae Roh, Ah-Ram Suh, Sang-Hoon Suh. 2009. Effects of the NADPH oxidase p22phox C242T polymorphism on endurance exercise performance and oxidative DNA damage in response to aerobic exercise training. *Molecules and Cells* **27**:5, 557-562. [CrossRef]
113. Yiqun Mo, Rong Wan, Sufan Chien, David J. Tollerud, Qunwei Zhang. 2009. Activation of endothelial cells after exposure to ambient ultrafine particles: The role of NADPH oxidase. *Toxicology and Applied Pharmacology* **236**:2, 183-193. [CrossRef]

114. Randall S. Frey , Masuko Ushio–Fukai , Asrar B. Malik . 2009. NADPH Oxidase-Dependent Signaling in Endothelial Cells: Role in Physiology and Pathophysiology. *Antioxidants & Redox Signaling* **11**:4, 791-810. [Abstract] [Full Text PDF] [Full Text PDF with Links]

115. Srikanth Pendyala , Peter V. Usatyuk , Irina A. Gorshkova , Joe G.N. Garcia , Viswanathan Natarajan . 2009. Regulation of NADPH Oxidase in Vascular Endothelium: The Role of Phospholipases, Protein Kinases, and Cytoskeletal Proteins. *Antioxidants & Redox Signaling* **11**:4, 841-860. [Abstract] [Full Text PDF] [Full Text PDF with Links]

116. Yingxiu Kang, Minghua Hu, Yanhui Zhu, Xin Gao, Ming-Wei Wang. 2009. Antioxidative effect of the herbal remedy Qin Huo Yi Hao and its active component tetramethylpyrazine on high glucose-treated endothelial cells. *Life Sciences* **84**:13-14, 428-436. [CrossRef]

117. Mona Sedeek, Richard L Hébert, Chris R Kennedy, Kevin D Burns, Rhian M Touyz. 2009. Molecular mechanisms of hypertension: role of Nox family NADPH oxidases. *Current Opinion in Nephrology and Hypertension* **18**:2, 122-127. [CrossRef]

118. S. Heymans, E. Hirsch, S. D. Anker, P. Aukrust, J.-L. Balligand, J. W. Cohen-Tervaert, H. Drexler, G. Filippatos, S. B. Felix, L. Gullestad, D. Hilfiker-Kleiner, S. Janssens, R. Latini, G. Neubauer, W. J. Paulus, B. Pieske, P. Ponikowski, B. Schroen, H.-P. Schultheiss, C. Tschope, M. Van Bilsen, F. Zannad, J. McMurray, A. M. Shah. 2009. Inflammation as a therapeutic target in heart failure? A scientific statement from the Translational Research Committee of the Heart Failure Association of the European Society of Cardiology. *European Journal of Heart Failure* **11**:2, 119-129. [CrossRef]

119. KA Jackman, AA Miller, TM De Silva, PJ Crack, GR Drummond, CG Sobey. 2009. Reduction of cerebral infarct volume by apocynin requires pretreatment and is absent in Nox2-deficient mice. *British Journal of Pharmacology* **156**:4, 680-688. [CrossRef]

120. L. Gao, G. E. Mann. 2009. Vascular NAD(P)H oxidase activation in diabetes: a double-edged sword in redox signalling. *Cardiovascular Research* **82**:1, 9-20. [CrossRef]

121. Po Sing Leung , Yuk Cheung Chan . 2009. Role of Oxidative Stress in Pancreatic Inflammation. *Antioxidants & Redox Signaling* **11**:1, 135-166. [Abstract] [Full Text PDF] [Full Text PDF with Links]

122. Adel Boueiz, Paul M. Hassoun. 2009. Regulation of endothelial barrier function by reactive oxygen and nitrogen species. *Microvascular Research* **77**:1, 26-34. [CrossRef]

123. Francesco Violi, Stefania Basili, Carmen Nigro, Pasquale Pignatelli. 2009. Role of NADPH oxidase in atherosclerosis. *Future Cardiology* **5**:1, 83-92. [CrossRef]

124. Hung-hsing Chao, Ju-chi Liu, Jia-wei Lin, Cheng-hsien Chen, Chieh-hsi Wu, Tzu-humg Cheng. 2008. Uric acid stimulates endothelin-1 gene expression associated with NADPH oxidase in human aortic smooth muscle cells. *Acta Pharmacologica Sinica* **29**:11, 1301-1312. [CrossRef]

125. U RESCH, Y SCHICHL, S SATTLER, R DEMARTIN. 2008. XIAP regulates intracellular ROS by enhancing antioxidant gene expression. *Biochemical and Biophysical Research Communications* **375**:1, 156-161. [CrossRef]

126. Wenyuan Zhao, Tieqiang Zhao, Yuanjian Chen, Robert A. Ahokas, Yao Sun. 2008. Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor-beta1 in hypertensive rats. *Molecular and Cellular Biochemistry* **317**:1-2, 43-50. [CrossRef]

127. Irina V. Gorudko, Inna V. Buko, Sergey N. Cherenkevich, Leonid Z. Polonetsky, Alexander V. Timoshenko. 2008. Lectin-induced Aggregates of Blood Cells from Patients with Acute Coronary Syndromes. *Archives of Medical Research* **39**:7, 674-681. [CrossRef]

128. Tankred Schewe, Yvonne Steffen, Helmut Sies. 2008. How do dietary flavanols improve vascular function? A position paper. *Archives of Biochemistry and Biophysics* **476**:2, 102-106. [CrossRef]

129. S LEE, H KIM, Y SONG, H JOO, J LEE, K LEE, E CHO, C CHO, J PARK, B JEON. 2008. Alteration of p66shc is associated with endothelial dysfunction in the abdominal aortic coarctation of rats. *FEBS Letters* **582**:17, 2561-2566. [CrossRef]

130. Cecilia Hidalgo , Paulina Donoso . 2008. Crosstalk Between Calcium and Redox Signaling: From Molecular Mechanisms to Health Implications. *Antioxidants & Redox Signaling* **10**:7, 1275-1312. [Abstract] [Full Text PDF] [Full Text PDF with Links]

131. J. David Lambeth, Karl-Heinz Krause, Robert A. Clark. 2008. NOX enzymes as novel targets for drug development. *Seminars in Immunopathology* **30**:3, 339-363. [CrossRef]

132. Biji T. Kurien, R. Hal Scofield. 2008. Autoimmunity and oxidatively modified autoantigens. *Autoimmunity Reviews* **7**:7, 567-573. [CrossRef]

133. Sara P. Alom-Ruiz , Narayana Anilkumar , Ajay M. Shah . 2008. Reactive Oxygen Species and Endothelial Activation. *Antioxidants & Redox Signaling* **10**:6, 1089-1100. [Abstract] [Full Text PDF] [Full Text PDF with Links]

134. Francisco R.M. Laurindo , Denise C. Fernandes , Angélica M. Amanso , Lucia R. Lopes , Célio X.C. Santos . 2008. Novel Role of Protein Disulfide Isomerase in the Regulation of NADPH Oxidase Activity: Pathophysiological Implications in Vascular Diseases. *Antioxidants & Redox Signaling* **10**:6, 1101-1114. [Abstract] [Full Text PDF] [Full Text PDF with Links]

135. Tetsuro Ago, Tong Liu, Peiyong Zhai, Wei Chen, Hong Li, Jeffery D. Molkentin, Stephen F. Vatner, Junichi Sadoshima. 2008. A Redox-Dependent Pathway for Regulating Class II HDACs and Cardiac Hypertrophy. *Cell* **133**:6, 978-993. [CrossRef]

136. Delphine Behr-Roussel, Alexandra Oudot, Stéphanie Caisey, Olivier L.E. Coz, Diane Gorny, Jacques Bernabé, Chris Wayman, Laurent Alexandre, François A. Giuliano. 2008. Daily Treatment with Sildenafil Reverses Endothelial Dysfunction and Oxidative Stress in an Animal Model of Insulin Resistance. *European Urology* **53**:6, 1272-1281. [CrossRef]

137. Agnes W. Boots, Guido R.M.M. Haenen, Aalt Bast. 2008. Health effects of quercetin: From antioxidant to nutraceutical. *European Journal of Pharmacology* **585**:2-3, 325-337. [CrossRef]

138. Roman Ginnan, Benjamin J. Guikema, Katharine E. Halligan, Harold A. Singer, David Jourd'heuil. 2008. Regulation of smooth muscle by inducible nitric oxide synthase and NADPH oxidase in vascular proliferative diseases. *Free Radical Biology and Medicine* **44**:7, 1232-1245. [CrossRef]

139. N FARAGO, G KOCSIS, L FEHER, T CSONT, L HACKLERJR, Z VARGAORVOS, C CSONKA, J KELEMEN, P FERDINANDY, L PUSKAS. 2008. Gene and protein expression changes in response to normoxic perfusion in mouse hearts. *Journal of Pharmacological and Toxicological Methods* **57**:2, 145-154. [CrossRef]

140. Jie Wang, Lingna Li, Hui Cang, Guiying Shi, Jing Yi. 2008. NADPH oxidase-derived reactive oxygen species are responsible for the high susceptibility to arsenic cytotoxicity in acute promyelocytic leukemia cells. *Leukemia Research* **32**:3, 429-436. [CrossRef]

141. Sashwati Roy, Savita Khanna, Chandan K. Sen. 2008. Redox regulation of the VEGF signaling path and tissue vascularization: Hydrogen peroxide, the common link between physical exercise and cutaneous wound healing. *Free Radical Biology and Medicine* **44**:2, 180-192. [CrossRef]

142. A. I. Kavalenka, G. N. Semenkova, S. N. Cherenkevich. 2007. Effects of hydrogen peroxide on neutrophil ability to generate reactive oxygen and chlorine species and to secrete myeloperoxidase in vitro. *Cell and Tissue Biology* **1**:6, 551-559. [CrossRef]

143. Jorge Gracia–Sancho, Bàrbara Laviña, Aina Rodríguez–Vilarrupla, Ralf P. Brandes, Mercedes Fernández, Jaume Bosch, Joan–Carles García–Pagán. 2007. Evidence Against a Role for NADPH Oxidase Modulating Hepatic Vascular Tone in Cirrhosis. *Gastroenterology* **133**:3, 959-966. [CrossRef]

144. M KANEGAE, L DAFONSECA, I BRUNETTI, S DEOLIVEIRASILVA, V XIMENES. 2007. The reactivity of ortho-methoxy-substituted catechol radicals with sulfhydryl groups: Contribution for the comprehension of the mechanism of inhibition of NADPH oxidase by apocynin. *Biochemical Pharmacology* **74**:3, 457-464. [CrossRef]

145. Pawel Niemiec, Iwona Zak, Krystian Wita. 2007. The 242T variant of the CYBA gene polymorphism increases the risk of coronary artery disease associated with cigarette smoking and hypercholesterolemia. *Coronary Artery Disease* **18**:5, 339-346. [CrossRef]

146. Subramaniam Pennathur , Jay W. Heinecke . 2007. Mechanisms for Oxidative Stress in Diabetic Cardiovascular Disease. *Antioxidants & Redox Signaling* **9**:7, 955-969. [Abstract] [Full Text PDF] [Full Text PDF with Links]

147. Grace Y. Sun, Lloyd A. Horrocks, Akhlaq A. Farooqui. 2007. The roles of NADPH oxidase and phospholipases A 2 in oxidative and inflammatory responses in neurodegenerative diseases. *Journal of Neurochemistry*, ahead of print070611013409004-???. [CrossRef]

148. Tetsuro Ago , Junichi Sadoshima . 2007. Thioredoxin1 as a Negative Regulator of Cardiac Hypertrophy. *Antioxidants & Redox Signaling* **9**:6, 679-687. [Abstract] [Full Text PDF] [Full Text PDF with Links]

149. Renata Laškaj, Dodig Slavica, Ivana #epelak, Ilija Kuzman. 2007. Gamma-Glutamyltransferase Activity and Total Antioxidant Status in Serum and Platelets of Patients with Community-acquired Pneumonia. *Archives of Medical Research* **38**:4, 424-431. [CrossRef]

150. Min Zhang, Ajay M. Shah. 2007. Role of reactive oxygen species in myocardial remodeling. *Current Heart Failure Reports* **4**:1, 26-30. [CrossRef]

151. Aina Rodríguez-Vilarrupla, Jaume Bosch, Joan-Carles García-Pagán. 2007. Potential role of antioxidants in the treatment of portal hypertension. *Journal of Hepatology* **46**:2, 193-197. [CrossRef]