MINIREVIEW

Hydrogen Peroxide as a Paracrine Vascular Mediator: Regulation and Signaling Leading to Dysfunction

NOELIA ARDANAZ AND PATRICK J. PAGANO[1]

Hypertension and Vascular Research Division, Henry Ford Health System, Detroit, Michigan 48202-2689

Numerous studies have demonstrated the ability of a variety of vascular cells, including endothelial cells, smooth muscle cells, and fibroblasts, to produce reactive oxygen species (ROS). Until recently, major emphasis was placed on the production of superoxide anion (O_2^-) in the vasculature as a result of its ability to directly attenuate the biological activity of endothelium-derived nitric oxide (NO). The short half-life and radius of diffusion of O_2^- drastically limit the role of this ROS as an important paracrine hormone in vascular biology. On the contrary, in recent years, the O_2^- metabolite hydrogen peroxide (H_2O_2) has increasingly been viewed as an important cellular signaling agent in its own right, capable of modulating both contractile and growth-promoting pathways with more far-reaching effects. In this review, we will assess the vascular production of H_2O_2, its regulation by endogenous scavenger systems, and its ability to activate a variety of vascular signaling pathways, thereby leading to vascular contraction and growth. This discussion will include the ability of H_2O_2 to (i) initiate calcium flux as well as (ii) stimulate pathways leading to sensitization of contractile elements to calcium. The latter involves a variety of protein kinases that have also been strongly implicated in vascular hypertrophy. Previous intensive study has emphasized the ability of NADPH oxidase-derived O_2^- and H_2O_2 to activate these pathways in cultured smooth muscle cells. However, growing evidence indicates a considerably more complex array of unique oxidase systems in the endothelium, media, and adventitia that appear to participate in these deleterious effects in a sequential and temporal manner. Taken together, these findings seem consistent with a paracrine effect of H_2O_2 across the vascular wall. Exp Biol Med 231:237–251, 2006

Key words: reactive oxygen species; hydrogen peroxide; blood vessel; signaling; hypertrophy; hypertension

Vascular Generation of H_2O_2

Reactive oxygen species (ROS) are a class of molecules that are derived from the metabolism of oxygen and include free radical and nonradical species that are generally capable of oxidizing molecular targets. H_2O_2 is a cell-permeant and highly stable ROS generated mainly by dismutation of superoxide (O_2^-) by superoxide dismutases (SOD). Although H_2O_2 is defined as an ROS, unlike O_2^- it is not a free radical, in that it does not possess an unpaired electron in its outer shell. This renders H_2O_2 more stable and less reactive with other tissue radicals and, thus, a more likely paracrine ROS. In the presence of iron, intracellular H_2O_2 can be converted to hydroxyl radical, which can oxidize molecular targets and cause lipid peroxidation and this may account for some of its local biological effects (1, 2). O_2^- is considered the precursor of all ROS because in most cases it is the first ROS produced by mammalian oxidases. This is clearly true in the vasculature, where a primary source of ROS has been identified as NAD(P)H oxidase (3–10). Our laboratory and those of Griendling and Wolin initially identified this major vascular source of ROS in vascular fibroblasts, vascular smooth muscle cells (VSMCs), and endothelial cells (3, 11–14). Our findings indicate that a substantial percentage of all O_2^--generating activity in rabbits, rats, and mice is traceable to adventitial fibroblast

This work was supported by the National Institutes of Health grants HL55425 and HL28982, American Heart Association grant 0151407Z and the Fund for Henry Ford Hospital. N.A. was also supported by an American Heart Association Fellowship 0520056Z. P.J.P. is an Established Investigator of the American Heart Association.

[1] To whom correspondence should be addressed at Hypertension and Vascular Research Division, RM 7044, E&R Building, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202-2689. E-mail: ppagano1@hfhs.org

1535-3702/06/2313-0237$15.00

NAD(P)H oxidase, which contains all four major neutrophil-like NAD(P)H oxidase components: nox2 (previously termed gp91-phox), $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$ (14). More recently, nox4 was found to be present in the adventitia (15). NAD(P)H oxidase in VSMCs has been extensively described and implicated in cell growth and impairment of endothelium-dependent relaxation (3, 12). VSMCs express critical phagocyte-like NAD(P)H oxidase components (9, 16–18) as well as homologues of essential phagocyte nox2, nox1, and nox4 that participate in O_2^- production (9, 19, 20). Whereas the rat and mouse aorta apparently possess a functional nox1 and nox4 but little or no nox2, nox2 appears to predominate in resistance artery smooth muscle cells (9). Likewise, endothelial cells contain phagocyte-like components including nox2 and at least one of its homologues, nox4, which are functionally involved in O_2^- production (11, 21–25) and endothelial dysfunction (25). While most studies confirm that NAD(P)H oxidases produce O_2^-, some contend that these enzymes can also produce H_2O_2 (Table 1; Refs. 26–29). Furthermore, rat and human fat cells possess a membrane-bound NAD(P)H oxidase that produces H_2O_2 as its initial product (27). Generally speaking, however, most H_2O_2 is derived from dismutation of O_2^-.

Other enzymes appear to be involved in the vascular production of O_2^-, although their contribution to vascular generation of ROS seems significantly lower than that of NAD(P)H oxidase. Even so, the activity of these other sources appears to be accentuated under pathological conditions. For instance, nitric oxide synthase (NOS) can generate not only NO but also O_2^- when concentrations of substrate or cofactors are compromised, leading to uncoupled NOS and increased O_2^- production (39, 40). The mitochondria seem to be a major source of O_2^- and H_2O_2 in the lung and heart *via* the mitochondrial respiratory chain complex I and III (41–43). Mitochondria do not appear to contribute significantly to total vascular ROS production (13, 14). This is most likely attributable to the relative metabolic inactivity of quiescent blood vessels. However, during oxygen delivery, rapid remodeling, or tissue damage, mitochondria would be expected to play a more significant role. Interestingly, the metabolic abnormalities seen in diabetes reveal a role for mitochondrial ROS in endothelial cells (44, 45).

Xanthine dehydrogenase is converted to xanthine oxidase by thiol oxidation or irreversible proteolytic cleavage, and its activity in endothelial cells seems to be increased in ischemia-reperfusion (46–48). One recent study showed that xanthine oxidase activity is upregulated in response to oscillatory shear stress, possibly implicating ROS derived from this source in the development of dysfunction and plaque formation at vascular regions of disturbed flow (49). Interestingly, in that same study (49), NADPH oxidase was described as an important modulator of xanthine oxidase expression and activity, suggesting a positive feedback loop between the two enzymes. Another intriguing study posits a link between xanthine oxidase and activation of cyclooxygenase 2, indicating a positive association in inflammation (50). Since cyclooxygenase has been described as a generator of vascular ROS, this interaction may participate in feed-forward generation of ROS, as suggested generally for NADPH oxidases (5, 8). Other studies have shown that cytochrome P450 and lipoxygenases are capable of releasing O_2^-, which may play an important role in vascular function (51, 52). Finally, other enzymes, including heme oxygenases and peroxidases, have also been implicated in O_2^- production (6). Although the preponderance of evidence appears to support a dominant role for NAD(P)H oxidases in the production of vascular ROS—given that inhibitors of other enzymatic sources appear to have negligible effects under most conditions—these enzymes in combination may contribute significantly to total ROS levels and alterations in tone and remodeling. Regardless of its source, O_2^- is spontaneously or efficiently converted to the stable and tissue-permeant H_2O_2 by abundant and ubiquitous intracellular and extracellular SODs (53, 54). The relevance of each class of SOD will depend, of course, on the location of O_2^- production in the cell. That is, manganese SOD (Mn-SOD) found in the mitochondria is critical to the proper handling of O_2^- derived from mitochondrial electron transport (55). Cytosolic copper/zinc (Cu/Zn)-SOD dismutes O_2^- derived from a variety of above-mentioned cytosolic oxidases, including VSMC NADPH oxidases (20, 56). Finally, the fate of extracellular production of O_2^- by leukocytes (and suggested for adventitial fibroblasts) is determined by the extracellular tethered form of Cu/Zn-SOD (ec-SOD) (57, 58). It has been suggested that endothelial cells can also produce extracellular O_2^- (59). As the expression of SOD is enhanced by inflammatory cytokines, in hypertension and in direct response to angiotensin II (Ang II) (60, 61), the

Table 1. Vascular Localization of NAD(P)H Oxidase Components

NAD(P)H oxidase component	Smooth muscle cells	Endothelium	Adventitia/fibroblasts
$p22^{phox}$	Yes (16, 22, 30)	Yes (21, 22)	Yes (14, 31, 32)
$p47^{phox}$	Yes (18)	Yes (21, 22)	Yes (14, 31, 33)
$p67^{phox}$	?	Yes (21, 22)	Yes (14, 31, 34, 35)
$p91^{phox}$(nox2)	No (19) Yes (9)	Yes (21, 22, 36, 37)	Yes (14, 31, 35)
nox1	Yes (19)	Yes (15, 38)	?
nox4	Yes (20)	Yes (25)	Yes (15)

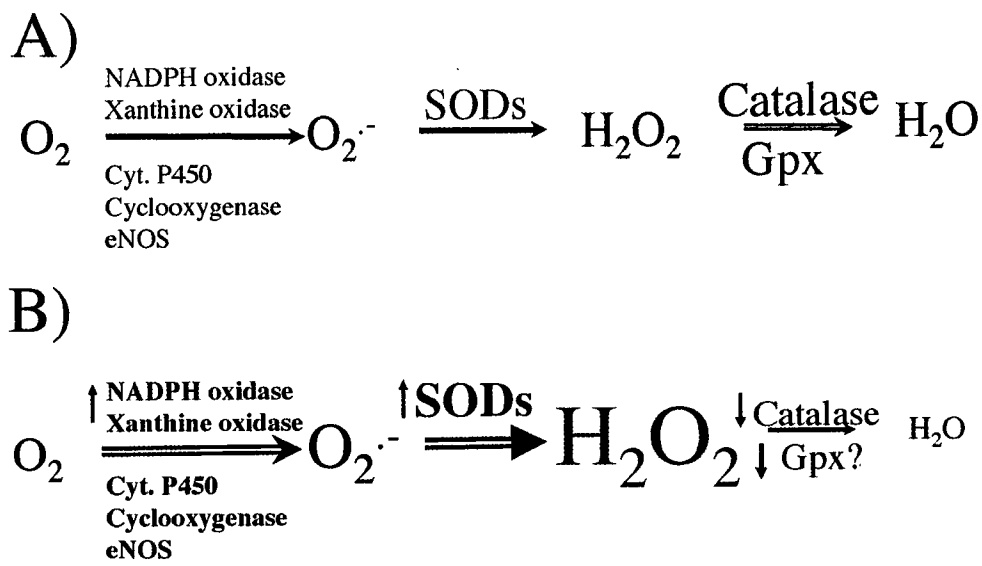

Figure 1. Scheme showing possible mechanisms of regulation of H_2O_2 in cardiovascular disease. Under normal conditions (A), constitutively active oxidases produce O_2^- that is rapidly converted by abundant superoxide dismutases to H_2O_2. Catalase and glutathione peroxidase inactivate H_2O_2. In hypertension, multiple oxidases leading to the production of O_2^- as well as SOD appear to be upregulated (B). Additionally, factors in cardiovascular disease causing reduced catalase and/or Gpx activity are expected to contribute to an increased steady-state level of H_2O_2.

conversion of O_2^- to H_2O_2 appears to be favored in cardiovascular disease (see Fig. 1). Given the stability of H_2O_2 and its plausibility as a paracrine mediator of vascular dysfunction, in this review we will focus on the regulation and biological activity of this important vascular ROS.

Endogenous Scavenger Systems that Regulate Vascular Levels of H_2O_2

Steady-state H_2O_2 levels in vascular tissue are tightly regulated by its endogenous scavengers catalase and glutathione peroxidase (Gpx1) (62, 63). Gpx1 is found in cellular cytosol and mitochondria and is a key enzyme for the cellular defense against oxidative stress, using glutathione to reduce H_2O_2 and lipid peroxides to their respective alcohols (64). Catalase is found primarily in peroxisomes and exclusively catalyzes the conversion of H_2O_2 to water. Although the contribution of these enzymes seems to vary in tissue, in vascular preparations catalase possesses a higher K_m for H_2O_2, compared to Gpx1, and may serve as an important intracellular defense against large amounts of H_2O_2 (63). Studies suggest that vascular Gpx1 is better suited to scavenge endogenous basal levels of H_2O_2 (63, 65). Moreover, the contribution of these enzymes to vascular ROS metabolism seems to change with age (66), and their relative contribution along the vasculature has not been established. Regardless of these distinctions, both enzymes are considered ubiquitously important in regulating endogenous H_2O_2. Interestingly, comparisons of aortas from populations of mice with high blood pressure versus normal blood pressure have revealed a reduction in catalase expression and activity concomitant with an increase in the activity of Mn-SOD and ec-SOD (67). It appears plausible, then, that an increase in the steady-state level of H_2O_2 in hypertension would be favored by such an imbalance (Fig. 1). In fact, it is known that in human patients with essential hypertension, plasma levels of H_2O_2 do rise (68, 69). Clearly, more studies will be necessary to examine the relationship of SODs versus catalase and Gpx1 in the vasculature in various cardiovascular diseases.

A relatively new class of antioxidant enzymes named peroxiredoxins have been shown to reduce H_2O_2 and, more recently, peroxynitrite using thioredoxin as the immediate electron donor (70, 71). Although their catalytic efficiency is less than that of Gpx-1 or catalase, these enzymes seem to regulate H_2O_2 signaling generated by different growth factors (72). Interestingly, a recent study showed that peroxiredoxin plays a role in platelet-derived growth factor signaling in VSMCs and appears to attenuate neointima formation (73).

H_2O_2 as a Vasoactive Substance

Although numerous studies have demonstrated an autocrine effect of H_2O_2 on cultured smooth muscle cell signaling and hypertrophy (6, 74), its role in vascular tone is not well understood. Studies have demonstrated both a contractile and relaxant response to H_2O_2 depending on the species, vascular bed, and contractile state (75–78). Clearly, H_2O_2 has been shown to cause constriction in a variety of vascular beds. Under quiescent conditions, H_2O_2 reportedly contracted the aorta, pulmonary artery, and superior mesenteric artery of the rat (79–84), the porcine pulmonary artery (85), and the canine basilar artery (86). The mechanism involved in H_2O_2-induced vasoconstriction seems to be Ca^{2+}-dependent in the rat aorta and dog basilar

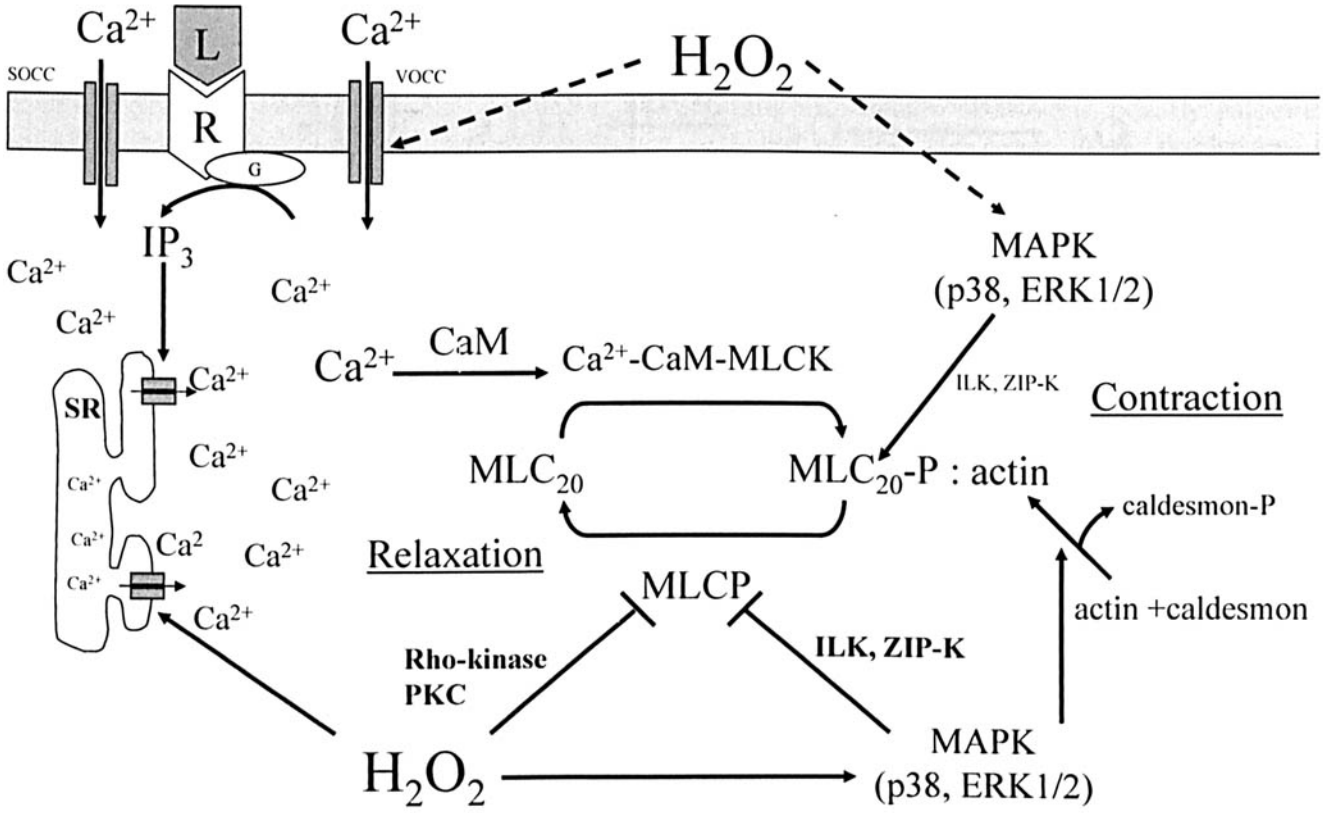

Figure 2. Signal transduction mechanisms of smooth muscle contraction and proposed mechanism of modulation by H_2O_2. Depolarization and activation of voltage-operated calcium channels (VOCC) or ligand-receptor interaction and stimulation of Ca^{2+} release from intracellular stores and secondary activation of store-operated Ca^{2+} channels (SOCC) produce increases in intracellular calcium that lead to activation of myosin light chain 20 (MLC_{20}) by Ca^{2+}-calmodulin-myosin light chain kinase (Ca^{2+}-CaM-MLCK). MLC_{20}-P binds to actin and contraction is produced. MLC_{20}-P is dephosphorylated by myosin light chain phosphatase (MLCP), leading to relaxation. H_2O_2 is able to increase intracellular calcium through VOCC and activation of Ca^{2+} release from intracellular stores. It can also activate MAPK and Rho-kinase, leading to enhanced vascular smooth muscle contraction. Ca^{2+} sensitization mechanisms enhance contraction independently of changes in intracellular Ca^{2+}. These mechanisms lead to an increase in MLC_{20}-P through direct phosphorylation or inhibition of MLCP. Rho-kinase phosphorylates and inactivates MLCP. Mitogen-activated protein kinase (MAPK) through activation of other kinases (ILK), or zipper-interacting protein kinase (ZIP-K) can phosphorylate MLC_{20} and also inactivate MLCP. Moreover, MAPK also seems to be involved in the phosphorylation of caldesmon, favoring the interaction MLC_{20}-P:actin.

artery (79, 81, 86). Ca^{2+}-dependent pathways lead to increased intracellular Ca^{2+} through voltage-gated Ca^{2+} channels as well as Ca^{2+} release from the sarcoplasmic reticulum (87) (Fig. 2). When Ca^{2+} levels are elevated above baseline, Ca^{2+} binds to calmodulin and activates myosin light chain kinase (MLCK), leading to phosphorylation of 20-kDa myosin light chains (MLC_{20}) and constriction. In fact, H_2O_2 can increase intracellular Ca^{2+} by mobilizing extracellular Ca^{2+} *via* activation of voltage-operated Ca^{2+} channels in rat mesenteric VSMCs (88), a mechanism that is also reportedly involved in rat aortic contraction (79, 81). Likewise, H_2O_2 can mobilize Ca^{2+} from the sarcoplasmic reticulum *via* ryanodine receptors (89), caffeine-sensitive stores (81), and inhibition of Ca^{2+}-ATPase (90), and by promoting inositol triphosphate (PI3)–induced calcium release (91). However, the role of both Ca^{2+}-ATPase and ryanodine receptors in H_2O_2-induced vascular constriction remains unclear (92–94). Moreover, H_2O_2 has been shown to elicit Ca^{2+} release from the mitochondria (95) *via* the production of hydroxyl radical (96).

H_2O_2 vasoconstriction is mediated by tyrosine kinase (79, 83, 86, 97), cyclooxygenase products (80, 82), protein kinase C (PKC) (79, 86), mitogen-activated protein kinases (MAPKs) (86, 97, 98), Rho-kinase (ROK) (99), and phosphorylated MLC_{20} (MLC_{20}-P) (83), although the role of PKC, MLC_{20}-P, and MAPK remains controversial (84, 85, 92). Some of these pathways have been described as Ca^{2+} sensitization pathways, defined as increases in generated force without increases in intracellular Ca^{2+} (100). These pathways lead to increases in MLC_{20}-P, mainly *via* inhibition of MLCP, but they may also be regulated by activation of MLCK, direct regulation by heat-shock proteins, and even by mechanisms involving thin filament regulation (caldesmon, calponin) (Fig. 2). MLCP is inhibited by phosphorylation by a variety of kinases, including integrin-linked kinase (ILK) (101, 102), PKC (103), and ROK (104–106). ROK has been shown to be activated by ROS (105) and has been directly implicated in

the constrictor effect of H_2O_2 in veins (99). Moreover, H_2O_2 has been demonstrated to cause vasoconstriction *via* MAPKs, including extracellular signal-regulated kinases (ERK1/2) and p38. In turn, ERK1/2 has been shown to modulate MLCK by increasing MLC_{20}-P (107, 108) and acts as an upstream mediator of ILK, which has also been shown to phosphorylate MLC_{20} in smooth muscle (102, 109) and inactivate MCLP (110). Additionally, ERK1/2 is known to phosphorylate caldesmon, an actin-binding protein that, once phosphorylated, inhibits actin-myosin interaction and thus contraction (111, 112).

p38 MAPK is involved in H_2O_2 vasoconstriction in KCl-preconstricted mesenteric arteries (113). In a few key studies, p38 MAPK-mediated phosphorylation of HSP27 has been described as modulating contraction of VSMCs (114–116). In fact, this mechanism could be involved in activation of zipper-interacting–like kinase (ZIP kinase) and phosphorylation of MLC_{20} as well as inactivation of MLCP. The relevance of Ca^{2+} sensitization pathways in H_2O_2-induced vasoconstriction is not fully understood, but because of the direct effect of H_2O_2 in the activation of MAPK and ROK, they may play an important role in the overall response to H_2O_2.

Interestingly, some findings have also indicated that H_2O_2 could induce constriction *via* Ca^{2+}-independent pathways in rat and rabbit pulmonary arteries (84, 85, 92). The latter reports of possible H_2O_2 constriction independent of calcium are provocative and may involve a direct effect of ROS on contractile elements. However, such discussion is currently beyond the scope of this review.

Lucchesi *et al.* (113) recently showed that H_2O_2 acts as a vasoconstrictor in mouse mesenteric resistance arteries depolarized with KCl. In that study, the same concentrations of H_2O_2 produced dilatation of phenylephrine-preconstricted vessels (113), implying that membrane potential of the vessel influences the effect of H_2O_2 on tone. Similarly, Sotnikova (81) found that increased extracellular potassium enhanced the vasoconstrictor effect of H_2O_2. We have obtained similar results in the abdominal aorta and superior mesenteric artery (i.e., when they were depolarized by KCl, H_2O_2-induced vasoconstriction was enhanced; unpublished data). It appears that extracellular potassium evokes two possible effects that contribute to H_2O_2-induced vasoconstriction: (i) blockade of K^+ channels induced by high levels of extracellular KCl reveals a contractile effect of H_2O_2 *via* suppression of H_2O_2-induced K^+ channel-mediated vasodilatation (117–120) and/or (ii) H_2O_2 activates Ca^{2+} sensitization pathways, enhancing the Ca^{2+}-dependent contraction secondary to depolarization. With regard to the physiologic relevance of these studies, vascular smooth muscle depolarization has been reported in different models of hypertension produced at least in part by alterations in K^+ channel activity (121–125). Decreased K^+ channel activity may contribute to increased vessel contractility (121) that in turn could help explain the increased vasoconstrictor response to H_2O_2 in hypertensive models (80, 126).

On the other hand, H_2O_2 elicits relaxation in agonist-constricted rat, mouse, and rabbit aortas (76, 127–129). This response has been observed in a variety of other vessels, including the human, mouse, rat, and rabbit mesenteric artery (76, 113, 120, 130, 131), porcine coronary artery (118, 132), human and porcine coronary arterioles (117, 133), canine cerebral (134) and basilar arteries (77), bovine and rabbit pulmonary arteries (135, 136), and rabbit iliac artery (137), among others; and the dilator response includes both endothelium-dependent (77, 127, 138, 139) and -independent mechanisms (75, 76, 113, 118, 133, 135). In some cases, both endothelium-dependent and -independent relaxation have been implicated in the effect of H_2O_2 (117, 129). The endothelium-dependent relaxation associated with H_2O_2 seems to be mediated by the NO/cyclic guanosine monophosphate (cGMP) pathway and may be related to an increase in intracellular Ca^{2+} (77, 127, 129, 138). P450 cytochrome metabolites are also plausible mediators of the endothelium-dependent response (117, 127). On the other hand, it has been suggested that H_2O_2-induced endothelium-independent relaxation is mediated through activation of guanylate cyclase and accumulation of cGMP (75, 76, 133, 135, 140), although the role of cGMP is questioned in other studies (117, 120, 134, 141). Another principal pathway that seems to be involved in H_2O_2 relaxation is activation of Ca^{2+}-dependent (117–120, 132, 134, 142), ATP-sensitive (120, 141), and voltage-dependent K^+ channels (120). In some cases, K^+ channel activation is preceded by release of arachidonic acid metabolites (118, 130, 134), so that, eicosanoids have been implicated in this response; however, the role of these metabolites in H_2O_2-induced relaxation is uncertain (120, 128). An interesting article published by Sato *et al.* (133) indicates that exogenous and endogenous H_2O_2 may elicit vasodilatation by different pathways depending on the origin and localization of the peroxide. In spontaneously hypertensive rats (SHR), attenuation of the relaxant response to H_2O_2 was linked to alterations in K^+ channel activity (126). Thus, alterations in K^+ channel activation may play a role in the development of hypertension both by decreasing vasorelaxant responses and by enhancing vasoconstrictor responses.

In accord with its ability to activate K^+ channels, multiple studies have proposed that H_2O_2 is an endothelium-derived hyperpolarizing factor (EDHF), including human and mouse mesenteric arteries (130, 131) and porcine and canine coronary arteries and arterioles (143, 144); however, some groups contradict this observation, as they were unable to demonstrate that EDHF is H_2O_2 (128, 137). It has been suggested that under conditions in which NO bioavailability is reduced, increased EDHF-induced relaxation compensates for the lack of response to NO and preserves endothelium-dependent relaxation (145, 146). In this regard, some authors postulate that in animal models in which endothelial NOS (eNOS) cofactors are compromised,

uncoupled eNOS can serve as a source of H_2O_2 that becomes a compensatory response for endothelium-dependent vasodilatation (39, 40, 147). Even though this increase may result acutely in a beneficial effect, we postulate that over time the effect will become detrimental.

H_2O_2 as a Mediator of Vascular Dysfunction

Despite the body of data suggesting an "acute" relaxant effect of H_2O_2, the effect of prolonged elevations of H_2O_2 (as observed in models of hypertension) on constriction is an important scientific question with regard to the *in vivo* vascular effects of H_2O_2. In fact, the ability of chronically elevated H_2O_2 to impair endothelium-dependent relaxation has been supported by a few key studies. Consistent with a role for endogenous H_2O_2 in impaired relaxation, Gpx1-deficient mice exhibit contraction of mesenteric arteries in response to the endothelium-dependent agonist methacholine, whereas wild-type mice exhibit dilatation (148). Furthermore, methacholine-induced relaxation of mesenteric arteries was converted to contraction in normal versus homocysteinemic mice, an effect that was reversed by Gpx1 overexpression (149). These authors observed a decreased release of bioactive NO from hyperhomocysteinemic endothelial cells, attributing this to homocysteine autooxidation to NO-inactivating peroxide radicals and/or to a specific decrease in cellular Gpx. Thus, overexpression of Gpx1 was capable of restoring NO bioactivity. A subsequent study by Dayal *et al.* (150) showed that deletion of the Gpx1 gene caused impairment of ACh-induced dilatation of the aorta compared to wild-type mice, an effect that was enhanced in hyperhomocysteinemic strains. Thus, these data are consistent with endogenous vascular H_2O_2 leading to impairment of endothelium-dependent relaxation. Interestingly, a recent study (151) showed that in mice, vascular overexpression of human catalase decreases systolic blood pressure and vascular constriction *per se*, suggesting the relevance of endogenous H_2O_2 as a vasoconstrictor and regulator of blood pressure.

However, the mechanisms by which elevated concentrations of H_2O_2 lead to vascular dysfunction remain unclear. One possibility is that sustained increases in H_2O_2 enhance the pathways involved in smooth muscle contraction (Ca^{2+}-dependent or sensitization pathways), leading to vascular dysfunction. Another possibility is that in pathological states such as hypertension, when reductions in K^+ channel activity prevail, these conditions will favor the vasoconstrictor effect of H_2O_2. A few studies show that H_2O_2 can reduce vascular NO production. One intriguing article by Wedgwood and Black (152) suggests that endothelin 1–induced stimulation of H_2O_2 release in pulmonary VSMCs decreases eNOS expression and activity in pulmonary endothelial cells. Another interesting study (153) demonstrated decreased gene expression of inducible NOS. Finally, Jaimes *et al.* (154) showed that H_2O_2 decreased NO production by inactivation of eNOS cofactors without affecting eNOS activity.

At this juncture, it is important to discuss recent findings revealing that H_2O_2 stimulates eNOS and SOD, resulting in higher NO levels (155, 156). One report by Cai *et al.* (155) intriguingly demonstrates that concomitant induction of NOS and SOD by H_2O_2 could explain the preservation of NO despite increased O_2^- and point to a compensatory mechanism of NO protection under some conditions and in some vascular beds. However, most reports, including our own, have suggested that O_2^- levels often outpace elevations in NO (148–150, 157–163). One mechanism by which H_2O_2 promotes such an increase is through dysfunction of NOS, just as it may stimulate phagocyte-like oxidases to produce more O_2^-, H_2O_2, and other ROS (see below). Taken together, these data appear to indicate that H_2O_2 plays an important role in the regulation of NOS activity and in the biological fate of NO, leading to an overall reduced NO bioactivity.

Ability of H_2O_2 to Activate Its Own Generation

Li *et al.* (164) showed that exogenous H_2O_2 activates cellular NAD(P)H oxidase to produce O_2^- and that this effect was independent of xanthine oxidase, cyclooxygenase, eNOS, and mitochondrial activation. These findings were recently corroborated by Seshiah *et al.* (5), who proposed that small amounts of H_2O_2 derived from NAD(P)H oxidase can promote sustained activation of NAD(P)H oxidase. The ability of elevations in ROS to promote oxidase activation has also been inferred from studies showing that an increase in ROS leads to increased p22-phox expression (16, 32). H_2O_2 can also increase transferrin receptor-dependent iron uptake, amplifying intracellular mitochondrial H_2O_2 generation (165). NADPH oxidase activation is required for xanthine oxidase activation and subsequent H_2O_2 formation in response to oscillatory shear stress (49). Finally, as noted earlier, H_2O_2 appears to be involved in the reduced bioavailability of eNOS cofactors, which may induce uncoupled eNOS and lead to increased H_2O_2 production.

H_2O_2 as a Mediator of VSMC Signal Transduction

In addition to the well-characterized involvement of ROS-insensitive pathways in VSMC signal transduction, numerous studies have demonstrated a role for ROS in the activation of both proximal and downstream MAPKs (6). H_2O_2 derived from NAD(P)H oxidase has been implicated in the activation of c-Src, which in turn transactivates receptor tyrosine kinases (5, 97, 166), a process that involves activation of phosphatidyl inositol (PI)3-kinase (5). Epidermal growth factor receptor (EGF-R) and platelet-derived growth factor receptor (PDGF-R) are tyrosine kinases that are both transactivated by Ang II, a process mediated by H_2O_2-derived from NAD(P)H oxidases (167, 168). The resulting tyrosine phosphorylation generally leads

to activation of Src homology complex-growth factor receptor–bound protein 2–son of sevenless complex that activates *ras*, leading to downstream activation of ROK, MAPKs, and transcription factors. Some of the key redox-sensitive kinases in these signaling pathways are extracellular-regulated kinase, c-Jun N-terminal kinases (JNK), big MAPK (ERK5), and p38 MAPK (169–172). In the case of p38 MAPK, activation of the Akt/protein kinase B pathway results in cellular hypertrophy (6, 172). H_2O_2 has been most clearly implicated in the activation of p38 MAPK and JNK (169, 173). In cultured SMCs, Ang II activation of ERK1/2 was shown to be H_2O_2–independent (172–174); however, exogenous H_2O_2 does modulate ERK1/2 phosphorylation in VSMCs and other tissues (97, 175, 176).

Vascular Tone: Role of MAPKs

Since multiple studies have confirmed that MAPK can be activated by H_2O_2, it seems plausible to infer that among these kinases, targets of paracrine activation by H_2O_2, p38, and ERK1/2 are most likely to participate in contraction. Supporting this notion are reports showing that H_2O_2 does in fact induce ERK1/2 activity in smooth muscle cells *via* a variety of mechanisms (175, 177). ERK1/2 has been proposed as a plausible MAPK mediator of vascular contraction in hypertension, since its activation has been shown to be associated with enhanced contraction in cultured vascular cells from SHR as well as aortas from SHR and DOCA-salt hypertensive rats (178). Several studies have shown that Ang II vasoconstriction is mediated by activation of ERK1/2 (179, 180). Touyz *et al.* (178) showed that in isolated SHR mesenteric arteries, an ERK1/2 inhibitor markedly reduced Ang II-induced contraction and ameliorated impaired endothelium-dependent relaxation (178). However, other studies demonstrate a dissociation between Ang II–induced contraction and ERK1/2 pathway activation in the rat aorta (181). On the other hand, with regard to serotonin-induced constriction of VSMCs, ERK1/2 activation, but not p38 MAPK or JNK, appears to be involved (182, 183).

p38 MAPK activity, however, does contribute to the contractile response of mesenteric and canine pulmonary arteries to catecholamines (115, 184). Both ERK1/2 and p38 MAPK partially regulate the endothelin-1–induced vasoconstriction in Wistar-Kyoto rats (185). Ushio-Fukai *et al.* (172) showed that NAD(P)H oxidase–derived H_2O_2 plays an important role in Ang II–induced p38 MAPK activation. They also demonstrated that overexpression of catalase with an adenoviral construct attenuated Ang II–induced H_2O_2 and p38 MAPK activation (172). These data were corroborated by Meloche *et al.* (116), who showed that Ang II–induced activation of p38 MAPK is H_2O_2-dependent and mediates vascular constriction.

ROS-Mediated Role of PI3- and Rho-Kinase

Studies have shown that ROS-sensitive kinases are involved in regulating PI3-kinase; thus, PI3 kinase has been described as a critical link in ROS-mediated signaling (5). The involvement of PI3 kinase in increased MLCK activity, calcium sensitivity, and the contractile state of the artery makes it an important potential upstream mediator of H_2O_2-dependent impaired relaxation and enhanced constriction. Vascular PI3-kinase is composed of a regulatory p85α subunit as well as various isoforms of the p110 subunit (p110$_\alpha$, p110$_\beta$, and p110δ, but not p110γ), all of which are reportedly present in the vasculature and are potentially involved in pathways leading to vasoconstriction (186).

Another influential upstream ROS-sensitive inhibitor of MLCP is ROK. ROK is activated by Rho, a GTP-exchange protein, which is a member of the Ras family of signaling molecules known to be ROS-sensitive (105). ROK has been shown to mediate the maintenance (tonic) phase of contraction in response to various agonists, including phenylephrine, serotonin, and endothelin-1 (99, 187). A recent study by Jin *et al.* (105) showed that ROS increase membrane-bound Rho associated with ROK activation during contraction of the rat aorta, suggesting that ROK activation is associated with contraction. Importantly, ROK has been shown to inhibit MLCP. Jin *et al.* went on to show that a ROK inhibitor prevented ROS-elicited MLCP inhibition and hence contraction. Thus, these data suggest an important role for ROS-activated ROK in pathways leading to vascular contraction.

Vascular Hypertrophy: Potential ROS-Mediated Role of p38 and JNK MAPKs

The literature suggests less complex involvement of the MAPKs p38 and JNK as ROS-sensitive pathways leading to hypertrophy. As mentioned earlier, EGF-R and PDGF-R are both transactivated by Ang II, a process mediated by ROS derived from NAD(P)H oxidases (167, 168). Thus, on the cellular level, H_2O_2 would be expected to synergize with the effects of growth factor receptor ligands and activate pathways leading to growth. The resulting tyrosine phosphorylation of these receptors leads to activation of a series of pathways, culminating in downstream activation of the MAPKs involved in growth signaling (6, 188). The key redox-sensitive kinases playing a role in this cascade seem to be JNK and p38 MAPK (169–171), which appear to activate the Akt/protein kinase B pathway and cause cellular hypertrophy (6, 172).

Paracrine Effect of H_2O_2 on Medial Smooth Muscle Hypertrophy

Clearly, endogenous H_2O_2 contributes to growth-related signaling (174). Zafari *et al.* (189) reported that H_2O_2 metabolized from NAD(P)H oxidase–derived O_2^- mediated Ang II–induced hypertrophy of cultured smooth muscle cells. Multiple studies indicate that Ang II exerts a direct hypertrophic effect on vascular smooth muscle (190, 191). In cultured smooth muscle cell preparations, NAD(P)H oxidase–derived ROS have been implicated in

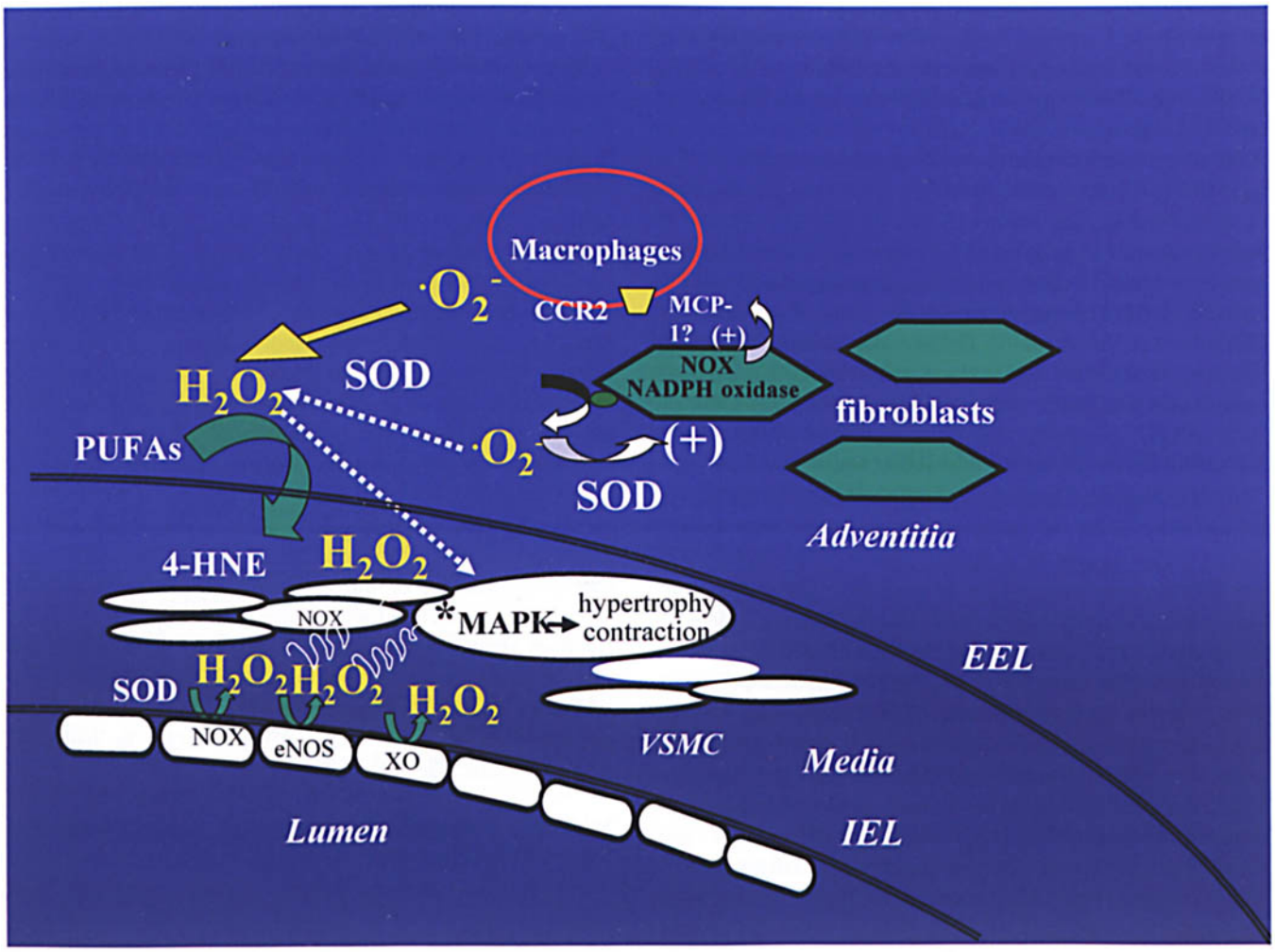

Figure 3. Potential vascular sites of hydrogen peroxide and proposed paracrine effect on medial contraction and hypertrophy. NADPH oxidase in fibroblasts produces O_2^- that is rapidly converted to H_2O_2 by SOD in the adventitia and may feed forward in the upregulation of greater oxidase expression. NADPH oxidase–derived ROS are chemotactic for macrophages *via* the possible release of monocyte chemoattractant peptide-1 (MCP-1) and its binding to the CCR2 receptor. Recruited cells exacerbate the production of ROS. Potential sources of ROS in the endothelium include NOXes, dysfunctional eNOS, xanthine oxidase, and mitochondria. These "initiator" peroxide producers either (i) directly activate VSMC kinases, leading to hypertrophy or contraction or (ii) activate local NOX to produce H_2O_2, which contributes to an enhanced effect.

hypertrophy (3, 16), activating signaling pathways and transcription factors involved in the growth response (6, 8, 74, 192, 193). Thus, ROS-dependent autocrine pathways are essential to smooth muscle growth in response to Ang II *in vitro*. A recent article by Zhang *et al.* (194) elegantly demonstrated that human catalase overexpression in VSMC decreases the hypertrophic effect of Ang II–induced hypertension, thereby indicating the important role of H_2O_2 *in vivo*. We and others have suggested a more complex *in vivo* scenario of paracrine, ROS-mediated influences on medial responsiveness and hypertrophy. A provocative study by Wang *et al.* (195) suggested a paracrine effect of ROS on medial hypertrophy. The authors showed that Ang II stimulates NAD(P)H oxidase–derived ROS in the aortic adventitia and intima, concomitant with medial hypertrophy, and that this stimulation was significantly reduced in gp91-phox–deficient mice with reduced intimal and adventitial NAD(P)H oxidase, suggesting a paracrine effect of adventitial and intimal NAD(P)H oxidase–derived ROS on medial hypertrophy (195). We postulated that adventitial NAD(P)H oxidase–derived ROS could influence medial hypertrophy. To test this hypothesis, we used an adenoviral construct targeting expression of an inhibitor of gp91-phox:p47-phox interaction (gp91ds) to the adventitia and confirmed expression of the inhibitor in adventitial fibroblasts and macrophages. Interestingly, this localized adventitial expression resulted in significant reduction in medial ROS detection and hypertrophy (196). Thus, these data support a paracrine effect of adventitial NAD(P)H oxidase–derived ROS on medial hypertrophy. It is important at this juncture to point out that in Ang II–induced hypertension, macrophages localize in the adventitia and

have been implicated in medial smooth muscle hypertrophy (197), yet the contribution of ROS or cytokines derived from these cells has not been delineated (198). It is tempting to speculate that even small amounts of ROS derived from adventitial fibroblasts may be chemotactic for macrophages (199, 200), which through their larger oxidase potential exacerbate ROS levels in the adventitia and enhance prohypertrophic mechanisms (Fig. 3).

We examined the effect of adventitia-targeted inhibitor expression on angioplasty-induced hyperplasia to study a potential paracrine effect of vascular ROS (201). In two consecutive studies, we compared the effect of localized adventitial expression of gp91ds and dominant negative p67-phox on neointima development in response to balloon angioplasty of the rat carotid artery (201) (unpublished observations). We found that gp91ds transfection to the adventitia inhibited neointimal hyperplasia to a greater degree than dominant negative p67-phox. These data are suggestive of a multicomponent adventitial NADPH oxidase having a paracrine effect on smooth muscle cell hyperplasia that in turn leads to neointimal growth. Since we also showed that these treatments had an inhibitory effect on fibroblast proliferation *in vitro*, we cannot rule out a direct role for fibroblasts in neointimal growth. However, taken together with the demonstrated importance of smooth muscle migration to neointimal growth, our findings suggest important paracrine influences of ROS, likely H_2O_2, compounding the effect of local medial production of ROS on vascular growth. In fact, we postulate that, as indicated in the activation of NADPH oxidase, vascular NADPH oxidases may be activated in series; that is, adventitial and endothelial NADPH oxidase–derived ROS may initiate activation of medial NADPH oxidase. Interestingly, this has been suggested by a number of authors who have shown that both the adventitia and endothelium are activated to produce greater amounts of ROS than the media under normal conditions and earlier in the disease process (14, 31, 202–205). Moreover, as mentioned above, there is burgeoning evidence of a temporal relationship among the various NADPH oxidase isoforms (206), such that one or more nox isoforms may be prominent early in the etiology of a particular disease and then taper off, whereas others predominate later. This relationship, the relative capacity of each of these isoforms to produce H_2O_2, and their location in the vascular wall all are likely to weigh heavily in the determination of vascular phenotype. This complex interplay among oxidase isoforms and the other metabolic pathways regulating H_2O_2 is expected to be the focus of intense future study.

In summary, NADPH oxidases and other ROS-generating enzymes are ubiquitous across the vascular wall. All of these sources appear to have the potential to contribute to the production of biologically active concentrations of H_2O_2 in muscle, which can promote vascular constriction and hypertrophy. Most certainly, under normal conditions, constitutive oxidase activities and endogenous scavenger systems, including catalase and glutathione peroxidases, maintain a homeostatic balance in favor of normal constrictor tone and wall thickness. Upon stimulation of the various oxidases by mechanical stretch and/or vasoactive hormones, increased production of peroxide and/or a deficiency in the capacity of the peroxidases lead to elevated prevailing levels of peroxide, which we postulate are free to traverse the radius of the blood vessel. While much emphasis has been placed on NADPH oxidase and H_2O_2 in cultured smooth muscle, and while these studies have afforded a detailed understanding of the downstream second messenger systems affected, the physiological role of vascular ROS is expected to be considerably more complex. Multiple oxidase systems in the endothelium, media, and adventitia are all expected to produce a significant share of biologically active ROS, including H_2O_2, that will eventually tip the balance in favor of a biological response. With growing evidence of a feed-forward relationship among the oxidases and an appreciation of H_2O_2's far-reaching vascular effects, careful study of the individual sources and their communication is expected to be an area of intense study.

1. Schubert J, Wilmer JW. Does hydrogen peroxide exist "free" in biological systems? Free Radic Biol Med 11:545–555, 1991.
2. Ruef J, Rao GN, Li F, Bode C, Patterson C, Bhatnagar A, Runge MS. Induction of rat aortic smooth muscle cell growth by the lipid peroxidation product 4-hydroxy-2-nonenal. Circulation 97:1071–1078, 1998.
3. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 74:1141–1148, 1994.
4. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, 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.
5. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, Griendling KK. Angiotensin II stimulation of NAD(P)H oxidase activity. Upstream mediators. Circ Res 91:406–413, 2002.
6. Griendling KK, Sorescu D, Lassègue B, 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.
7. Touyz RM, Schiffrin EL. Ang II-stimulated superoxide production is mediated via phospholipase D in human vascular smooth muscle cells. Hypertension 34:976–982, 1999.
8. Touyz RM, Yao G, Schiffrin EL. c-Src induces phosphorylation and translocation of P47phox. Role in superoxide generation by angiotensin II in human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 23:981–987, 2003.
9. Touyz RM, Chen X, Tabet F, Yao G, He G, Quinn MT, Pagano PJ, 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.
10. Touyz RM, Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. Pharmacol Rev 52:639–672, 2000.
11. Mohazzab-H KM, Kaminski PM, Wolin MS. NADH oxidoreductase

is a major source of superoxide anion in bovine coronary artery endothelium. Am J Physiol 266:H2568–H2572, 1994.

12. Mohazzab-H KM, Wolin MS. Sites of superoxide anion production detected by lucigenin in calf pulmonary artery smooth muscle. Am J Physiol 267:L815–L822, 1994.
13. Pagano PJ, Ito Y, Tornheim K, Gallop PM, Tauber AI, Cohen RA. An NADPH oxidase superoxide-generating system in the rabbit aorta. Am J Physiol 268:H2274–H2280, 1995.
14. Pagano PJ, Clark JK, Cifuentes-Pagano ME, Clark SM, Callis GM, Quinn MT. Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. Proc Natl Acad Sci U S A 94:14483–14488, 1997.
15. Sorescu D, Weiss D, Lassègue B, Clempus RE, Szöcs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, Griendling KK. Superoxide production and expression of nox family proteins in human atherosclerosis. Circulation 105:1429–1435, 2002.
16. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, Griendling KK. P22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. J Biol Chem 271: 23317–23321, 1996.
17. Lavigne MC, Malech HL, Holland SM, Leto TL. Genetic demonstration of P47phox-dependent superoxide anion production in murine vascular smooth muscle cells. Circulation 104:79–84, 2001.
18. Patterson C, Ruef J, Madamanchi NR, Barry-Lane P, Hu Z, Horaist C, Ballinger CA, Brasier AR, Bode C, 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.
19. Suh Y-A, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, Lambeth JD. Cell transformation by the superoxide-generating oxidase Mox1 (letter). Nature 401:79–82, 1999.
20. Lassègue B, Sorescu D, Szöcs K, Yin Q, Akers M, Zhang Y, Grant SL, Lambeth JD, 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.
21. Jones SA, O'Donnell VB, Wood JD, Broughton JP, Hughes EJ, Jones OTG. Expression of phagocyte NADPH oxidase components in human endothelial cells. Am J Physiol 271:H1626–H1634, 1996.
22. Meyer JW, Holland JA, Ziegler LM, Chang M-M, Beebe G, 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.
23. Smith KR, Klei LR, Barchowsky A. Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells. Am J Physiol Lung Cell Mol Physiol 280:L442–L449, 2001.
24. Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, Dikalov S, Giddens DP, Griendling KK, Harrison DG, Jo H. Oscillatory shear stress stimulates endothelial production of O_2^- from $P47^{phox}$-dependent NAD(P)H oxidases, leading to monocyte adhesion. J Biol Chem 278:47291–47298, 2003.
25. Ago T, Kitazono T, Ooboshi H, Iyama T, Han YH, Takada J, Wakisaka M, Ibayashi S, Utsumi H, Iida M. Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase. Circulation 109:227–233, 2004.
26. Thannickal VJ, Fanburg BL. Activation of an H_2O_2-generating NADH oxidase in human lung fibroblasts by transforming growth factor B1. J Biol Chem 270:30334–30338, 1995.
27. Krieger-Brauer HI, Kather H. Human fat cells possess a plasma membrane-bound H_2O_2-generating system that is activated by insulin via a mechanism bypassing the receptor kinase. J Clin Invest 89: 1006–1013, 1992.
28. Geiszt M, Witta J, Baffi J, Lekstrom K, Leto TL. Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. FASEB J 17:1502–1504, 2003.
29. Mahadev K, Motoshima H, Wu X, Ruddy JM, Arnold RS, Cheng G, Lambeth JD, Goldstein BJ. The NAD(P)H oxidase homolog nox4 modulates insulin-stimulated generation of H_2O_2 and plays an integral role in insulin signal transduction. Mol Cell Biol 24:1844–1854, 2004.
30. Fukui T, Lassegue B, Kai H, Alexander RW, Griendling KK. Cytochrome *B*-558 α-subunit cloning and expression in rat aortic smooth muscle cells. Biochim Biophys Acta 1231:215–219, 1995.
31. Wang HD, Hope S, Du Y, Quinn MT, Cayatte A, Pagano PJ, 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.
32. Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers Q IV, Taylor WR, Harrison DG, de Leon H, Wilcox JN, Griendling KK. P22phox MRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. Circ Res 80:45–51, 1997.
33. Jones SA, Wood JD, Coffey MJ, Jones OTG. The functional expression of P47-*Phox* and P67-*Phox* may contribute to the generation of superoxide by an NADPH oxidase-like system in human fibroblasts. FEBS Lett 355:178–182, 1994.
34. Pagano PJ, Chanock SJ, Siwik DA, Colucci WS, Clark JK. Angiotensin II induces $P67^{phox}$ MRNA expression and NADPH oxidase superoxide generation in rabbit aortic adventitial fibroblasts. Hypertension 32:331–337, 1998.
35. Cifuentes ME, Rey FE, Carretero OA, Pagano PJ. Upregulation of $P67^{phox}$ and $Gp91^{phox}$ in aortas from angiotensin II-infused mice. Am J Physiol Heart Circ Physiol 279:H2234–H2240, 2000.
36. Görlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, Busse R. A Gp91phox 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.
37. Bayraktutan U, Blayney L, 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.
38. Sorescu GP, Song H, Tressel SL, Hwang J, Dikalov S, Smith DA, Boyd NL, Platt MO, Lassègue B, Griendling KK, 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.
39. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 111:1201–1209, 2003.
40. Cosentino F, Barker JE, Brand MP, Heales SJ, Werner ER, Tippins JR, West N, Channon KM, Volpe M, Luscher TF. Reactive oxygen species mediate endothelium-dependent relaxations in tetrahydrobiopterin-deficient mice. Arterioscler Thromb Vasc Biol 21:496–502, 2001.
41. Archer SL, Huang J, Henry T, Peterson D, Weir EK. A redox-based O_2 sensor in rat pulmonary vasculature. Circ Res 73:1100–1112, 1993.
42. Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191:421–427, 1980.
43. Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnefsky EJ. Production of reactive oxygen species by mitochondria: central role of complex III. J Biol Chem 278:36027–36031, 2003.
44. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes H-P, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage (letter). Nature 404:787–790, 2000.

45. Yamagishi S, Edelstein D, Du X, Kaneda Y, Guzmán M, Brownlee M. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem 276: 25096–25100, 2001.
46. Zweier JL, Broderick R, Kuppusamy P, Thompson-Gorman S, Lutty GA. Determination of the mechanism of free radical generation in human aortic endothelial cells exposed to anoxia and reoxygenation. J Biol Chem 269:24156–24162, 1994.
47. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol 555:589–606, 2004.
48. Rieger JM, Shah AR, Gidday JM. Ischemia-reperfusion injury of retinal endothelium by cyclooxygenase- and xanthine oxidase-derived superoxide. Exp Eye Res 74:493–501, 2002.
49. McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H, Harrison DG. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. Am J Physiol Heart Circ Physiol 285:H2290–H2297, 2003.
50. Ohtsubo T, Rovira II, Starost MF, Liu C, Finkel T. Xanthine oxidoreductase is an endogenous regulator of cyclooxygenase-2. Circ Res 95:1118–1124, 2004.
51. Kukreja RC, Kontos HA, Hess ML, Ellis EF. PGH synthase and lipoxygenase generate superoxide in the presence of NADH or NADPH. Circ Res 59:612–619, 1986.
52. Fleming I, Michaelis UR, Bredenkotter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R. Endothelium-derived hyperpolarizing factor synthase (cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. Circ Res 88:44–51, 2001.
53. Marklund SL. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. Biochem J 222:649–655, 1984.
54. Jung O, Marklund SL, Geiger H, Pedrazzini T, Busse R, Brandes RP. Extracellular superoxide dismutase is a major determinant of nitric oxide bioavailability. In vivo and ex vivo evidence from EcSOD-deficient mice. Circ Res 93:622–629, 2003.
55. Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med 33:337–349, 2002.
56. Hilenski LL, Clempus RE, Quinn MT, Lambeth JD, Griendling KK. Distinct subcellular localizations of nox1 and nox4 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 24:677–683, 2004.
57. Stralin P, Karlsson K, Johansson BO, Marklund SL. The interstitium of the human arterial wall contains very large amounts of extracellular superoxide dismutase. Arterioscler Thromb Vasc Biol 15:2032–2036, 1995.
58. Rey FE, Li X-C, Carretero OA, Garvin JL, Pagano PJ. Perivascular superoxide anion contributes to impairment of endothelium-dependent relaxation. Role of $Gp91^{phox}$. Circulation 106:2497–2502, 2002.
59. Zhang H, Schmeisser A, Garlichs CD, Plotze K, Damme U, Mugge A, Daniel WG. Angiotensin II-induced superoxide anion generation in human vascular endothelial cells: role of membrane-bound NADH-/NADPH-oxidases. Cardiovasc Res 44:215–222, 1999.
60. Marklund SL. Regulation by cytokines of extracellular superoxide dismutase and other superoxide dismutase isoenzymes in fibroblasts. J Biol Chem 267:6696–6701, 1992.
61. Fukai T, Siegfried MR, Ushio-Fukai M, Griendling KK, Harrison DG. Modulation of extracellular superoxide dismutase expression by angiotensin II and hypertension. Circ Res 85:23–28, 1999.
62. Aruoma OI, Halliwell B. Molecular Biology of Free Radicals in Human Diseases. London: OICA International, 1998.
63. Suttorp N, Toepfer W, Roka L. Antioxidant defense mechanisms of endothelial cells: glutathione redox cycle versus catalase. Am J Physiol 251:C671–C680, 1986.
64. Ursini F, Maiorino M, Brigelius-Flohé R, Aumann KD, Roveri A, Schomburg D, Flohé L. Diversity of glutathione peroxidases. Methods Enzymol 252:38–53, 1995.
65. Harlan JM, Levine JD, Callahan KS, Schwartz BR. Glutathione redox cycle protects cultured endothelial cells against lysis by extracellularly generated hydrogen peroxide. J Clin Invest 73:706–713, 1984.
66. Demaree SR, Lawler JM, Linehan J, Delp MD. Ageing alters aortic antioxidant enzyme activities in Fischer-344 rats. Acta Physiol Scand 166:203–208, 1999.
67. Uddin M, Yang H, Shi M, Polley-Mandal M, Guo Z. Elevation of oxidative stress in the aorta of genetically hypertensive mice. Mech Ageing Dev 124:811–817, 2003.
68. Lacy F, Kailasam MT, O'Connor DT, Schmid-Schönbein GW, Parmer RJ. Plasma hydrogen peroxide production in human essential hypertension. Role of heredity, gender, and ethnicity. Hypertension 36:878–884, 2000.
69. Lacy F, O'Connor DT, Schmid-Schönbein GW. Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. J Hypertens 16:291–303, 1998.
70. Rhee SG, Chae HZ, Kim K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. Free Radic Biol Med 38:1543–1552, 2005.
71. Dubuisson M, Vander Stricht D, Clippe A, Etienne F, Nauser T, Kissner R, Koppenol WH, Rees JF, Knoops B. Human peroxiredoxin 5 is a peroxynitrite reductase. FEBS Lett 571:161–165, 2004.
72. Kang SW, Chae HZ, Seo MS, Kim K, Baines IC, Rhee SG. Mammalian peroxiredoxin isoforms can reduce hydrogen peroxide generated in response to growth factors and tumor necrosis factor-alpha. J Biol Chem 273:6297–6302, 1998.
73. Choi MH, Lee IK, Kim GW, Kim BU, Han YH, Yu DY, Park HS, Kim KY, Lee JS, Choi C, Bae YS, Lee BI, Rhee SG, Kang SW. Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. Nature 435:347–353, 2005.
74. Rao GN, Berk BC. Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression. Circ Res 70:593–599, 1992.
75. Burke-Wolin TM, Abate CJ, Wolin MS, Gurtner GH. Hydrogen peroxide-induced pulmonary vasodilation: role of guanosine 3', 5'-cyclic monophosphate. Am J Physiol 5:L393–L398, 1991.
76. Fujimoto S, Asano T, Sakai M, Sakurai K, Takagi D, Yoshimoto N, Itoh T. Mechanisms of hydrogen peroxide-induced relaxation in rabbit mesenteric small artery. Eur J Pharmacol 412:291–300, 2001.
77. Yang Z, Zhang A, Altura BT, Altura BM. Endothelium-dependent relaxation to hydrogen peroxide in canine basilar artery: a potential new cerebral dilator mechanism. Brain Res Bull 47:257–263, 1998.
78. Gil-Longo J, Gonzalez-Vazquez C. Characterization of four different effects elicited by H_2O_2 in rat aorta. Vascul Pharmacol 43:128–138, 2005.
79. Yang Z, Zheng T, Zhang A, Altura BT, Altura BM. Mechanisms of hydrogen peroxide-induced contraction of rat aorta. Eur J Pharmacol 344:169–181, 1998.
80. Rodriguez-Martinez MA, Garcia-Cohen EC, Baena AB, Gonzalez R, Salaices M, Marin J. Contractile responses elicited by hydrogen peroxide in aorta from normotensive and hypertensive rats. Endothelial modulation and mechanism involved. B J Pharmacol 125:1329–1335, 1998.
81. Sotnikova R. Investigation of the mechanisms underlying H_2O_2-evoked contraction in the isolated rat aorta. Gen Pharmacol 31:115–119, 1998.
82. Gao YJ, Lee RM. Hydrogen peroxide induces a greater contraction in mesenteric arteries of spontaneously hypertensive rats through thromboxane A(2) production. Br J Pharmacol 134:1639–1646, 2001.
83. Jin N, Rhoades RA. Activation of tyrosine kinases in H_2O_2-induced

contraction in pulmonary artery. Am J Physiol 272:H2686–H2692, 1997.

84. Pelaez NJ, Braun TR, Paul RJ, Meiss RA, Packer CS. H_2O_2 mediates Ca^{2+}- and MLC_{20} phosphorylation-independent contraction in intact and permeabilized vascular muscle. Am J Physiol Heart Circ Physiol 279:H1185–H1193, 2000.
85. Pelaez NJ, Osterhaus SL, Mak AS, Zhao Y, Davis HW, Packer CS. MAPK and PKC activity are not required for H_2O_2-induced arterial muscle contraction. Am J Physiol Heart Circ Physiol 279:H1194–H1200, 2000.
86. Yang ZW, Zheng T, Wang J, Zhang A, Altura BT, Altura BM. Hydrogen peroxide induces contraction and raises [Ca2+]i in canine cerebral arterial smooth muscle: participation of cellular signaling pathways. Naunyn Schmiedebergs Arch Pharmacol 360:646–653, 1999.
87. Horowitz A, Menice CB, Laporte R, Morgan KG. Mechanisms of smooth muscle contraction. Physiol Rev. 76:967–1003, 1996.
88. Tabet F, Savoia C, Schiffrin EL, Touyz RM. Differential calcium regulation by hydrogen peroxide and superoxide in vascular smooth muscle cells from spontaneously hypertensive rats. J Cardiovasc Pharmacol 44:200–208, 2004.
89. Favero TG, Zable AC, Abramson JJ. Hydrogen peroxide stimulates the Ca2+ release channel from skeletal muscle sarcoplasmic reticulum. J Biol Chem 270:25557–25563, 1995.
90. Grover AK, Samson SE, Fomin VP. Peroxide inactivates calcium pumps in pig coronary artery. Am J Physiol 263:H537–H543, 1992.
91. Grover AK, Samson SE. Effect of superoxide radical on Ca^{2+} pumps of coronary artery. Am J Physiol 255:C297–C303, 1988.
92. Sheehan DW, Giese EC, Gugino SF, Russell JA. Characterization and mechanisms of H_2O_2-induced contractions of pulmonary arteries. Am J Physiol 264:H1542–H1547, 1993.
93. Shen JZ, Zheng XF, Wei EQ, Kwan CY. Evidence against inhibition of sarcoplasmic reticulum Ca2+-pump as mechanism of H_2O_2-induced contraction of rat aorta. Acta Pharmacol Sin 22:498–504, 2001.
94. Suzuki YJ, Ford GD. Inhibition of Ca(2+)-ATPase of vascular smooth muscle sarcoplasmic reticulum by reactive oxygen intermediates. Am J Physiol 261:H568–H574, 1991.
95. Redondo PC, Salido GM, Rosado JA, Pariente JA. Effect of hydrogen peroxide on Ca2+ mobilisation in human platelets through sulphydryl oxidation dependent and independent mechanisms. Biochem Pharmacol 67:491–502, 2004.
96. Roychoudhury S, Ghosh SK, Chakraborti T, Chakraborti S. Role of hydroxyl radical in the oxidant H_2O_2-mediated Ca2+ release from pulmonary smooth muscle mitochondria. Mol Cell Biochem 159:95–103, 1996.
97. Oeckler-RA, Kaminski PM, 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.
98. Oeckler RA, Arcuino E, Ahmad M, Olson SC, Wolin MS. Cytosolic NADH redox and thiol oxidation regulate pulmonary arterial force through ERK MAP kinase. Am J Physiol Lung Cell Mol Physiol 288: L1017–L1025, 2005.
99. Thakali K, Demel SL, Fink GD, Watts SW. Endothelin-1-induced contraction in veins is independent of hydrogen peroxide. Am J Physiol Heart Circ Physiol 289:H1115–H1122, 2005.
100. Somlyo AP, Somlyo AV. Ca2+ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. Physiol Rev. 83:1325–1358, 2003.
101. Muranyi A, MacDonald JA, Deng JT, Wilson DP, Haystead TA, Walsh MP, Erdodi F, Kiss E, Wu Y, Hartshorne DJ. Phosphorylation of the myosin phosphatase target subunit by integrin-linked kinase. Biochem J 366:211–216, 2002.
102. Deng JT, Van Lierop JE, Sutherland C, Walsh MP. Ca2+-independent smooth muscle contraction. A novel function for integrin-linked kinase. J Biol Chem 276:16365–16373, 2001.
103. Li L, Eto M, Lee MR, Morita F, Yazawa M, Kitazawa T. Possible involvement of the novel CPI-17 protein in protein kinase C signal transduction of rabbit arterial smooth muscle. J Physiol 508:871–881, 1998.
104. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, Iwamatsu A, Kaibuchi K. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science 273:245–248, 1996.
105. Jin L, Ying Z, Webb RC. Activation of Rho/Rho kinase signaling pathway by reactive oxygen species in rat aorta. Am J Physiol Heart Circ Physiol 287:H1495–H1500, 2004.
106. Koyama M, Ito M, Feng J, Seko T, Shiraki K, Takase K, Hartshorne DJ, Nakano T. Phosphorylation of CPI-17, an inhibitory phosphoprotein of smooth muscle myosin phosphatase, by Rho-kinase. FEBS Lett 475:197–200, 2000.
107. D'Angelo G, Adam LP. Inhibition of ERK attenuates force development by lowering myosin light chain phosphorylation. Am J Physiol Heart Circ Physiol 282:H602–H610, 2002.
108. Klemke RL, Cai S, Giannini AL, Gallagher PJ, de Lanerolle P, Cheresh DA. Regulation of cell motility by mitogen-activated protein kinase. J Cell Biol 137:481–492, 1997.
109. Kim N, Cao W, Song IS, Kim CY, Harnett KM, Cheng L, Walsh MP, Biancani P. Distinct kinases are involved in contraction of cat esophageal and lower esophageal sphincter smooth muscles. Am J Physiol Cell Physiol 287:C384–C394, 2004.
110. Harnett KM, Biancani P. Calcium-dependent and calcium-independent contractions in smooth muscles. Am J Med 115(Suppl 3A):24S–30S, 2003.
111. Dessy C, Kim I, Sougnez CL, Laporte R, Morgan KG. A role for MAP kinase in differentiated smooth muscle contraction evoked by alpha-adrenoceptor stimulation. Am J Physiol 275:C1081–C1086, 1998.
112. Zhang Y, Moreland S, Moreland RS. Regulation of vascular smooth muscle contraction: myosin light chain phosphorylation dependent and independent pathways. Can J Physiol Pharmacol 72:1386–1391, 1994.
113. Lucchesi PA, Belmadani S, Matrougui K. Hydrogen peroxide acts as both vasodilator and vasoconstrictor in the control of perfused mouse mesenteric resistance arteries. J Hypertens 23:571–579, 2005.
114. Ibitayo AI, Sladick J, Tuteja S, Louis-Jacques O, Yamada H, Groblewski G, Welsh M, Bitar KN. HSP27 in signal transduction and association with contractile proteins in smooth muscle cells. Am J Physiol 277:G445–G454, 1999.
115. Yamboliev IA, Hedges JC, Mutnick JL, Adam LP, Gerthoffer WT. Evidence for modulation of smooth muscle force by the P38 MAP kinase/HSP27 pathway. Am J Physiol Heart Circ Physiol 278:H1899–H1907, 2000.
116. Meloche S, Landry J, Huot J, Houle F, Marceau F, Giasson E. P38 MAP kinase pathway regulates angiotensin II-induced contraction of rat vascular smooth muscle. Am J Physiol Heart Circ Physiol 279: H741–H751, 2000.
117. Thengchaisri N, Kuo L. Hydrogen peroxide induces endothelium-dependent and -independent coronary arteriolar dilation: role of cyclooxygenase and potassium channels. Am J Physiol Heart Circ Physiol 285:H2255–H2263, 2003.
118. Barlow RS, White RE. Hydrogen peroxide relaxes porcine coronary arteries by stimulating BK_{Ca} channel activity. Am J Physiol 275: H1283–H1289, 1998.
119. Krippeit-Drews P, Haberland C, Fingerle J, Drews G, Lang F. Effects of H_2O_2 on membrane potential and [Ca2+]i of cultured rat arterial smooth muscle cells. Biochem Biophys Res Commun 209:139–145, 1995.
120. Gao Y-J, Hirota S, Zhang D-W, Janssen LJ, Lee RMKW.

Mechanisms of hydrogen-peroxide-induced biphasic response in rat mesenteric artery. Br J Pharmacol 138:1085–1092, 2003.

121. Martens JR, Gelband CH. Ion channels in vascular smooth muscle: alterations in essential hypertension. Proc Soc Exp Biol Med 218:192–203, 1998.
122. Bratz IN, Falcon R, Partridge LD, Kanagy NL. Vascular smooth muscle cell membrane depolarization after NOS inhibition hypertension. Am J Physiol Heart Circ Physiol 282:H1648–H1655, 2002.
123. Martens JR, Gelband CH. Alterations in rat interlobar artery membrane potential and K+ channels in genetic and nongenetic hypertension. Circ Res 79:295–301, 1996.
124. Cox RH, Lozinskaya I, Matsuda K, Dietz NJ. Ramipril treatment alters Ca(2+) and K(+) channels in small mesenteric arteries from Wistar-Kyoto and spontaneously hypertensive rats. Am J Hypertens 15:879–890, 2002.
125. Amberg GC, Bonev AD, Rossow CF, Nelson MT, Santana LF. Modulation of the molecular composition of large conductance, Ca(2+) activated K(+) channels in vascular smooth muscle during hypertension. J Clin Invest 112:717–724, 2003.
126. Gao YJ, Zhang Y, Hirota S, Janssen LJ, Lee RM. Vascular relaxation response to hydrogen peroxide is impaired in hypertension. Br J Pharmacol 142:143–149, 2004.
127. Yang Z, Zhang A, Altura BT, Altura BM. Hydrogen peroxide-induced endothelium-dependent relaxation of rat aorta. Involvement of Ca^{2+} and other cellular metabolites. Gen Pharmacol 33:325–336, 1999.
128. Ellis A, Pannirselvam M, Anderson TJ, Triggle CR. Catalase has negligible inhibitory effects on endothelium-dependent relaxations in mouse isolated aorta and small mesenteric artery. Br J Pharmacol 140: 1193–1200, 2003.
129. Zembowicz A, Hatchett RJ, Jakubowski AM, Gryglewski RJ. Involvement of nitric oxide in the endothelium-dependent relaxation induced by hydrogen peroxide in the rabbit aorta. Br J Pharmacol 110: 151–158, 1993.
130. Matoba T, Shimokawa H, Kubota H, Morikawa K, Fujiki T, Kunihiro I, Mukai Y, Hirakawa Y, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. Biochem Biophys Res Commun 290:909–913, 2002.
131. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J Clin Invest 106:1521–1530, 2000.
132. Barlow RS, El Mowafy AM, White RE. H_2O_2 opens BK_{Ca} channels via the PLA_2-arachidonic acid signaling cascade in coronary artery smooth muscle. Am J Physiol Heart Circ Physiol 279:H475–H483, 2000.
133. Sato A, Sakuma I, Gutterman DD. Mechanism of dilation to reactive oxygen species in human coronary arterioles. Am J Physiol Heart Circ Physiol 285:H2345–H2354, 2003.
134. Iida Y, Katusic ZS. Mechanisms of cerebral arterial relaxations to hydrogen peroxide. Stroke 31:2224–2230, 2000.
135. Burke TM, Wolin MS. Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation. Am J Physiol 252:H721–H732, 1987.
136. Saito T, Ito K, Kurasaki M, Fujimoto S, Kaji H, Saito K. Determination of true specific activity of superoxide dismutase in human erythrocytes. Clin Sci 63:251–255, 1982.
137. Chaytor AT, Edwards DH, Bakker LM, Griffith TM. Distinct hyperpolarizing and relaxant roles for gap junctions and endothelium-derived H_2O_2 in NO-independent relaxations of rabbit arteries. Proc Natl Acad Sci U S A 100:15212–15217, 2003.
138. Hirai T, Tsuru H, Tanimitsu N, Takumida M, Watanabe H, Yajin K, Sasa M. Effect of hydrogen peroxide on guinea pig nasal mucosa vasculature. Jpn J Pharmacol 84:470–473, 2000.
139. Bharadwaj L, Prasad K. Mediation of H_2O_2-induced vascular relaxation by endothelium-derived relaxing factor. Mol Cell Biochem 149–150:267–270, 1995.
140. Fujimoto S, Mori M, Tsushima H. Mechanisms underlying the hydrogen peroxide-induced, endothelium-independent relaxation of the norepinephrine-contraction in guinea-pig aorta. Eur J Pharmacol 459:65–73, 2003.
141. Wei EP, Kontos HA, Beckman JS. Mechanisms of cerebral vasodilation by superoxide, hydrogen peroxide, and peroxynitrite. Am J Physiol 271:H1262–H1266, 1996.
142. Hayabuchi Y, Nakaya Y, Matsuoka S, Kuroda Y. Hydrogen peroxide-induced vascular relaxation in porcine coronary arteries is mediated by Ca^{2+}-activated K^+ channels. Heart Vessels 13:9–17, 1998.
143. Matoba T, Shimokawa H, Morikawa K, Kubota H, Kunihiro I, Urakami-Harasawa L, Mukai Y, Hirakawa Y, Akaike T, Takeshita A. Electron spin resonance detection of hydrogen peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary microvessels. Arterioscler Thromb Vasc Biol 23:1224–1230, 2003.
144. Yada T, Shimokawa H, Hiramatsu O, Kajita T, Shigeto F, Goto M, Ogasawara Y, Kajiya F. Hydrogen peroxide, an endogenous endothelium-derived hyperpolarizing factor, plays an important role in coronary autoregulation in vivo. Circulation 107:1040–1045, 2003.
145. Sofola OA, Knill A, Hainsworth R, Drinkhill M. Change in endothelial function in mesenteric arteries of Sprague-Dawley rats fed a high salt diet. J Physiol 543:255–260, 2002.
146. Sendao Oliveira AP, Bendhack LM. Relaxation induced by acetylcholine involves endothelium-derived hyperpolarizing factor in 2-kidney 1-clip hypertensive rat carotid arteries. Pharmacology 72:231–239, 2004.
147. Cosentino F, Katusic ZS. Tetrahydrobiopterin and dysfunction of endothelial nitric oxide synthase in coronary arteries. Circulation 91: 139–144, 1995.
148. Forgione MA, Cap A, Liao R, Moldovan NI, Eberhardt RT, Lim CC, Jones J, Goldschmidt-Clermont PJ, Loscalzo J. Heterozygous cellular glutathione peroxidase deficiency in the mouse. Abnormalities in vascular and cardiac function and structure. Circulation 106:1154–1158, 2002.
149. Weiss N, Zhang Y-Y, Heydrick S, Bierl C, Loscalzo J. Overexpression of cellular glutathione peroxidase rescues homocyst(e)ine-induced endothelial dysfunction. Proc Natl Acad Sci U S A 98:12503–12508, 2001.
150. Dayal S, Brown KL, Weydert CJ, Oberley LW, Arning E, Bottiglieri T, Faraci FM, Lentz SR. Deficiency of glutathione peroxidase-1 sensitizes hyperhomocysteinemic mice to endothelial dysfunction. Arterioscler Thromb Vasc Biol 22:1996–2002, 2002.
151. Suvorava T, Lauer N, Kumpf S, Jacob R, Meyer W, Kojda G. Endogenous vascular hydrogen peroxide regulates arteriolar tension in vivo. Circulation 112:2487–2495, 2005.
152. Wedgwood S, Black SM. Endothelin-1 decreases endothelial NOS expression and activity through ETA receptor-mediated generation of hydrogen peroxide. Am J Physiol Lung Cell Mol Physiol 288:L480–L487, 2005.
153. Guikema BJ, Ginnan R, Singer HA, Jourd'heuil D. Catalase potentiates interleukin-1beta-induced expression of nitric oxide synthase in rat vascular smooth muscle cells. Free Radic Biol Med 38:597–605, 2005.
154. Jaimes EA, Sweeney C, Raij L. Effects of the reactive oxygen species hydrogen peroxide and hypochlorite on endothelial nitric oxide production. Hypertension 38:877–883, 2001.
155. Cai H, Li Z, Dikalov S, Holland SM, Hwang J, Jo H, Dudley SC Jr, Harrison DG. NAD(P)H oxidase-derived hydrogen peroxide mediates endothelial nitric oxide production in response to angiotensin II. J Biol Chem 277:48311–48317, 2002.
156. Laude K, Cai H, Fink B, Hoch N, Weber DS, McCann L, Kojda G, Fukai T, Schmidt HHHW, Dikalov S, Ramasamy S, Gamez G, Griendling KK, Harrison DG. Hemodynamic and biochemical

adaptations to vascular smooth muscle overexpression of $P22^{phox}$ in mice. Am J Physiol Heart Circ Physiol 288:H7–H12, 2005.

157. Pagano PJ, Griswold MC, Najibi S, Marklund SL, Cohen RA. Resistance of endothelium-dependent relaxation to elevation of O_2^- levels in rabbit carotid artery. Am J Physiol 277:H2109–H2114, 1999.
158. Bouloumié A, Bauersachs J, Linz W, Schölkens BA, Wiemer G, Fleming I, Busse R. Endothelial dysfunction coincides with an enhanced nitric oxide synthase expression and superoxide anion production. Hypertension 30:934–941, 1997.
159. Darley-Usmar VM, Hogg N, O'Leary VJ, Wilson MT, Moncada S. The simultaneous generation of superoxide and nitric oxide can initiate lipid peroxidation in human low density lipoprotein. Free Radic Res Commun 17:9–20, 1992.
160. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 320:454–456, 1986.
161. Gutierrez JA, Clark SG, Giulumian AD, Fuchs LC. Superoxide anions contribute to impaired regulation of blood pressure by nitric oxide during the development of cardiomyopathy. J Pharmacol Exp Ther 282:1643–1649, 1997.
162. Guzik TJ, West NEJ, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM. Vascular superoxide production by NAD(P)H oxidase. Association with endothelial dysfunction and clinical risk factors. Circ Res 86:e85–e90, 2000.
163. Pritchard KA Jr, Ackerman AW, Gross ER, Stepp DW, Shi Y, Fontana JT, Baker JE, Sessa WC. Heat shock protein 90 mediates the balance of nitric oxide and superoxide anion from endothelial nitric-oxide synthase. J Biol Chem 276:17621–17624, 2001.
164. Li W-G, Miller FJ Jr, Zhang HJ, Spitz DR, Oberley LW, Weintraub NL. H_2O_2-induced O2 production by a non-phagocytic NAD(P)H oxidase causes oxidant injury. J Biol Chem 276:29251–29256, 2001.
165. Tampo Y, Kotamraju S, Chitambar CR, Kalivendi SV, Keszler A, Joseph J, Kalyanaraman B. Oxidative stress-induced iron signaling is responsible for peroxide-dependent oxidation of dichlorodihydrofluorescein in endothelial cells: role of transferrin receptor-dependent iron uptake in apoptosis. Circ Res 92:56–63, 2003.
166. Griendling KK, Ushio-Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. Regul Pept 91:21–27, 2000.
167. Ushio-Fukai M, Griendling KK, Becker PL, Hilenski H, Halleran S, Alexander RW. Epidermal growth factor receptor transactivation by angiotensin II requires reactive oxygen species in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 21:489–495, 2001.
168. Heeneman S, Haendeler J, Saito Y, Ishida M, Berk BC. Angiotensin II induces transactivation of two different populations of the platelet-derived growth factor β receptor. Key role for the P66 adaptor protein Shc. J Biol Chem 275:15926–15932, 2000.
169. Silver MA, Pick R, Brilla CG, Jalil JE, Janicki JS, Weber KT. Reactive and reparative fibrillar collagen remodelling in the hypertrophied left ventricle: two experimental models of myocardial fibrosis. Cardiovasc Res 24:741–747, 1990.
170. Yoshizumi M, Abe J, Haendeler J, Huang Q, Berk BC. Src and Cas mediate JNK activation but not ERK1/2 and P38 kinases by reactive oxygen species. J Biol Chem 275:11706–11712, 2000.
171. Abe J, Kusuhara M, Ulevitch RJ, Berk BC, Lee J-D. Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. J Biol Chem 271:16586–16590, 1996.
172. Ushio-Fukai M, Alexander RW, Akers M, Griendling KK. P38 mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. Role in vascular smooth muscle cell hypertrophy. J Biol Chem 273:15022–15029, 1998.
173. Viedt C, Soto U, Krieger-Brauer HI, Fei J, Elsing C, Kübler W, Kreuzer J. Differential activation of mitogen-activated protein kinases in smooth muscle cells by angiotensin II. Involvement of P22phox and reactive oxygen species. Arterioscler Thromb Vasc Biol 20:940–948, 2000.
174. Baas AS, Berk BC. Differential activation of mitogen-activated protein kinases by H_2O_2 and O_2^- in vascular smooth muscle cells. Circ Res 77:29–36, 1995.
175. Blanc A, Pandey NR, Srivastava AK. Distinct roles of Ca^{2+}, calmodulin, and protein kinase C in H_2O_2-induced activation of ERK1/2, P38 MAPK, and protein kinase B signaling in vascular smooth muscle cells. Antioxid Redox Signal 6:353–366, 2004.
176. Frank GD, Eguchi S, Yamakawa T, Tanaka S, Inagami T, Motley ED. Involvement of reactive oxygen species in the activation of tyrosine kinase and extracellular signal-regulated kinase by angiotensin II. Endocrinology 141:3120–3126, 2000.
177. Lee K, Esselman WJ. Inhibition of PTPs by H_2O_2 regulates the activation of distinct MAPK pathways. Free Radic Biol Med 33: 1121–1132, 2002.
178. Touyz RM, Deschepper C, Park JB, He G, Chen X, Neves MFT, Virdis A, Schiffrin EL. Inhibition of mitogen-activated protein/extracellular signal-regulated kinase improves endothelial function and attenuates Ang II-induced contractility of mesenteric resistance arteries from spontaneously hypertensive rats. J Hypertens 20:1127–1134, 2002.
179. Matrougui K, Eskildsen-Helmond YEG, Fiebeler A, Henrion D, Levy BI, Tedgui A, Mulvany MJ. Angiotensin II stimulates extracellular signal-regulated kinase activity in intact pressurized rat mesenteric resistance arteries. Hypertension 36:617–621, 2000.
180. Massett M., Ungvari Z, Csiszar A, Kaley G, Koller A. Different roles of PKC and MAP kinases in arteriolar constrictions to pressure and agonists. Am J Physiol Heart Circ Physiol 283:H2282–H2287, 2002.
181. Watts SW, Florian JA, Monroe KM. Dissociation of angiotensin II-stimulated activation of mitogen-activated protein kinase kinase from vascular contraction. J Pharmacol Exp Ther 286:1431–1438, 1998.
182. Banes AKL, Loberg RD, Brosius FC III, Watts SW. Inability of serotonin to activate the C-Jun N-terminal kinase and P38 kinase pathways in rat aortic vascular smooth muscle cells. BMC Pharmacol 1:8, 2001.
183. Watts SW. Serotonin activates the mitogen-activated protein kinase pathway in vascular smooth muscle: use of the mitogen-activated protein kinase kinase inhibitor PD098059. J Pharmacol Exp Ther 279: 1541–1550, 1996.
184. Ohanian J, Cunliffe P, Ceppi E, Alder A, Heerkens E, Ohanian V. Activation of P38 mitogen-activated protein kinases by endothelin and noradrenaline in small arteries, regulation by calcium influx and tyrosine kinases, and their role in contraction. Arterioscler Thromb Vasc Biol 21:1921–1927, 2001.
185. Kwon S, Fang LH, Kim B, Ha TS, Lee SJ, Ahn HY. P38 mitogen-activated protein kinase regulates vasoconstriction in spontaneously hypertensive rats. J Pharmacol Sci 95:267–272, 2004.
186. Northcott CA, Poy MN, Najjar SM, Watts SW. Phosphoinositide 3-kinase mediates enhanced spontaneous and agonist-induced contraction in aorta of deoxycorticosterone acetate-salt hypertensive rats. Circ Res 91:360–369, 2002.
187. Swärd K, Mita M, Wilson DP, Deng JT, Susnjar M, Walsh MP. The role of RhoA and Rho-associated kinase in vascular smooth muscle contraction. Curr Hypertens Rep 5:66–72, 2003.
188. Rey RE, Pagano PJ. The reactive adventitia. Fibroblast oxidase in vascular function. Arterioscler Thromb Vasc Biol 22:1962–1971, 2002.
189. Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, Taylor WR, Griendling KK. Role of NADH/NADPH oxidase-derived H_2O_2 in angiotensin II-induced vascular hypertrophy. Hypertension 32:488–495, 1998.
190. Geisterfer AAT, Peach MJ, Owens GK. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. Circ Res 62:749–756, 1998.

191. Griffin SA, Brown WCB, MacPherson F, McGrath JC, Wilson VG, Korsgaard N, Mulvany MJ, Lever AF. Angiotensin II causes vascular hypertrophy in part by a non-pressor mechanism. Hypertension 17: 626–635, 1991.
192. Wolin MS. Reactive oxygen species and vascular signal transduction mechanisms. Microcirculation 3:1–17, 1996.
193. Ushio-Fukai M, Alexander RW, Akers M, Yin Q, Fujio Y, Walsh K, 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.
194. Zhang Y, Griendling KK, Dikalova A, Owens GK, Taylor WR. Vascular hypertrophy in angiotensin II-induced hypertension is mediated by vascular smooth muscle cell-derived H_2O_2. Hypertension 46:732–737, 2005.
195. Wang HD, Xu S, Johns DG, Du Y, Quinn MT, Cayatte AJ, 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.
196. Liu J, Ormsby A, Oja-Tebbe N, 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.
197. Bush E, Maeda N, Kuziel WA, Dawson TC, Wilcox JN, DeLeon H, Taylor WR. CC chemokine receptor 2 is required for macrophage infiltration and vascular hypertrophy in angiotensin II-induced hypertension. Hypertension 36:360–363, 2000.
198. Janabi M, Yamashita S, Hirano K, Matsumoto K, Nozaki S, Matsuzawa Y. Reduced secretion of tumor necrosis factor-α and interleukin-1β from monocyte-derived macrophages of CD36-deficient subjects in response to oxidized LDL. In: Kita T, Yokode M, Eds. Lipoprotein Metabolism and Atherogenesis. Tokyo: Springer-Verlag, pp227–229, 2000.
199. Kiarash A, Carretero OA, Pagano PJ. A competitive inhibitor of NADPH oxidase reduces ICAM-1 expression and macrophage infiltration in the rat thoracic aorta (abstract). Free Radic Biol Med 29(Suppl 1):S48, 2000.
200. Liu J, Yang F, Yang X-P, Jankowski M, Pagano PJ. NAD(P)H oxidase mediates angiotensin II-induced vascular macrophage infiltration and medial hypertrophy. Arterioscler Thromb Vasc Biol 23:776–782, 2003.
201. Dourron HM, Jacobson GM, Park JL, Liu J, Reddy DJ, Scheel ML, Pagano PJ. Perivascular gene transfer of NADPH oxidase inhibitor suppresses angioplasty-induced neointimal proliferation of rat carotid artery. Am J Physiol Heart Circ Physiol 288:H946–H953, 2005.
202. Wang HD, Johns DG, Xu S, Cohen RA. Role of superoxide anion in regulating pressor and vascular hypertrophic response to angiotensin II. Am J Physiol Heart Circ Physiol 282:H1697–H1702, 2002.
203. Azumi H, Inoue N, Takeshita S, Rikitake Y, Kawashima S, Hayashi Y, Itoh H, Yokoyama M. Expression of NADH/NADPH oxidase $P22^{phox}$ in human coronary arteries. Circulation 100:1494–1498, 1999.
204. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 91:2546–2551, 1993.
205. Shi Y, Niculescu R, Wang D, Patel S, Davenpeck KL, 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.
206. Szöcs K, Lassègue B, Sorescu D, Hilenski LL, Valppu L, Couse TL, Wilcox JN, Quinn MT, Lambeth JD, Griendling KK. Upregulation of nox-based NAD(P)H oxidases in restenosis after carotid injury. Arterioscler Thromb Vasc Biol 22:21–27, 2002.