ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 7 & 8, 2006

Forum Review

Mechanosensitive Production of Reactive Oxygen Species in Endothelial and Smooth Muscle Cells: Role in Microvascular Remodeling?

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ABSTRACT

Changes in the hemodynamic environment (*e.g.*, hypertension, increased blood flow/shear stress) are known to lead to vascular remodeling; however, the underlying mechanisms by which hemodynamic forces control gene expression in vascular cells are not yet completely understood. This review considers how mechanosensitive generation of reactive oxygen species (ROS) by NAD(P)H oxidases and other sources interacts with downstream signaling systems [including activation of nuclear factor kappa B (NF-κB) and AP-1] that modulate the phenotype of endothelial and smooth muscle cells, leading to vascular remodeling. We propose a model for an interaction between direct mechanosensitive ROS signaling and pathways activated by pressure-induced upregulation of prooxidant paracrine signaling mechanisms [local renin–angiotensin system, TNF-α–converting enzyme (TACE)/tumor necrosis factor α (TNF-α) system, and endothelin signaling]. *Antioxid. Redox Signal.* 8, 1121–1129.

INTRODUCTION

CHANGES IN HEMODYNAMIC FORCES acting on endothelial and smooth muscle cells in hypertension lead to adaptive remodeling in resistance arteries. The cellular processes underlying vascular remodeling involve smooth muscle hypertrophy, hyperplasia, migration, and differentiation, as well as enhanced collagen decomposition and extracellular matrix (ECM) reorganization. Another pathophysiologic mechanism contributing to remodeling is inflammation, associated with an increased expression of redox-sensitive proinflammatory genes. Increasing evidence indicates that production of reactive oxygen species (ROS), including $O_2^{\cdot -}$ and H_2O_2, through activation of vascular NAD(P)H oxidase (Nox) plays a central role in vascular remodeling. Among the mechanisms involved in increased ROS production and arterial remodeling, circulating factors such as angiotensin II have been well characterized. The present review discusses mechanosensitive production of ROS in endothelial and smooth muscle cells, focusing on the role of ROS in activation of signaling pathways involved in vascular growth and inflammation in hypertension.

Microvascular Nox oxidases

Potential vascular sources of $O_2^{\cdot -}$ include Nox oxidases, nitric oxide synthase (NOS), xanthine oxidase, cytochrome P450, cyclooxygenase, and mitochondria. Recent studies suggest that in most vascular beds, including coronary arteries and arterioles (45, 46), Nox oxidases are the predominant source of $O_2^{\cdot -}$, whereas the activities of other oxidases in the vessel wall are below the range that appears to influence signaling systems under baseline physiologic conditions.

Endothelial and smooth muscle cells express different Nox that consist of multiple oxidase and regulatory subunits. In phagocytic cells, gp91^{phox} (Nox-2) oxidase subunit has been reported to be activated by stimulation of the assembly of p47^{phox}, p67^{phox}, and p40^{phox} subunits and activation of the small G protein rac, which bind to cell membrane–bound

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gp91*phox*-p22*phox* complex to promote high rates of NADPH-dependent $O_2^{\cdot -}$ generation. The vascular Nox oxidases differ from the neutrophil NAD(P)H oxidase in several important respects. The neutrophil oxidase releases large amounts of $O_2^{\cdot -}$ in bursts, whereas the vascular Nox oxidases continuously produce low levels of $O_2^{\cdot -}$. Many of the Nox oxidase subunits expressed in neutrophils, including p22*phox*, p47*phox*, p67*phox*, gp91*phox*, and Rac are present in vascular endothelial and smooth muscle cells. Vascular cells were reported to express both Nox-2 and the gp91phox homologues Nox-1 or Nox-4 oxidase subunits or both (18, 20, 67). The Nox system present in vascular smooth muscle shows a basal oxidase activity supported by both NADH and NADPH (18, 20, 46). Although both Nox-1 and Nox-4 generate ROS in cultured vascular smooth muscle cells, their activation and response to growth factors may differ. Activation of the Nox-1, similar to gp91*phox*, may involve binding of p47*phox* and p67*phox* to the membrane oxidase complex (52), a process stimulated by protein kinase C (PKC) phosphorylation of p47*phox* (4, 23, 61, 68, 70). In contrast, Nox-4 does not seem to have sites that could be regulated by these mechanisms involving p47*phox*-related subunit binding. Recently hypotheses were put forward that different compartmentalization of Nox-1 and Nox-4 may also underlie their opposing functions (26). With immunofluorescent labeling, Nox-1 was shown to colocalize with caveolin (26). It can be hypothesized that the presence of Nox-1 in caveolae (in close proximity to protein kinase C and Src-family kinases) could be involved in growth-promoting actions by receptor stimulation (4). In contrast, Nox-4 was reported to colocalize with vinculin in focal adhesions (26), suggesting a role in integrin-linked mechanotransduction of cellular stretch. Nox-4 also appears to be present in submembrane vesicular structures (21). Although the Nox-4 protein seems to contain an endoplasmic reticulum retention signal (the di-lysine KKXX motif near the C-terminus), the significance of these observations has not been established.

Activation of Nox oxidases in endothelial and smooth muscle cells by hemodynamic forces

Several lines of evidence suggest that hemodynamic forces, either directly or indirectly, can activate vascular Nox oxidase–derived ROS production. Ample evidence indicates that the hypertension is associated with an increased activity of vascular Nox oxidases in both conduit arteries and arteriolar microvessels (reviewed in references 4 and 34), including vessels from the coronary circulation (Fig. 1). The vascular effects of hypertension are complex and are likely to be induced, at least in part, by increased levels of neurohumoral factors. Among them, angiotensin II has been suggested to increase $O_2^{\cdot -}$ generation in vascular cells (13, 47, 54). However, oxidative stress seems to be present in virtually all forms of hypertension (2, 54) [including low-renin hypertension (37, 55), genetic hypertension, angiotensin II–induced hypertension (47, 54), renovascular hypertension (23, 25), and pheochromocytoma-related hypertension (24)] despite the differences in plasma levels of circulating factors (for a detailed discussion of the topic, see references 34, 68, and 70).

Vascular cells are equipped with various sensors that enable them to detect and respond to alterations of hemodynamic forces. The structural components of vascular endothelial and smooth muscle cells (focal adhesion sites, integrins, cellular junctions, and the extracellular matrix) have an established role in mechanotransduction, transmitting tension within the cells. Hemodynamic forces can initiate complex signal-transduction cascades via these structural sensors (for an excellent review, see references 34 and 40), which may involve ROS as mediators. The hypothesis that high intraluminal pressure itself promotes vascular Nox oxidase–dependent $O_2^{\cdot -}$ generation is supported by the observations that in aortic banded rats (in which blood vessels proximal to the coarctation are exposed to high pressure, whereas in distal vascular beds, pressure does not exceed normotensive levels) in the presence of the same circulating factors, regional increases in blood pressure result in selective increases in vascular $O_2^{\cdot -}$ production (70). In some models of hypertension, high pressure seems to be associated with an upregulation of Nox oxidase(s). However, increased expression of the oxidases is likely not a prerequisite for hypertension-related oxidative stress. For example, in the hypertensive vessels of aortic banded rats, expression of Nox oxidase subunits nox-1, p22*phox*, p47*phox*, and p67*phox* is normal (65), yet Nox oxidase activity is significantly increased. Recent microarray gene-expression profiling of small coronary arteries of hypertensive rats also showed unaltered (or even slightly decreased) expression of Nox-1, Nox-4, and gp91*phox*, in the presence of an increased Nox oxidase activity (Fig. 1C).

Several mechanisms intrinsic to the vascular wall indirectly can upregulate Nox oxidase function, including the local renin–angiotensin system, the endothelin system, and the TNFα-converting enzyme (TACE)–TNF-α–TNF receptor (TNFR1) axis (1, 16, 17, 67). It seems that prolonged presence of high pressure can simultaneously activate these paracrine regulatory mechanisms. For example, in the coronary circulation, high pressure tends to upregulate ACE, angiotensinogen and TNF-α (Fig. 1C). ACE activity also is increased in hypertensive forelimb arteries, but not in normotensive hindlimb arteries, of aortic banded rats (70). Recent studies also suggest that an enhanced endothelin synthesis may contribute to vascular $O_2^{\cdot -}$ production in some models of hypertension (41). It is likely that simultaneous (even mild) increases in these prooxidant paracrine factors will have an additive effect, which can significantly contribute to the increased Nox oxidase activity in hypertensive vessels.

In addition to the effects of local angiotensin II, endothelin, and TNF-α, high pressure is likely to stimulate vascular ROS generation directly. Early studies revealed that short-term increases in pressure both *in vivo* impair endothelial function (10, 15, 74). In a landmark study, Dr. Akos Koller's laboratory demonstrated that *in vitro* exposure of isolated arterioles to high pressure, in the absence of circulating factors, results in impaired flow-induced dilations that could be prevented by antioxidant treatment (30). Direct evidence for high pressure–induced, Nox oxidase–dependent oxidative stress came from our recent studies showing that increases in wall tension due to the exposure of isolated arteries to high pressure (160 mm Hg) in a vessel culture system elicited significant $O_2^{\cdot -}$ production (69). Importantly, in instrumented conscious dogs, a temporary increase in coronary perfusion

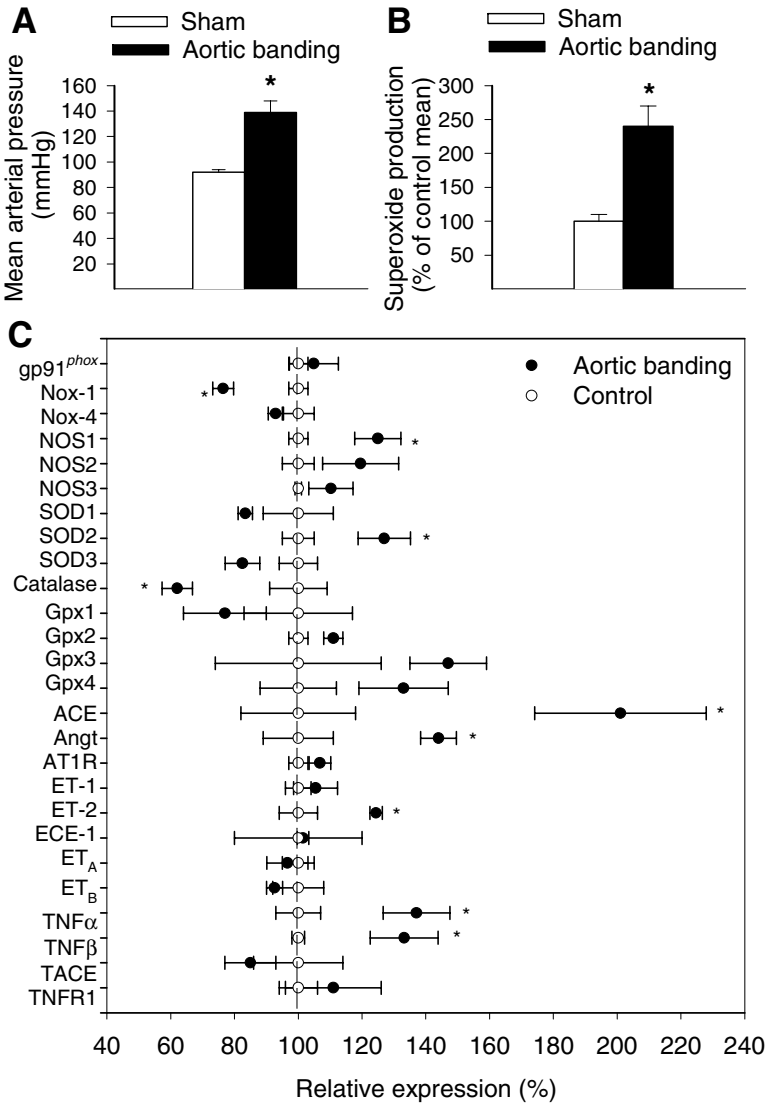

FIG. 1. **A**: **Mean arterial pressure in rats with aortic banding–induced hypertension (70). B:** Increased superoxide production in coronary arteries of aortic banded rats (*$p < 0.05$). **C:** Gene-expression profiling in coronary arteries of normotensive ($n = 5$) and aortic-banded hypertensive ($n = 3$) Wistar rats was performed by using Affymetrix U230A micoarrays. Expression of NAD(P)H oxidase subunits, NO synthase (NOS) isoforms, antioxidant enzymes [including catalase, superoxide dismutase (SOD) isoforms and glutathione peroxidases (Gpx)] and components of vascular paracrine signaling systems [including the local renin–angiotensin system, TNF-α–converting enzyme (TACE)–TNF axis and endothelin (ET) system] is shown (values are expressed as percentage of control mean ± SEM; *$p < 0.05$.) (ACE, angiotensin-converting enzyme; Angt, angiotensinogen; AT1R, angiotensin II receptor-1; ECE, endothelin-converting enzyme; TNFR, TNF-α receptor-1).

pressure resulted in a significant microvascular endothelial dysfunction that could be prevented by inhibitors of Nox-oxidase function, suggesting that similar pressure-sensitive mechanisms are operational in both large arteries and arteriolar microvessel (33). It is likely that wall tension–dependent cellular stretch is the primary mechanical stimulus for Nox-oxidase activation, because exposure of isolated arterial rings to *in vitro* stretching also activates vascular Nox oxidase–dependent $O_2^{\cdot -}$ generation (52), mimicking the effects of high pressure. Increased production of ROS also has been detected in cultured endothelial and smooth muscle cells subjected to *in vitro* stretching (19, 27, 28). Vascular cells abundantly express SOD isoforms, which catalyze the removal of $O_2^{\cdot -}$ with a rate constant of 2×10^9 mol/L/s. Thus, a significant portion of pressure-induced Nox oxidase–derived $O_2^{\cdot -}$ is likely dismutated to H_2O_2. Increased H_2O_2 levels have been demonstrated both in high pressure–exposed arteries (8) and arteriolar microvessel (51). The underlying mechanisms by which high pressure/wall tension–related cell stretch elicits Nox-oxidase activation likely involves increases in $[Ca^{2+}]_i$ and phosphorylation and activation of PKC-α (69–71). PKC-dependent serine phosphorylation of the regulatory p47*phox* subunit (68) results in its translocation from the cytosol to the membrane oxidase subunits (52), which activates NAD(P)H oxidase function. Prolonged presence of high pressure also may affect the expression of certain PKC isoforms in some vascular beds (53), including coronary arteries (Fig. 2). Importantly, the intracellular signaling pathways activated by angiotensin II, TNF-α, and endothelins (most notably the activation of PKC) overlap with that activated by pressure/cell stretch. Thus, we propose that even mild increases in these paracrine signaling pathways sensitize the vascular cells toward the effects of high pressure/cell stretch (Fig. 3.).

In addition to the effects of pressure, ROS signaling also is likely to participate in the mechanotransduction of other modalities of blood flow, such as pulsatility and shear stress. Indeed, evidence suggests that pulsatile stretch increases $O_2^{\cdot -}$ production in human coronary artery smooth muscle cells (27). In pulsatile flow-exposed porcine coronary arterioles, administration of SOD improved bioavailability of NO (56).

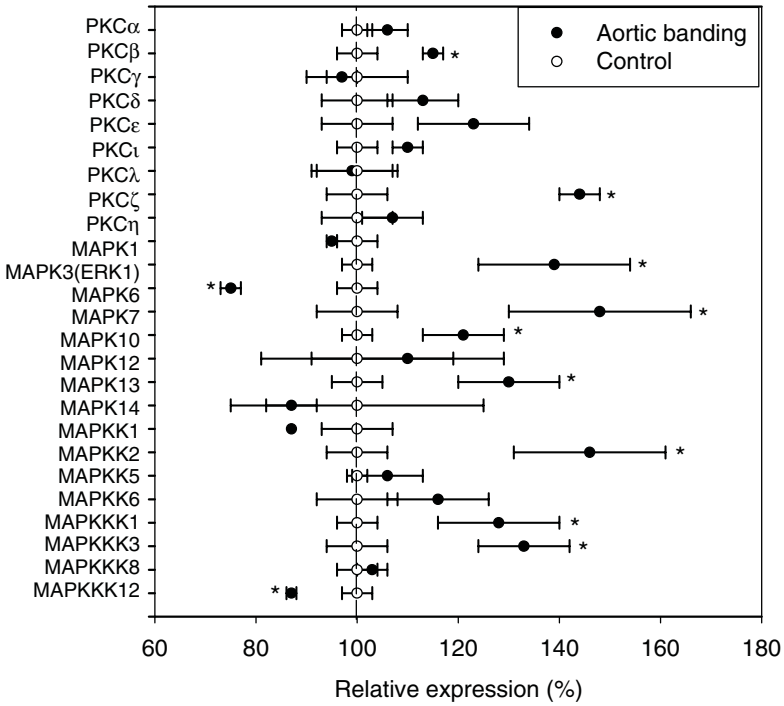

FIG. 2. Expression of protein kinase (PKC) isoforms and components of MAP kinase (MAPK) pathways in coronary arteries of normotensive (n = 5) and aortic-banded hypertensive (n = 3) Wistar rats (Affymetrix U230A; values are expressed as percentage of control mean ± SEM; *p < 0.05). (MAPKK, MAP kinase kinase; MAPKKK, MAP kinase kinase kinase).

Further studies are needed to characterize the role for pulsatility-related ROS production in vascular remodeling.

It is well documented that shear stress stimulates the release of the free radical NO from the endothelium (9, 12, 14, 35, 36, 49, 57, 59, 64, 66), which exerts antiinflammatory, antiproliferative effects. However, some studies show that changes in shear stress may also increase endothelial ROS production (29, 38, 58), likely via stimulation of Nox oxidases (31). It is generally accepted that oscillatory shear stress, at least in cultured endothelial cells, is a particularly potent stimulus of $O_2^{\cdot-}$ production (11, 22, 31, 43). Multiple mechanisms may contribute to the more prominent prooxidant effect of oscillatory shear stress (43), including differential regulation of the expression/activity of SOD (5, 11, 50, 60) and Nox oxidase by laminar and oscillatory shear stresses. Interestingly, recent studies raised the possibility that in human coronary arterioles (isolated from hearts of cardiac patients), laminar shear stress elicits the release of

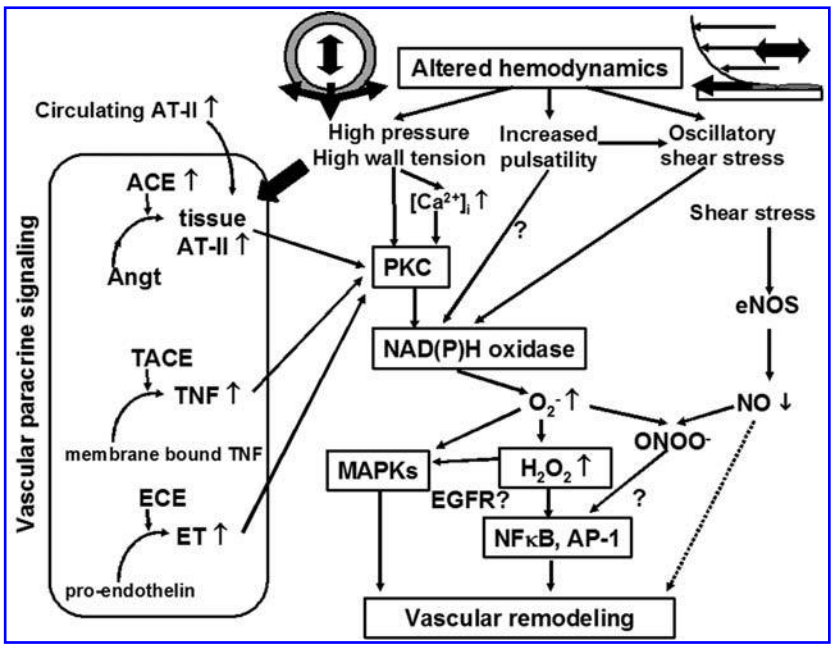

FIG. 3. Proposed scheme for mechanosensitive activation of NAD(P)H oxidase–dependent ROS production in endothelial and smooth muscle cells by altered hemodynamic forces that activate signaling pathways, leading to microvascular remodeling. We propose that indirect upregulation of vascular paracrine signaling systems (including the local renin–angiotensin system, TACE–TNF axis, and endothelin system) by high pressure together with an increased concentration of prooxidant circulating factors (angiotensin II) sensitize the vascular cells toward the direct prooxidant effects of hemodynamic forces.

substantial amounts of H_2O_2 that act as an endothelium-derived relaxing factor (44). However, mitochondria appear to be a source of H_2O_2 that is released from the human coronary arteries when exposed to increased flow (42). Whether Nox oxidase activation or ROS production or both exert vasoactive effects in healthy blood vessels *in vivo* is still under scrutiny. Although it is generally accepted that short-term administration of H_2O_2 can elicit vasodilation (76), it is likely that prolonged presence of subthreshold concentrations H_2O_2 is sufficient to activate signaling pathways (*e.g.*, NF-κB, MAP kinases) involved in cell proliferation and structural remodeling.

Signaling pathways activated by Nox oxidases: role in vascular remodeling

Previous studies have established that Nox-derived $O_2^{\cdot-}$ or H_2O_2 or both function as signaling molecules eliciting various biologic responses (reviewed in references 75 and 77). Accumulating evidence from experimental and clinical studies indicates that NAD(P)H oxidase activation plays a central role in vascular remodeling in hypertension and other pathophysiologic conditions (73), likely by regulating cell proliferation and inflammatory gene expression. The role for H_2O_2 generated by Nox oxidase in angiotensin II–induced smooth muscle hypertrophy is particularly well documented (16, 17, 72, 80). For example, angiotensin-induced cellular hypertrophy can be inhibited by the flavoprotein inhibitor DPI (16), knockdown of $p22^{phox}$ (72), and by catalase overexpression (79). In transgenic mice that overexpress $p22^{phox}$ in the vascular smooth muscle cells, an increased H_2O_2 is associated with vascular hypertrophy (73). It seems that enhanced NAD(P)H oxidase–dependent oxidative stress in human smooth muscle cells also is associated with Ang II–induced vascular remodeling in essential hypertension (61).

ROS are likely to activate multiple signaling pathways including p42/44 and p38 MAP kinases (16, 17, 39, 52, 63), tyrosine kinases, and the transcription factor AP-1 (reviewed in references 3 and 62). In chronic hypertension, increased vascular oxidative stress is associated with increased activity of MAP kinases (32), which are important in cell growth and differentiation (42). Evidence indicates that cellular stretch–induced MAP kinase activation in bovine coronary arteries is mediated by Nox-derived ROS (52). Pulsatility-induced activation of p42/44 MAP kinase also appears to be mediated by ROS in cultured rabbit aorta (39). Some data link stretch- and H_2O_2-induced p42/44 signaling to EGF-receptor phosphorylation (52). In addition, multiple components of MAP kinase–dependent signaling pathways (*e.g.*, ERK1) appear to be transcriptionally regulated in hypertensive coronary arteries (Fig. 2), which is likely to sensitize cellular signaling systems to the effects of ROS.

Recent evidence suggests that high pressure–induced Nox-derived H_2O_2 activates NF-κB (7), which contributes to a proinflammatory shift in vascular phenotype. Accordingly, pulsatile stretch *in vitro* also can activate NF-κB in smooth muscle cells (6). The activity of NF-κB is tightly regulated by interaction with inhibitory IκB proteins. Signals that lead to activation of NF-κB converge on a high-molecular-weight complex that contains a serine-specific IκB kinase (IKK). The IKK is an unusual kinase in that it contains two related kinases, IKKα and IKKβ, which are active as a dimer. Activation of IKK leads to the phosphorylation of two specific serines near the N terminus of IκBα, which targets IκBα for ubiquitination and degradation by the proteasome, allowing the unmasked NF-κB enter the nucleus to activate target gene expression. Previous studies have reported ROS-mediated targeted degradation of IκB in various cell types. These findings have important clinical relevance, as NF-κB has been linked to vascular remodeling and microvascular damage in hypertension (48). AP-1 binding sites are present in the promoter region of many known mechanical stress-response genes and in cultured cells, stretching was shown to activate AP-1, likely via stimulating oxidative stress (78). The mechanisms by which ROS activate AP-1 likely involve upregulation of c-*fos* and c-*jun*, which form protein homo- or heterodimers, comprising AP-1, or by phosphorylation of c-*jun* by JNK or both. ROS may also modulate vascular remodeling by altering deposition of extracellular matrix proteins. Collagen degradation depends on the activity of enzymes known as matrix metalloproteinases (MMPs) secreted by smooth muscle cells in an inactive form. Numerous studies have linked vascular oxidative stress to MMP activation, suggesting that this pathway also may be involved in NAD(P)H oxidase–dependent vascular remodeling.

Perspectives

Taken together, we propose that mechanosensitive activation of Nox-dependent ROS production in endothelial and smooth muscle cells by altered hemodynamic forces (interacting pressure/wall tension and shear stress) activates signaling pathways, which leads to microvascular remodeling. As shown in Fig. 3, even short-term presence of high pressure/increased wall tension elicits increases in $[Ca^{2+}]_I$ and PKC activation, stimulating Nox-dependent $O_2^{\cdot-}$ and H_2O_2 production in both endothelial and smooth muscle cells. In the endothelial cells, laminar shear stress stimulates eNOS-dependent production of NO, which exerts antiproliferative, antiinflammatory effects on the smooth muscle cells. In contrast, oscillatory/pulsatile shear stress increases Nox-derived ROS generation, which results in the formation of $ONOO^-$, decreasing the bioavailability of NO. Prolonged presence of disturbed hemodynamic conditions may lead to upregulation of paracrine signaling systems (including the local renin–angiotensin system, TACE–TNF axis, and endothelin system) that are known to regulate cell growth. Increased local angiotensin II, endothelin, or TNFs levels (or a combination of these) in the vascular wall together with an increased concentration of prooxidant circulating factors (most important, angiotensin II) sensitize the vascular cells toward the direct effects of hemodynamic forces. The reactive oxygen and nitrogen species generated in response to these direct and indirect effects of changes in hemodynamic forces initiate a diversity of signaling processes that control vascular smooth muscle proliferation, inflammatory phenotypic changes, and extracellular matrix homeostasis that underlies microvascular remodeling in hypertension and other pathophysiological states.

ACKNOWLEDGMENT

This work was supported by grants from the American Heart Association 0430108N and 0435140N, American Federation for Aging Research Philip Morris USA Inc. and Philip Morris International, and NIH HL077 256, HL31069, HL-43023 and HL-66331.

ABBREVIATIONS

ACE, angiotensin-converting enzyme; Angt, angiotensinogen; AT1R, angiotensin receptor type 1; ECE, endothelin-converting enzyme; $ET_{A,}$ endothelin receptor type A; $ET_{B,}$ endothelin receptor type B; Gpx, glutathione peroxidase; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; NOS, nitric oxide synthase; Nox, NAD(P)H oxidase; PKC, protein kinase C; ROS, reactive oxygen species; SOD, superoxide dismutase; TACE, TNF-α–converting enzyme; TNFR1, TNF receptor type 1; TNF-α, tumor necrosis factor-α.

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Date of first submission to ARS Central, December 14, 2005; date of acceptance, January 7, 2006.

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