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Oxidative Stress in Arterial Hypertension
Role of NAD(P)H Oxidase

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Abstract—Increased vascular reactive oxygen species production, especially superoxide anion, contributes significantly in the functional and structural alterations present in hypertension. An enhanced superoxide production causes a diminished NO bioavailability by an oxidative reaction that inactivates NO. Exaggerated superoxide levels and a low NO bioavailability lead to endothelial dysfunction and hypertrophy of vascular cells. It has been shown that the enzyme NAD(P)H oxidase plays a major role as the most important source of superoxide anion in vascular cells. Several experimental observations have shown an enhanced superoxide generation as a result of the activation of vascular NAD(P)H oxidase in hypertension. Although this enzyme responds to stimuli such as vasoactive factors, growth factors, and cytokines, some recent data suggest the existence of a genetic background modulating the expression of its different components. New polymorphisms have been identified in the promoter of the $p22^{phox}$ gene, an essential subunit of NAD(P)H oxidase, influencing the activity of this enzyme. Genetic investigations of these polymorphisms will provide novel markers for determination of genetic susceptibility to oxidative stress in hypertension. **(*Hypertension*. 2001;38: 1395-1399.)**

Key Words: angiotensin II ■ genetics ■ hypertension, arterial ■ stress ■ free radicals

Large amounts of reactive oxygen species (ROS), resulting from oxygen, are produced in vascular cells, including superoxide anion ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2), and act as important intracellular signals. Oxidative stress describes the injury caused to cells by the oxidizing of macromolecules resulting from increased formation of ROS and/or decreased antioxidant reserve. Recent works have reported that all types of vascular cells generate ROS. A growing number of reports have provided a critical role for oxidative stress in the pathogenesis of cardiovascular diseases, including hypertension.[1]

An enhanced production of ROS contributes to the dysregulation of physiological processes, which leads to structural and functional alterations in hypertension.[2] Two characteristic alterations of the vascular wall in hypertension are endothelial dysfunction and vascular smooth muscle cell (VSMC) hypertrophy. An enhanced production of ROS causes a loss of NO bioavailability, which impairs endothelial function, causing (among others) a decreased endothelium-dependent vasodilation.[3] Among these ROS, $\cdot O_2^-$ is critically involved in the breakdown of NO.[4] Thus, a diminished availability of NO can be the result of a decreased activity from the NO-production pathway or the result of an increase in the oxidative inactivation of NO by $\cdot O_2^-$. Recently, we have shown that endothelial dysfunction is associated with an excess of $\cdot O_2^-$ generation rather than a diminished NO production in the aorta of adult spontaneously hypertensive rats (SHR).[5] The presence of unpaired electrons causes $\cdot O_2^-$ to be chemically unstable and highly reactive. The reaction of $\cdot O_2^-$ with NO leads to the production of peroxynitrite,[6] a potent oxidant believed to be responsible for tissue injury. Peroxynitrite induces the oxidation of proteins, DNA, and lipids in vascular cells.[7] On the other hand, recent findings suggest that increased ROS may stimulate VSMC hypertrophy and hyperplasia.[8] Li et al[9] has shown that $\cdot O_2^-$ induces the proliferation of VSMCs, and Zafari et al[10] has proposed a role for $\cdot O_2^-$ and H_2O_2 in angiotensin II–induced VSMC hypertrophy. ROS are also involved in several signal pathways and in the activation of redox-sensitive transcriptional factors, such as nuclear factor (NF)-kB.[11] It has been shown recently that angiotensin II activates NF-κB in VSMCs.[12] Furthermore, NF-κB has been implicated in the transcription of a number of vascular genes.[13] Finally, NF-κB seems to play a pivotal role in angiotensin II–stimulated ROS generation and inflammatory mechanisms (see review[14]).

Vascular NAD(P)H Oxidase

Enzymatic sources of ROS in the vascular wall playing a functional role in hypertension are NAD(P)H oxidase, NO synthase, xanthine oxidase, and cyclooxygenase. Vascular

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Expression of NAD(P)H Oxidase Components in Vascular Cells

	Endothelial Cells	Fibroblasts	VSMCs
$p22^{phox}$	+	+	+
$gp91^{phox}$	+	+	−
$p67^{phox}$	+	+	−
$p47^{phox}$	+	+	+
rac	+	+	+

NAD(P)H oxidase, which is to some extent similar to the previously reported neutrophil NADPH oxidase, is the most important source of $\cdot O_2^-$ in vascular cells.[15–18] The structure and function of this vascular oxidase has been recently reviewed.[8] At present, response to extracellular NAD(P)H is one of the major unanswered questions concerning membrane orientation and function of this oxidase.[19] Vascular NAD(P)H oxidase consists of a cytochrome *b*558, composed of $p22^{phox}$ and $gp91^{phox}$ subunits and 3 cytosolic components, $p47^{phox}$, $p67^{phox}$, and rac. The Table summarizes the expression of these components in vascular cells. Transfection of antisense $p22^{phox}$ demonstrated this subunit of the cytochrome to be essential for functionality of NAD(P)H oxidase.[20] Disruption of $gp91^{phox}$ and $p47^{phox}$ subunits lowers vascular $\cdot O_2^-$ production, without significant alterations in basal blood pressure.[21,22] Thus, the existence of compensatory mechanisms regulating blood pressure in this knockout mouse cannot be discarded. Although the $gp91^{phox}$ subunit is absent in VSMCs, the presence of functional isoforms, *Nox1* and *Nox4*, has been reported.[23,24] Recently, it has been shown that *Nox1* mediates angiotensin II–induced $\cdot O_2^-$ formation and redox-sensitive signaling pathways in VSMCs.[24] Vascular NAD(P)H oxidase is a constitutive enzyme, but it can be also regulated by humoral factors, such as angiotensin II, platelet-derived growth factor, thrombin, tumor growth factor-α, and glucocorticoids,[18,25–28] and hemodynamic forces, including laminar and oscillatory shear stress.[29]

NAD(P)H Oxidase in Experimental Hypertension

Angiotensin II–Induced Hypertension

Rajagopalan et al[30] demonstrated that chronic infusion of angiotensin II in rats resulted in hypertension in correlation with an increased NAD(P)H oxidase-derived $\cdot O_2^-$ generation. In the same study, these alterations were corrected by pretreatment of the rats with losartan. Fukui et al[31] reported that increased activity of NAD(P)H oxidase in angiotensin II–induced hypertension activated NAD(P)H oxidase by upregulating the $p22^{phox}$ mRNA levels, a critical component of this oxidase.[20] Infusion of recombinant heparin-binding superoxide dismutase (SOD) decreased both blood pressure and $p22^{phox}$ mRNA expression.[31]

Recent evidence also suggests the involvement of other subunits in angiotensin II–induced hypertension. Thus, in aortas from angiotensin II–infused mice, there is an increased NAD(P)H-driven $\cdot O_2^-$ production concomitant with increased protein levels of $p67^{phox}$ and $gp91^{phox}$ subunits that is associated with the elevation of blood pressure.[32] Furthermore, these angiotensin II–induced increases were normalized by simultaneous treatment with losartan.

DOCA-Salt and Renovascular Hypertension

Somers et al[33] showed an enhanced vascular $\cdot O_2^-$ production associated with impaired endothelium-dependent relaxation in deoxycorticosterone acetate (DOCA)-salt rats, a hypertension model characterized by a low plasma renin activity. Recently, Wu et al[34] have reported that the enhanced $\cdot O_2^-$ production present in the aorta of DOCA-salt hypertensive rats is associated with an increased NADH oxidase activity. It seems that this increased oxidase activity is independent of the rise in blood pressure. It has been suggested that an increased vascular angiotensin II release as a consequence of nephrectomy is the origin of the increased NADH oxidase activity in these rats.

Renovascular hypertension in the 2-kidney, 1-clip rat model depends on an increase in circulating angiotensin II levels.[35] In this model, NO production is increased,[36] and a potential role for $\cdot O_2^-$ in enhanced NO breakdown has been suggested. Heitzer et al[37] showed an increased aortic $\cdot O_2^-$ generation in this hypertension model associated with an overactivity of NAD(P)H oxidase. Although the mechanism whereby angiotensin II activates NAD(P)H oxidase is still unclear, it might involve a protein kinase C–dependent process.

Genetic Hypertension

Several works have recently provided evidence confirming the pathophysiological function of ROS in the SHR. Suzuki et al[38] showed an increased $\cdot O_2^-$ generation in venules and arterioles in these hypertensive rats. Furthermore, Nakazono et al[39] demonstrated that administration within the vessel wall of heparin-binding SOD normalized the blood pressure of SHR. Recently, we reported an enhanced NAD(P)H oxidase–driven $\cdot O_2^-$ production associated with an upregulated $p22^{phox}$ mRNA expression in the aorta of adult SHR with endothelial dysfunction and vascular wall hypertrophy.[40]

In the same work, NAD(P)H oxidase–driven $\cdot O_2^-$ production was not increased in young SHR, which discards a critical role of hypertension in the regulation of oxidase. In this regard, it has been reported that in norepinephrine-induced hypertension, neither $\cdot O_2^-$ production nor NAD(P)H oxidase is increased.[30] Interestingly, we found that both $p22^{phox}$ mRNA expression and NAD(P)H oxidase activity were normalized in adult SHR treated with the angiotensin II type 1 (AT_1) receptor antagonist irbesartan.[40] This suggests a critical role of angiotensin II in the upregulation of this oxidase in the adult SHR. This possibility is further supported by the fact that enhanced expression of both AT_1 receptor and ACE have been reported in vessels of adult SHR.[41] As a consequence of an overactivity of the renin-angiotensin system, changes in the degree of activation of vascular cells can regulate $p22^{phox}$ expression. In this regard, we observed that differences in the VSMC phenotype were correlated with changes in the $p22^{phox}$ gene promoter activity.[42] Thus, $p22^{phox}$ gene promoter activity was increased in VSMCs isolated from adult SHR compared with those obtained from normotensive Wistar-Kyoto rats (WKY).

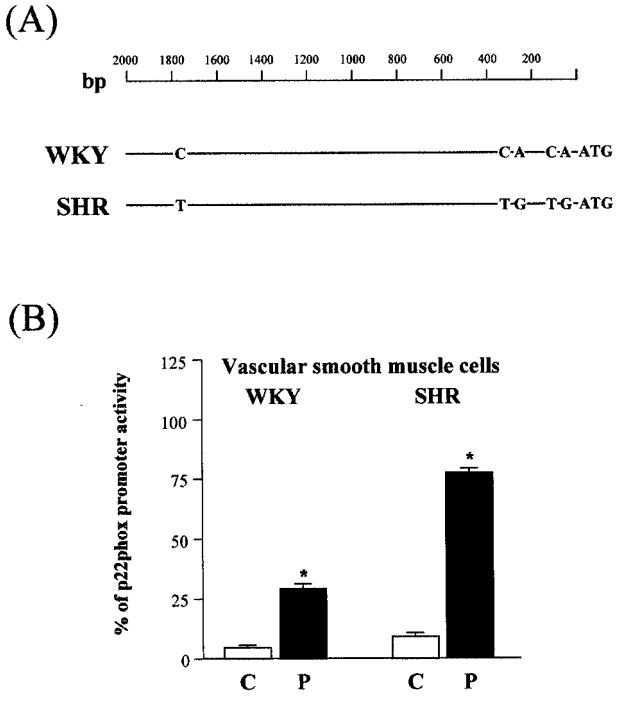

Figure 1. Influence of cellular phenotype and gene polymorphisms on p22^{phox} subunit expression. A, Existence of polymorphisms between normotensive WKY and SHR p22^{phox} promoter. ATG represents the translation initiation codon. B, Transfection experiments with the SHR polymorphic promoter (P) and the WKY control promoter (C) into VSMCs from WKY and SHR. Histograms express relative luciferase activity of the p22^{phox} promoter. *$P<0.05$ vs WKY control promoter, by Student's t test. (This figure is an adaptation.[42])

On the other hand, upregulation of the oxidase p22^{phox} subunit in the SHR may be consequence of alterations in the sequence of the p22^{phox} gene. In this way we identified 5 polymorphisms in the promoter region of the SHR p22^{phox} gene (Figure 1). Interestingly, the polymorphic SHR promoter possessed functional significance, suggesting that these polymorphisms might be involved in overexpression of the p22^{phox} gene in the vascular wall of the SHR.[42] Taken together, these findings suggest that besides changes in degree of activation of VSMCs associated with the development of hypertension in SHR, the presence of several polymorphisms in the promoter region of the p22^{phox} gene might contribute to the upregulation of p22^{phox} in the vessel wall of SHR. Increased p22^{phox} expression is attenuated by SOD in hypertensive animals, suggesting a role for $\cdot O_2^-$ itself in the regulation of p22^{phox} expression.[31] Interestingly, we have described 2 putative consensus binding sites for NF-κB in the strong positive regulatory region of the rat p22^{phox} promoter.[42]

In another model of genetic hypertension, Kerr et al[43] showed a diminished NO bioavailability as a consequence of an enhanced vascular $\cdot O_2^-$ production in 12- to 16-week-old stroke-prone SHR (SHR-SP) and suggested a critical role of the endothelium and endothelial NO synthase as sources of the $\cdot O_2^-$ generation. Hamilton et al[44] have recently described similar results in old (9- to 12-month) SHR-SP. Interestingly, in this last report, they showed that apocynin, a specific inhibitor of NAD(P)H oxidase subunit assembly, decreased the enhanced $\cdot O_2^-$ production present in the aortic wall of both 3- to 4-month-old and 9- to 12-month-old SHR-SP. From these results, a contributing role of NAD(P)H oxidase in vascular $\cdot O_2^-$ generation in this model of hypertension could be hypothesized.

NAD(P)H Oxidase in Human Essential Hypertension

Clinical studies have shown the occurrence of increased ROS production in humans with essential hypertension.[45,46] In physiological conditions, $\cdot O_2^-$ levels are modulated by endogenous scavenging systems, such as SOD. It seems that in essential hypertension, it should be an unbalance between an enhanced $\cdot O_2^-$ generation and a decreased antioxidant activity. In fact, the levels of ROS scavengers, such as vitamin E, glutathione, and SOD, have been reported to be depressed in hypertensive patients.[47] Furthermore, vitamin C recovers endothelial function by restoring the NO-mediated vasodilation of the endothelium in hypertensive patients.[48]

Berry et al[49] have demonstrated that NAD(P)H oxidase is a source of basal $\cdot O_2^-$ production in human internal mammary arteries and saphenous veins. The same authors have reported that angiotensin II increases $\cdot O_2^-$ in human arteries. This effect is mediated by NAD(P)H oxidase and is completely inhibited by the AT_1 receptor antagonist losartan. Higher basal $\cdot O_2^-$ concentration in arteries, compared with that in veins, was maintained after endothelial denudation by rubbing, suggesting that VSMCs might be an important source of $\cdot O_2^-$ generation in the human arterial wall. Up to now, no studies have been published dealing with vascular NAD(P)H oxidase activity in human hypertension.

Although the relationship between AT_1 receptor and NAD(P)H oxidase activity is fascinating, several studies do not show a beneficial effect of ACE inhibitors and AT_1 antagonists on endothelial function in patients with essential hypertension.[50,51] On the other hand, results with these drugs are more convincing in patients with coronary artery disease.[52] Thus, the possibility exists that NAD(P)H oxidase could play a role in patients with a greater cardiovascular risk.

Guzik et al[53] have reported a functional effect of the C242T p22^{phox} polymorphism in the p22^{phox} gene on NAD(P)H oxidase–driven $\cdot O_2^-$ production in the vascular wall of patients with atherosclerosis. Recently, Schachinger et al[54] described an association of the C242T p22^{phox} polymorphism with coronary endothelial vasodilator function. Gardemann et al[55] showed that the association of the A640G polymorphism in the p22^{phox} gene with the presence and extent of coronary artery disease was stronger in hypertensive than in normotensive subjects. Thus, the role of p22^{phox} polymorphisms via NAD(P)H oxidase–mediated $\cdot O_2^-$ production in the development of atherosclerosis in essential hypertension can be hypothesized.

Conclusion and Perspectives

Arterial hypertension is associated with an enhanced vascular production of ROS, namely, $\cdot O_2^-$. Overactivity of NAD(P)H oxidase may be critically involved in such an alteration (Figure 2). Thus, this enzyme may play a role in endothelial dysfunction and vascular hypertrophy present in hypertension

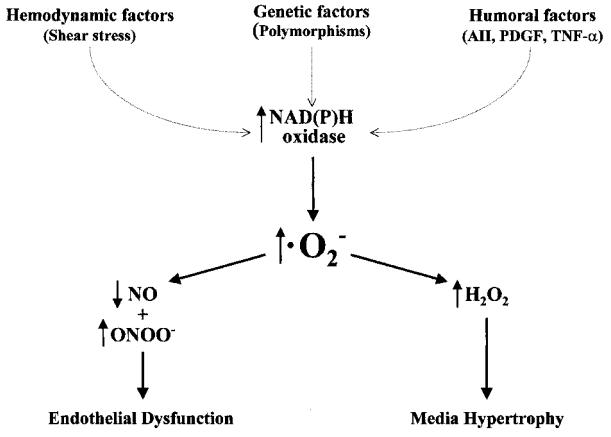

Figure 2. NAD(P)H oxidase activation and functional consequences in arterial hypertension. AII indicates angiotensin II; PDGF, platelet-derived growth factor; and TNF-α, tumor necrosis factor-α. Multiple humoral agonists and hemodynamic forces activate NAD(P)H oxidase. Genetic changes may be involved by modulating the expression of the components of NAD(P)H oxidase. An enhanced superoxide anion production driven by NAD(P)H oxidase activation is involved in endothelial dysfunction by decreasing NO bioavailability and is involved in media hypertrophy through the production of H_2O_2.

(Figure 2). Besides hemodynamic factors, humoral factors such as angiotensin II may be responsible for altered NAD(P)H oxidase in hypertension (Figure 2), thus allowing for specific pharmacological interventions aimed to reduce oxidative stress in hypertension. The possibility also exists that $p22^{phox}$ gene promoter polymorphisms might regulate NAD(P)H oxidase–driven $\cdot O_2^-$ production in hypertensive patients. Nevertheless, to confirm that these polymorphisms of the $p22^{phox}$ gene are novel markers for hypertensive oxidative stress, investigations in large populations are necessary.

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