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

Guillermo Zalba, Gorka San José, María U. Moreno, María A. Fortuño, Ana Fortuño, Francisco J. Beaumont and Javier Díez

Hypertension 2001;38;1395-1399
DOI: 10.1161/hy1201.099611
Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0194-911X. Online ISSN: 1524-4563

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

Guillermo Zalba, Gorka San José, María U. Moreno, María A. Fortuño, Ana Fortuño, Francisco J. Beaumont, Javier Díez

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

Received August 8, 2001; first decision August 15, 2001; revision accepted September 10, 2001.

From the Division of Cardiovascular Pathophysiology, School of Medicine (G.Z., G.S.J., M.U.M., M.A.F., A.F., F.J.B., J.D.), and the Department of Cardiology and Cardiovascular Surgery, University Clinic (J.D.), University of Navarra, Pamplona, Spain.

Correspondence to Guillermo Zalba, PhD, División de Fisiopatología Vascular, Facultad de Medicina, C/Irunlarrea s/n, 31080 Pamplona, Spain. E-mail gzalba@unav.es

Hypertension **is available at http://www.hypertensionaha.org**

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).

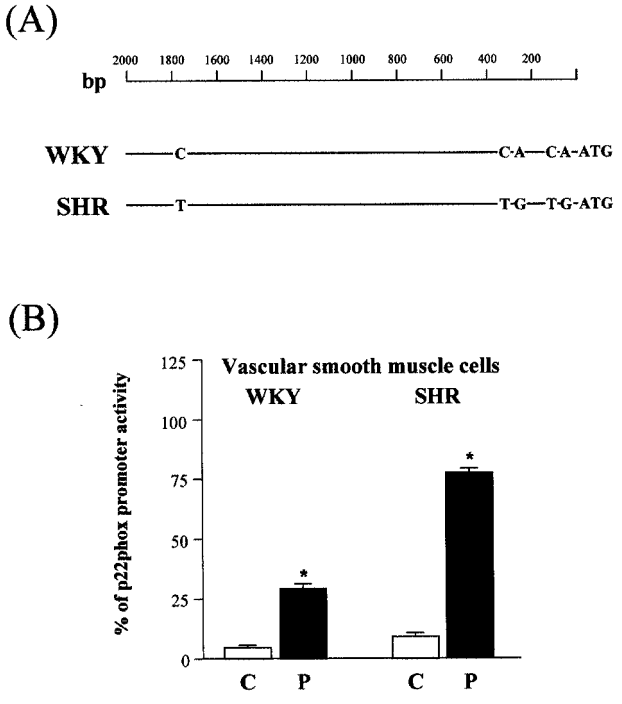

Figure 1. Influence of cellular phenotype and gene polymorphisms on p22^{phox} subunit expression. A, Existence of polymorphisms between normotensive WKY and SHR p22^{phox} promoter. ATG represents the translation initiation codon. B, Transfection experiments with the SHR polymorphic promoter (P) and 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

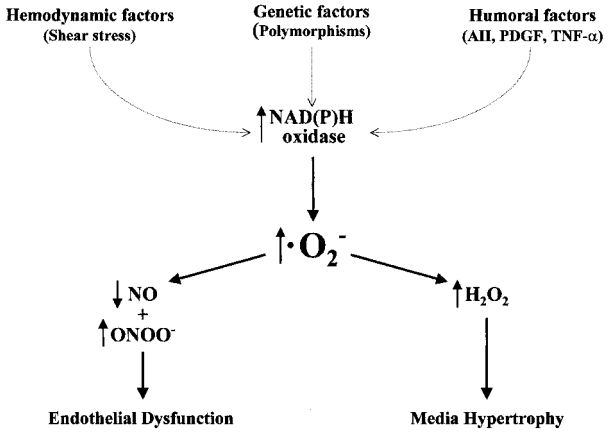

Figure 2. NAD(P)H oxidase activation and functional consequences in arterial hypertension. AII indicates angiotensin II; PDGF, platelet-derived growth factor; and TNF-α, tumor necrosis factor-α. Multiple humoral agonists and hemodynamic forces activate NAD(P)H oxidase. Genetic changes may be involved 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.

References

1. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res*. 2000;87:840–844.
2. Zalba G, Beaumont J, San José G, Fortuño MA, Fortuño A, Díez J. Vascular oxidant stress: molecular mechanisms and pathophysiological implications. *J Physiol Biochem*. 2000;56:57–64.
3. Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*. 1990;323:22–27.
4. Gryglewski RJ, Palmer RM, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature*. 1986;320:454–456.
5. Zalba G, Beaumont FJ, San José G, Fortuño A, Fortuño MA, Díez J. Is the balance between nitric oxide and superoxide altered in spontaneously hypertensive rats with endothelial dysfunction? *Nephrol Dial Transplant*. 2001;16(suppl 1):2–5.
6. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A*. 1990;87:1620–1624.
7. Ballinger SW, Patterson C, Yan CN, Doan R, Burow DL, Young CG, Yakes FM, Van Houten B, Ballinger CA, Freeman BA, et al. Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. *Circ Res*. 2000;86:960–966.
8. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res*. 2000;86:494–501.
9. Li PF, Dietz R, von Harsdorf R. Differential effect of hydrogen peroxide and superoxide anion on apoptosis and proliferation of vascular smooth muscle cells. *Circulation*. 1997;96:3602–3609.
10. 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*. 1998;32:488–495.
11. Irani K. Oxidant signaling in vascular cell growth, death, and survival: a review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. *Circ Res*. 2000;87:179–183.
12. Ruiz-Ortega M, Lorenzo O, Rupérez M, König S, Wittig B, Egido J. Angiotensin II activates nuclear transcription factor κB through AT_1 and AT_2 in vascular smooth muscle cells: molecular mechanisms. *Circ Res*. 2000;86:1266–1272.
13. Brasier AR, Li J. Mechanisms for inducible control of angiotensinogen gene transcription. *Hypertension*. 1996;27:465–475.
14. Luft FC. Mechanisms and cardiovascular damage in hypertension. *Hypertension*. 2001;37:594–598.
15. Mohazzab KM, Kaminski PM, Wolin MS. NADH oxidoreductase is a major source of superoxide anion in bovine coronary artery endothelium. *Am J Physiol*. 1994;266:H2568–H2572.
16. Pagano P, Ito Y, Tornheim K, Gallop P, Tauber A, Cohen R. An NADPH oxidase superoxide-generating system in the rabbit aorta. *Am J Physiol*. 1995;268:H2274–H2280.
17. Jones SA, O'Donnell VB, Wood JD, Broughton JP, Hugher EJ, Jones OT. Expression of phagocyte NADPH oxidase components in human endothelial cells. *Am J Physiol*. 1996;271:H1626–H1634.
18. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res*. 1994;74:1141–1148.
19. Sorescu D, Somers MJ, Lassègue B, Grant S, Harrison DG, Griendling KK. Electron spin resonance characterization of the NAD(P)H oxidase in vascular smooth muscle cells. *Free Radic Biol Med*. 2001;30:603–612.
20. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, Griendling KK. $p22^{phox}$ 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*. 1996;271:23317–23321.
21. Hsich E, Segal BH, Pagano PJ, Rey FE, Paigen B, Deleonardis J, Hoyt RF, Holland SM, Finkel T. Vascular effects following homozygous disruption of $p47^{phox}$: an essential component of NADPH oxidase. *Circulation*. 2000;101:1234–1236.
22. Archer SL, Reeve HL, Michelakis E, Puttagunta L, Waite R, Nelson DP, Dinauer MC, Weir EK. O_2 sensing is preserved in mice lacking the gp91 phox subunit of NADPH oxidase. *Proc Natl Acad Sci U S A*. 1999;96:7944–7949.
23. Suh Y, Arnold RS, Lassègue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, Lambeth JD. Cell transformation by the superoxide-generating oxidase mox1. *Nature*. 1999;401:79–82.
24. Lassegue B, Sorescu D, Szocs 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*. 2001;88:888–894.
25. De Keulenauer GW, Alexander RW, Ushio-Kukai M, Ishizaka N, Griendling KK. Tumor necrosis factor α activates a $p22^{phox}$ based NADH oxidase in vascular smooth muscle. *Biochem J*. 1998;329:653–657.
26. 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*. 1999;274:19814–19822.
27. Marumo T, Schini-Kerth VB, Fisslthaler B, Busse R. Platelet-derived growth factor–stimulated superoxide anion production modulates activation of transcription factor NF-kB and expression of monocyte chemoattractant protein-1 in human aortic smooth muscle cells. *Circulation*. 1997;96:2361–2367.
28. Marumo T, Schini-Kerth VB, Brandes RP, Busse R. Glucocorticoids inhibit superoxide anion production and $p22^{phox}$ mRNA expression in human aortic smooth muscle cells. *Hypertension*. 1998;32:1083–1088.
29. De Keulenaer GW, Chappell DC, Ishizaka N, Nerem RM, Alexander RW, Griendling KK. Oscillatory and steady laminar shear stress differentially affect human endothelial redox state. *Circ Res*. 1998;82:1094–1101.

30. 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*. 1996;97:1916–1923.
31. Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers Q IV, Taylor WR, Harrison DG, de Leon H, Wilcox JN, Griendling KK. $p22^{phox}$ mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. *Circ Res*. 1997;80:45–51.
32. Cifuentes ME, Rey FE, Carretero OA, Pagano JP. Upregulation of $p67^{phox}$ and $gp91^{phox}$ in aortas from angiotensin II-infused mice. *Am J Physiol*. 2000;279:H2234–H2240.
33. Somers MJ, Mavromatis K, Galis ZS, Harrison DG. Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate–salt. *Circulation*. 2000;101:1722–1728.
34. Wu R, Millete E, Wu L, de Champlain J. Enhanced superoxide anion formation in vascular tissues from spontaneously hypertensive and deoxycorticosterone acetate-salt hypertensive rats. *J Hypertens*. 2001;19:741–748.
35. Ploth D. Angiotensin-dependent renal mechanisms in two-kidney, one-clip renal vascular hypertension. *Am J Physiol*. 1983;245:F131–F141.
36. Sigmon DH, Beierwaltes WH. Nitric oxide influences blood flow distribution in renovascular hypertension. *Hypertension*. 1994;23:134–139.
37. Heitzer T, Wenzel U, Hink U, Krollner D, Skatchkov M, Stahl RAK, Macharzina R, Bräsen JH, Meinertz T, Münzel T. Increased NAD(P)H oxidase–mediated superoxide production in renovascular hypertension: evidence for an involvement of protein kinase C. *Kidney Int*. 1999;55:252–260.
38. Suzuki H, Swei A, Zweifach BW, Schmid-Schonbein GW. In vivo evidence for microvascular oxidative stress in spontaneously hypertensive rats. *Hypertension*. 1995;25:1083–1089.
39. Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M. Does superoxide underlie the pathogenesis of hypertension? *Proc Natl Acad Sci U S A*. 1991;88:10045–10048.
40. Zalba G, Beaumont FJ, San José G, Fortuño A, Fortuño MA, Etayo JC, Díez J. Vascular NADH/NADPH oxidase is involved in enhanced superoxide production in spontaneously hypertensive rats. *Hypertension*. 2000;35:1055–1061.
41. Otsuka S, Sugano M, Makino N, Sawada S, Hata T, Niho Y. Interaction of mRNAs for angiotensin II type 1 and type 2 receptors to vascular remodeling in spontaneously hypertensive rats. *Hypertension*. 1998;32:467–472.
42. Zalba G, San José G, Beaumont FJ, Fortuño MA, Fortuño A, Díez J. Polymorphisms and promoter overactivity of the $p22^{phox}$ gene in vascular smooth muscle cells from SHR. *Circ Res*. 2001;88:217–222.
43. Kerr S, Brosnan MJ, McIntyre M, Reid JL, Dominiczak AF, Hamilton CA. Superoxide anion production is increased in a model of genetic hypertension: the role of the endothelium. *Hypertension*. 1999;33:1353–1358.
44. Hamilton CA, Brosnan MJ, McIntyre M, Graham D, Dominiczak AF. Superoxide excess in hypertension and aging: a common cause of endothelial dysfunction. *Hypertension*. 2001;37:529–534.
45. Mehta JL, Lopez LM, Chen L, Cox OE. Alterations in nitric oxide synthase activity, superoxide anion generation, and platelet aggregation in systemic hypertension, and effects of celiprolol. *Am J Cardiol*. 1994;74:901–905.
46. Lacy F, O'Connor DT, Schmid-Schonbein GW. Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. *J Hypertens*. 1998;16:291–303.
47. Sagar S, Kallo IJ, Kaul N, Ganguly NK, Sharma BK. Oxygen free radicals in essential hypertension. *Mol Cell Biochem*. 1992;111:103–108.
48. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation*. 1998;97:2222–2229.
49. Berry C, Hamilton CA, Brosnan MJ, Magill FG, Berg GA, McMurray JJV, Dominiczak AF. Investigation into the sources of superoxide in human blood vessels: angiotensin II increases superoxide production in human internal mammary arteries. *Circulation*. 2000;101:2206–2212.
50. Kiowski W, Linder L, Nuesch R, Martina B. Effects of cilazapril on vascular structure and function in essential hypertension. *Hypertension*. 1996;27:371–376.
51. Ghiadoni L, Virdis A, Magagna A, Taddei S, Salvetti A. Effect of the angiotensin II type 1 receptor blocker candesartan on endothelial function in patients with essential hypertension. *Hypertension*. 2000;35:501–506.
52. Hornig B, Landmesser U, Kohler C, Ahlersmann D, Spiekermann S, Christoph A, Tatge H, Drexler H. Comparative effect of ACE inhibition and angiotensin II type 1 receptor antagonism on bioavailability of nitric oxide in patients with coronary artery disease: role of superoxide dismutase. *Circulation*. 2001;103:799–805.
53. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Chanon KM. Functional effect of the C242T polymorphism in the NAD(P)H oxidase $p22^{phox}$ gene on vascular superoxide production in atherosclerosis. *Circulation*. 2000;102:1744–1747.
54. Schachinger V, Britten MB, Dimmeler S, Zeiher AM. NADH/NADPH oxidase $p22^{phox}$ gene polymorphism is associated with improved coronary endothelial vasodilator function. *Eur Heart J*. 2001;22:96–101.
55. Gardemann A, Mages P, Katz N, Tillmanns H, Haberbosch W. The $p22^{phox}$ A640G gene polymorphism but not the C242T gene variation is associated with coronary heart disease in younger individuals. *Atherosclerosis*. 1999;145:315–323.