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Review

Reactive Oxygen Species in Hypertension

An Update

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Hypertension is associated with an elevation of reactive oxygen species (ROS) and frequently also with an impairment of endogenous antioxidant mechanisms. Experimental manipulation of the redox state in vivo shows that ROS can be a cause of hypertension. During the development of the disease, ROS are generated by endogenous sources, notably the NADPH oxidase enzyme family and uncoupled nitric oxide synthase, due to a mutual reinforcement between ROS and humoral factors. The ROS affect multiple tissues, either directly or through nitric oxide depletion. In the vasculature, they induce contraction and endothelial dysfunction. In blood vessels and myocardium, they cause hypertrophic remodeling. In the kidneys, ROS promote salt reabsorption, decrease glomerular filtration, and lead to tissue damage. Finally, they also increase efferent sympathetic activity from the central nervous system. Progress in our understanding of the mechanisms of ROS formation and their plethora of pathophysiologic effects is expected to lead from simple antioxidant therapy to specific antihypertensive treatments. Am J Hypertens 2004;17:852–860 © 2004 American Journal of Hypertension, Ltd.

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Reactive oxygen species (ROS), long known as microbicide agents produced by phagocytes or undesirable by-products of aerobic metabolism, have recently acquired the status of ubiquitous signaling molecules. Alterations in the biochemical pathways leading to their production and metabolism have been implicated in pathologies as varied as cancer and diabetes. Here we will review recent evidence linking ROS with hypertension and evaluate their possible causal contribution to the development of the disease. After describing the molecular sources of ROS in hypertension and the factors that trigger their formation, we will concentrate on their targets in the organism.

Effect of Hypertension on Reactive Oxygen Species

Reactive Oxygen Species and Oxidation Markers

Experimental hypertension is frequently induced by stimulation of the renin-angiotensin system: by chronic angiotensin II (Ang II) infusion, overexpression of the renin and angiotensinogen genes, or coarctation of the renal artery with or without uninephrectomy. Angiotensin II is also involved in hypertension development in the spontaneously hypertensive rat (SHR). In these models, ROS such as superoxide and hydrogen peroxide (H_2O_2), as well as oxidation markers such as tyrosine nitration of proteins, production and excretion of 8-isoprostaglandin $F_{2\alpha}$ (8-iso), malonyl dialdehyde, and thiobarbituric acid reactive substances, are increased in vessels, heart, and kidneys. In addition, renal blood flow and glomerular filtration are decreased in SHR.[1–5] Similar effects are observed with a high salt diet, notably in obesity-prone and Dahl salt-sensitive rats.[6,7]

Hypertension models with low renin and angiotensin are obtained, for example, by infusion of endothelin-1 (ET-1) or by uninephrectomy and administration of deoxycorticosterone and high dietary salt (DOCA-salt). Here also, vascular superoxide and oxidation markers are elevated.[5,8–13] Thus, in many experimental models, hypertension is associated with increased ROS production.

Antioxidant Defense Mechanisms

The ROS abundance in hypertension could result from increased production (see Molecular Sources) or impaired degradation. Very active antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, convert superoxide to H_2O_2 and water. Expression of extracellular

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(ec)SOD in kidney is decreased by Ang II infusion.[2] CuZn-SOD and MnSOD activities are similarly lower in vessels and kidney in subtotal nephrectomy,[14] Dahl salt-sensitive, renal artery stenosis, and DOCA-salt.[15] The MnSOD activity is markedly decreased in the kidney by Ang II infusion due to tyrosine nitration.[1] Similarly, kidney catalase and glutathione peroxidase are decreased after renal artery stenosis. Aortic catalase is also downregulated in genetically hypertensive mice.[16] Thus, in these animal models, downregulation of endogenous antioxidant enzymes may contribute to the elevation of ROS in hypertension.

The Superoxide/Nitric Oxide Balance

Superoxide is degraded by reaction with nitric oxide (NO) three times faster than by SOD, leading to loss of NO and generation of peroxynitrite, a harmful ROS. To assess the relevance of this reaction in hypertension, the concentration of NO and its metabolites, nitrite and nitrate (NO_x), have been measured. Urinary excretion of NO_x is decreased in renin and angiotensinogen transgenics,[3] Dahl salt-sensitive, obesity, and subtotal nephrectomy.[14] This NO decrease is not due to a downregulation of NO synthase (NOS) in the SHR vasculature, for instance, as endothelial NOS (eNOS) activity is increased despite the reduced agonist-induced NO production.[4,17,18]

The loss of NO in hypertension leads to impaired endothelium-dependent relaxation in various models, such as rats infused with Ang II,[5,19] rats and mice overexpressing renin and angiotensinogen,[3,5] SHR,[4,18] rats on a high salt diet with or without nephrectomy, and DOCA-salt.[13] Similarly, patients with essential hypertension also present a decreased endothelial-dependent relaxation and a diminished effect of NOS antagonists.[20] Thus, by consuming NO, ROS are not only associated with hypertension, but also contribute to the disease (Fig. 1).

Causal Role of Reactive Oxygen Species in Hypertension

Treatments Increasing Reactive Oxygen Species

Exposure of cells or tissues to exogenous oxidants mimics events that contribute to hypertension development. Generators of superoxide abolish endothelium-dependent relaxation of aortic rings from SHR,[18] induce contraction of mesenteric beds,[21] and increase chloride reabsorption by isolated thick ascending limbs of the loop of Henle.[22] Similarly, tert-butyl hydroperoxide (t-BOOH) induces contraction of isolated vessels.[15] In whole rats, infusion of H_2O_2 in the renal medulla increases blood pressure (BP) and decreases blood and urine flows, as well as sodium excretion.[23]

Inhibition of endogenous antioxidants increases ROS with similar effects on the hypertensive phenotype. For instance, incubation of isolated vessels with the CuZnSOD inhibitor diethyldithiocarbamate (DETC) impairs both endothelium-dependent and endothelium-independent relaxations.[5] In vivo, DETC increases BP after intravenous administration or infusion into the kidney medulla.[24] Sur-

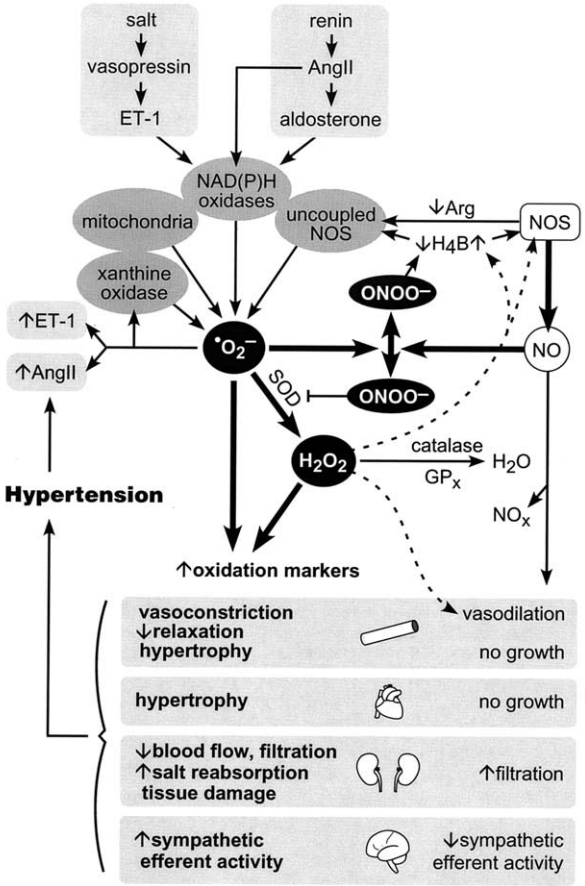

FIG. 1. Major pathways leading to hypertension via reactive oxygen species formation. Reactive oxygen species, such as superoxide ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$), are increased in target tissues, directly or indirectly, by hypertensive agents, such as salt, vasopressin, endothelin-1 (ET-1), renin, angiotensin II (AngII), or aldosterone. These proximal agents increase superoxide production through NAD(P)H oxidases, uncoupled nitric oxide synthase (NOS), xanthine oxidase, and mitochondria. NOS can be uncoupled by decreased availability of its substrate L-arginine (Arg) or its cofactor tetrahydrobiopterin (H_4B). In the presence of sufficient substrate and cofactor, NOS generates the potent antihypertensive agent nitric oxide (NO), often measured by assay of its metabolites nitrite and nitrate (NO_x). The relative abundance of superoxide and NO, termed "superoxide/NO balance," controls the proportion of NO degraded by reaction with superoxide to form the noxious peroxynitrite. This reactive oxygen species, which can alter proteins by nitration on tyrosine, is responsible for NOS uncoupling and inhibition of manganese superoxide dismutase (SOD). The SOD enzymes convert superoxide to hydrogen peroxide, which may be further degraded to water by catalase or glutathione peroxidase (GP_x). Hydrogen peroxide can increase NOS activity directly or by increased H_4B synthesis, and act as an endothelium-derived relaxing factor in vessels (dotted arrows). Superoxide and its metabolites can induce vasoconstriction, vascular and myocardial hypertrophy, decrease kidney function, and increase sympathetic efferent activity from the central nervous system. All these effects, antagonized by NO, contribute to the development of hypertension. Note the presence of closed loops, leading to uncoupling of NOS, activation of xanthine oxidase, and generation of ET-1 and Ang II. These are possible self-reinforcing mechanisms that can lead to persistent hypertension.

prisingly, BP is not elevated by disruption of the CuZn-SOD gene in mice, in spite of increases in vascular superoxide and contractile responses to vasoconstrictors, as well as impaired endothelial-dependent relaxation.[25] This paradoxical effect may be due to compensatory mechanisms. Deletion of the ecSOD gene does not affect BP, but increases susceptibility to ROS-increasing hypertensive treatments.[26] However, BP and oxidation markers are elevated in rats treated with an inhibitor of glutathione synthesis, whereas NO and NO_x excretion are reduced.[27] Overall, vascular reactivity and BP are usually adversely affected by exogenous ROS and by inhibition of endogenous ROS degradation.

Treatments Decreasing Reactive Oxygen Species

Administration of exogenous antioxidants, such as the SOD mimetic tempol, is frequently used to assess the role of ROS in experimental hypertension. Tempol improves hypertension, oxidation markers, endothelium-dependent relaxation, media/lumen ratio, neuronal NOS (nNOS) activity, kidney damage, glomerular filtration, and NO_x excretion in models such as Ang II infusion,[28] SHR,[29] nephrectomy,[14] ET-1 infusion,[10] DOCA-salt,[13] and Dahl salt-sensitive.[6] Tempol also reduces CuZnSOD inactivation and vessel contraction induced by ET-1 or t-BOOH in DOCA-salt animals.[9,15] Similar improvements in BP and associated aberrations are seen with dietary antioxidant vitamins C and E in experimental hypertension models such as SHR and Dahl salt-sensitive, but not DOCA-salt.[17,30]

Boosting endogenous antioxidants to reduce ROS is also beneficial. For example, exogenous SOD improves endothelium-dependent and endothelium-independent relaxations in isolated vessels from DOCA-salt animals or rats with subtotal nephrectomy.[5] Similarly, addition of SOD with catalase normalizes elevated ROS and impaired endothelium-dependent relaxation in microvessels from rats fed a high salt diet. Overexpression of SOD reduces hypertension and oxidation markers, increases available NO and endothelium-dependent relaxation in SHR,[31] renin and angiotensinogen transgenics,[3] and Ang II infusion.[5] Likewise, increases in BP, heart rate, and drinking behavior induced in mice by intracerebroventricular injection of Ang II are abolished by prior injection of MnSOD or CuZnSOD in adenoviral vectors.[32]

Addition of catalase to tempol is required to reduce hypertension induced by DETC infusion in the kidney medulla.[23] Likewise, catalase overexpression in transgenic mice reduces hypertension after acute or chronic norepinephrine or Ang II administration.[33]

The elevation of BP by oxidants and its amelioration by antioxidants strongly supports a causal role of ROS in hypertension. However, hypertension can also increase ROS. For instance, aortic coarctation in rats increases oxidation markers in tissues located above, but not below the stricture, supporting a direct mechanical effect.[34] Therefore, the mutual amplification of ROS and hypertension may contribute to the development of chronic disease.

Molecular Sources of Reactive Oxygen Species in Hypertension

NAD(P)H Oxidases

The NAD(P)H oxidase (nox) family, including the phagocyte oxidase (phox), is a ubiquitous source of superoxide. In each cell type, a specific complement of subunits may be assembled into different enzymes. In the cardiovascular system, the major catalytic subunits are nox1, gp91phox (a.k.a. nox2), and nox4, and the regulatory subunits include p22phox, p47phox, p67phox, and rac.[5,35]

A role for these oxidases in hypertension is suggested by their regulation in experimental conditions similar to disease. The four phox subunits are upregulated in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) from small vessels exposed to Ang II,[35–37] and ROS production is reduced by transfection of antisense p47phox, abolished by deletion of the p47phox gene, and rescued by expression of p47phox.[38] Similarly, in VSMC exposed to cyclic stretch, nox1 mRNA is upregulated and the ROS increase is abolished by p47phox disruption.[39] Superoxide is also reduced in vitro by the oxidase assembly inhibitor apocynin in vessels exposed to ET-1 or isolated from SHR or DOCA-salt.[8,9,13] Furthermore, Ang II-induced H_2O_2 production is increased in VSMC isolated from hypertensive patients.[40]

In vivo, gp91phox is upregulated in kidney and liver after subtotal nephrectomy.[14] Upregulation of aortic gp91phox by Ang II infusion is decreased by a protein kinase C inhibitor that also lowers BP.[5] However, disruption of the gp91phox gene does not prevent hypertension induced by Ang II infusion, but lowers basal BP, reduces aortic adventitial nitrotyrosine accumulation, and medial hyperplasia,[5] suggesting that it may have a role in chronic, rather than acute hypertension. The nox1 subunit is upregulated in vessels and renal cortex by renin overexpression or Ang II infusion,[2,5] and reduced in vessels by administration of a protein kinase C inhibitor to Ang II-infused rats[5] or a statin to SHR.[5] Thus, regulation of the catalytic oxidase subunits in vivo appears to be linked to hypertension.

Expression of regulatory NAD(P)H subunits also correlates with hypertension and ROS production in vessels[5,18,41] and kidney.[2] The G allele of a p22phox promoter polymorphism is more frequent in hypertensive patients.[42] Moreover, functional animal studies show that an inhibitor of p47phox translocation abolishes vascular superoxide and reduces hypertension after Ang II infusion.[5] Similarly, disruption of the p47phox gene blocks superoxide elevation and reduces hypertension in DOCA-salt and Ang II-infused mice.[5,12] Apocynin also reduces vascular superoxide and hypertension in DOCA-salt ani-

mals.[5] Both in vitro and in vivo evidence strongly suggest that NAD(P)H oxidases contribute to hypertension.

Nitric Oxide Synthase

All three forms of NOS, eNOS, nNOS, and inducible (iNOS) are potentially important in BP regulation.[43] The NOS enzymes consume arginine to produce the potent vasodilator NO. Low availability of the tetrahydrobiopterin (H_4B) cofactor or arginine leads to NOS uncoupling, which then produces superoxide, rather than NO. By reaction with superoxide, NO is further decreased and generates peroxynitrite. This latter ROS can oxidize H_4B, leading to additional NOS uncoupling.[44]

Hypertension is induced by deletion of the eNOS gene, but is surprisingly almost absent after inactivation of other forms of NOS,[43] probably due to compensatory mechanisms. However, BP is increased by NOS inhibition,[21] genetic H_4B deficiency,[45] or inhibitors of H_4B synthesis.[46] In this latter case, endothelium-dependent relaxation is improved by SOD and restored by an H_4B precursor.[46] Similarly, in rats with subtotal nephrectomy, hypertension is corrected by administration of arginine or H_4B, which also increases mesenteric eNOS expression.[47] In SHR, superoxide is elevated and NO production reduced, in spite of increased expression and activity of NOS. The balance between superoxide and NO is restored or improved by SOD, NOS inhibitors, ascorbate, or H_4B.[4,17]

In carotid arteries from DOCA-salt rats, NO and H_4B are decreased, and overexpression of the limiting enzyme in H_4B biosynthesis decreases superoxide and improves NO release and endothelium-dependent relaxation.[13] Similarly, in DOCA-salt mice, hypertension, superoxide, H_4B, and NO are improved or restored by treatment with H_4B, eNOS gene deletion, apocynin, or p47phox gene deletion, suggesting that NADPH oxidases are responsible for NOS uncoupling.[12,13] It is therefore apparent that NOS uncoupling actually occurs in vivo and that it is an important and self-sustained source of ROS in hypertension. Nevertheless, other possible sources of superoxide have been proposed.

Other Sources of Reactive Oxygen Species

Mitochondria In physiologic conditions, electron leakage from the mitochondrial electron transport chain constitutively produces superoxide, usually rapidly degraded in situ by MnSOD. However, mitochondria isolated from hypertensive rats hearts produce excess superoxide.[48] As mentioned above, MnSOD is inactivated by tyrosine nitration and its activity is markedly decreased in Ang II infusion,[1] subtotal nephrectomy,[14] and DOCA-salt.[15] In this latter model, ex-vivo transfection with MnSOD reduces superoxide,[8] suggesting that mitochondria may be a significant source of superoxide in hypertension.

Xanthine Oxidase The possible contribution of xanthine oxidase to ROS elevation in hypertension has been assessed using specific inhibitors. Such treatments normalize ROS in microvessels from rats fed a high salt diet,[7] and increase endothelial-dependent relaxation in arteries from SHR[18] and rats overexpressing renin and angiotensinogen genes,[3] suggesting that xanthine oxidase is another source of elevated ROS in hypertension that can impair vascular function. In ECs, incubation with apocynin or disruption of the p47phox gene markedly decreases xanthine oxidase expression and activity, suggesting that this enzyme is upregulated by NAD(P)H oxidase activation.[49] Future studies will be required to determine whether this effect is important in hypertension. Overall, it is apparent that multiple endogenous sources of ROS are activated in hypertension.

Interaction of Antagonists and Reactive Oxygen Species in Hypertension

Angiotensin II Infusion and Spontaneously Hypertensive Rat Models

In experimental hypertension with elevated renin or angiotensin, a host of ROS-related changes occur. As mentioned, the NAD(P)H oxidase is upregulated in the kidney and cardiovascular system,[2,19] resulting in increased ROS and oxidation markers.[2,3,19] Endogenous antioxidant enzymes are inhibited,[2] whereas NO and endothelial-dependent relaxation are decreased.[3,19] In addition, vascular hypertrophy[19] and kidney damage[6] can lead to chronic disease. Most of these effects are blocked by AT_1 antagonists[2,3] and enhanced by AT_2 antagonists,[2] indicating that AT_2 is a moderator, but is superseded by AT_1 in disease. A mineralocorticoid receptor antagonist also improves BP and other vascular alterations, suggesting that part of the effect of Ang II is mediated by its stimulation of aldosterone production.[19]

The AT_1 antagonists are beneficial in other hypertension models as well. For example, in the SHR, they improve BP, superoxide, oxidation markers, vascular p22phox, media size, eNOS, NO availability, endothelium-dependent relaxation, and restore the sensitivity of tubuloglomerular feedback to nNOS inhibitors.[29] Similarly, AT_1 antagonists prevent hypertension and superoxide induced by glutathione depletion or NOS inhibition. The importance of Ang II in ROS production is, therefore, evident even when hypertension is not directly originating from the renin-angiotensin system, suggesting that a positive feedback loop between Ang II and ROS exists in vivo.

Endothelin-1, Vasopressin, DOCA-salt

Low renin angiotensin hypertension involves other mediators whose relationships to ROS were first assessed in vitro. In VSMCs and isolated vessels, ET-1 induces an increase in superoxide, which is reduced by NAD(P)H oxidase inhibitors.[8] Moreover, in isolated arteries vaso-

pressin increases ET-1 and the subsequent increase in superoxide is inhibited by an ET-1 receptor (ET_A) antagonist.[11] Similarly, ET-1 is increased in vessels from DOCA-salt rats[8,11,50] and superoxide is normalized by treatment with vasopressin receptor (V1) or ET_A antagonists, apocynin or tempol.[11,13] Furthermore, exposing VSMCs or ECs to a superoxide generator increases expression of ET-1 and endothelin converting enzyme,[51] suggesting that ROS and ET-1 mutually reinforce each other.

In vivo, ET-1 infusion in rats increases BP, vascular and kidney superoxide, and oxidation markers and decreases renal blood flow. These effects are inhibited by tempol.[10] In the DOCA-salt, vascular vasopressin is elevated[11] and V1 or ET_A antagonists improve BP, vascular superoxide, oxidation markers, and endothelium-dependent relaxation,[8,9,52] suggesting that part of the hypertension is mediated by sequential increases in salt, vasopressin, ET-1, and superoxide. Similarly, ET_A antagonists also inhibit Ang II-induced hypertension, indicating that its effect is also mediated in part by ET-1.[53] It is clear that, whether the renin-angiotensin system is involved or not, hypertension is controlled, at least in part, by the activities and reciprocal amplification of ROS and specific sets of agonists. However, these effects must be mediated by functional alterations of particular targets.

Tissue Targets of Reactive Oxygen Species in Hypertension

Blood Vessels

Contraction Besides decreasing NO, ROS can induce hypertension by inducing vascular contraction. In VSMCs, ROS increase inositol trisphosphate, cytoplasmic calcium concentration,[54] inhibit the calcium reuptake pump,[55] and decrease cyclic GMP. Similarly, in isolated vessels, t-BOOH, superoxide generators, or H_2O_2 induce a contraction,[15] which is increased in SHR arteries and inhibited by tempol[15] or SOD. Furthermore, SOD or hydroxyl radical scavengers inhibit contractions induced by ET-1,[9] serotonin, and hypoxia. However, catalase inhibits contraction in some, but not all instances,[15] and exogenous superoxide can inhibit agonist-induced contraction in VSMCs.[56] Thus, the vasoconstrictor effects of ROS support their role in hypertension, but additional factors modulate their function. This is not surprising given the large number of vasoactive mediators with opposing effects present in the vessel wall.

Relaxation The paradoxical effect of catalase just mentioned suggests that H_2O_2 may be a vasodilator. Exogenous addition of H_2O_2 to arteries or VSMCs induces relaxation,[57] hyperpolarization, and inhibition of agonist-induced contraction. Furthermore, catalase inhibits endothelial-dependent relaxation of arteries by acetylcholine or flow,[57] as well as bradykinin-induced vasodilation in the presence of both NOS and cyclooxygenase inhibitors, indicating that H_2O_2 is an endothelium-derived hyperpolarizing factor.[58] The inhibition of endothelial-dependent relaxation by catalase is more pronounced in SHR[4] and DOCA-salt,[12] suggesting that H_2O_2 serves as a relaxing factor in hypertension with NO depletion.

Another mechanism by which H_2O_2 induces relaxation is through activation of eNOS and increasing NO release in ECs, with a peak at 5 min.[59] Prolonged incubation with H_2O_2 additionally upregulates eNOS expression and activity and increases H_4B synthesis.[60] Although this upregulation of eNOS may alleviate NO degradation, the deficiency of endothelial function in hypertension suggests that this compensation is incomplete.

Growth The vascular hypertrophy observed in hypertension suggests that ROS directly promote VSMC growth. In vitro, stimulation of VSMCs with agents such as Ang II, serotonin, ET-1, platelet-derived growth factor (PDGF), or serum increases ROS formation and growth in the form of hyperplasia or hypertrophy. These effects are mimicked by addition of H_2O_2 and antagonized by NAD(P)H oxidase inhibitors, catalase, or antioxidants.[5,61] Furthermore, exposure to Ang II, superoxide, or H_2O_2 induces transactivation of the epidermal growth factor (EGF) and PDGF receptors, leading to growth.[5,62]

In hypertensive models such as Ang II infusion, SHR and DOCA-salt, vascular remodeling is inhibited by tempol, a diet rich in vitamins C or E,[30] or NAD(P)H oxidase inhibition.[5] Similarly, indirect antioxidants, such as peroxisome proliferator-activated receptor activators[63] and AT_1 antagonists also inhibit hypertrophy.

Vascular remodeling requires activation of specific extracellular endopeptidases known as matrix metalloproteinases (MMPs). Exposing isolated arteries to increased transmural pressure for 48 hours activates MMP-2 and MMP-9.[64] In vitro, their activity is increased by incubation of VSMCs with t-BOOH, peroxynitrite, or superoxide.[64] Treatments that increase ROS in VSMC, IL-1β or cyclic stretch, also activate the same MMPs.[39] Conversely, an NO donor inhibits MMP-9 activation by IL-1β, and deletion of the p47phox gene abolishes MMP-2 upregulation and activation by cyclic stretch.[39] Interestingly, in VSMCs an MMP inhibitor blocks activation of EGF receptors by H_2O_2,[65] suggesting that MMPs are involved in growth signaling.

Although prolonged incubations with high concentrations of ROS can lead to apoptosis,[66] it appears that in hypertension ROS are usually generated in moderate amounts, promoting VSMC growth and vascular hypertrophy,[19] as suggested by the protective effect of antioxidants. Hypertrophy contributes to the hypertensive effect of ROS, but is only one of their mechanisms of action.

Heart

Hypertension is also correlated with ROS increases in the heart. For example, in glucose-induced hypertension in

rats, cardiac mitochondrial superoxide production is increased and prevented by dietary supplementation with the antioxidant α-lipoic acid.[48] Experimental hypertension induces oxidative alterations in the myocardium, such as elevated malonyl dialdehyde and lowered vitamin E and glutathione.[67] Depending on the model, endogenous antioxidant enzymes may be reduced, as glutathione peroxidase in the Dahl salt-sensitive rat,[67] or elevated, as MnSOD, CuZnSOD, and γ-glutamylcysteine synthetase (the source of glutathione) in the SHR,[68] presumably to degrade excess ROS generated in this model by upregulation of xanthine oxidase.[69] Nitric oxide synthase inhibitors also markedly increase ROS in rat myocardium, with elevation of oxidation markers, glutathione depletion, upregulation of xanthine oxidase, and downregulation of catalase, glutathione peroxidase, and γ-glutamylcysteine synthetase.[70,71] Although SOD activity is increased after 1 week, both MnSOD and CuZnSOD are downregulated by an 8-week treatment.[70,71] The NO depletion induces severe necrotic lesions in the myocardium that can be prevented by NO donors.[72–74] However, these lesions may be secondary to decreased coronary circulation. Heart rate is increased, suggesting that ROS stimulate heart function.[70] This interpretation is supported by the observation that exogenous H_2O_2 increases inotropic stimulations by α-adrenergic agonists or ET-1 in isolated rat atria.[75]

Hypertension also induces myocardial hypertrophy as noted in the SHR,[68,76] Dahl salt-sensitive,[67] NO depleted,[73] or Ang II-infused rats.[77] In this latter case, hypertrophy is blocked by a statin, a drug known to inhibit NAD(P)H oxidase activation, suggesting that it is due to ROS. Hypertrophy is frequently associated with fibrosis, as in the DOCA-salt[78] or NO-depleted rat.[73,74,76] The fact that hypertrophy is decreased by angiotensin-converting enzyme inhibitors in the SHR, but not when NOS is blocked, suggests that Ang II and NOS are antagonistic forces in myocardium growth, presumably through the superoxide/NO balance.[76] It can be argued that hypertrophy is a reaction to increased BP, independent of ROS. However, equivalent increases in BP are produced in rats by a 10-day infusion of either a low dose of Ang II with normal sodium, or a high dose of Ang II with a low-sodium diet. In the second group, myocardial superoxide is reduced and hypertrophy abolished, suggesting that remodeling is due to ROS, rather than pressure.[79] Similarly, cardiac hypertrophy is reduced in aortic-banded guinea pigs by administration of angiotensin-converting enzyme inhibitors or vitamin C, without reduction of BP.[80]

In summary, current evidence suggests that the superoxide/NO balance is an important factor in maintaining heart function. In the short term, ROS can increase BP by stimulation of heart rate and force. Reactive oxygen species may also contribute to chronic hypertension by inducing myocardial hypertrophy. In extreme cases, oxidative stress may be responsible for the appearance of stenotic lesions and fibrosis.

Kidneys

The kidneys are important BP regulators that are also likely targets of ROS. In hypertension, renal ROS and oxidation markers are elevated,[1] urinary NO_x are decreased,[3,14] and associated tissue damage[6] results in protein excretion. These alterations are improved by treatment with tempol[6,14] or antioxidants. Furthermore, in experimental hypertension, xanthine oxidase activity[3] and the NAD(P)H oxidase subunits nox1, gp91phox, p22phox, and p47phox, are upregulated.[2,14] In addition, SODs are inactivated[1] or downregulated.[2,14,15]

Functional studies indicate that superoxide increases chloride reabsorption in isolated thick ascending limbs of the loop of Henle.[22] Infusion of ROS generators and inhibitors through a catheter implanted in the renal medullary interstitium shows that H_2O_2 decreases blood and urine flows, and inhibits sodium excretion.[23] In hypertension, nNOS activity is impaired and the effect of NO donors is reduced,[29] leading to increased tubuloglomerular feedback, decreased renal blood flow, and glomerular filtration.[10,29] These alterations are improved or normalized by tempol, suggesting that superoxide consumes NO and uncouples nNOS, which is required to maintain perfusion. Overall, these functional changes cooperatively increase BP acutely, whereas long-term hypertension is likely due to tissue damage and remodeling.

Central Nervous System

Blood pressure is regulated centrally by control of efferent sympathetic activity. Responsible structures include the hypothalamus and nuclei of the lower brain stem, such as the rostral ventrolateral medulla (RVLM) and the nucleus of the solitary tract (NTS), which controls baroreflex pathways. The NO generated by neurons in these nuclei decreases sympathetic activity, thus tonically lowering BP. Nitric oxide also decreases the formation of Ang II, itself responsible for stimulation of sympathetic activity, vasopressin production, and drinking behavior.[81]

Given this pressure-lowering effect of NO, production of ROS in the central nervous system could contribute to hypertension. Administration of H_2O_2 to the thoracic spine in rats, produces an increase in heart rate and BP inhibited by the antioxidant *N*-acetyl cysteine.[82] This is consistent with the downregulation of endogenous antioxidants observed in SHR brain. MnSOD is affected in all areas and CuZnSOD in various structures, including the hypothalamus.[83] Furthermore, microinjection of SOD into the RVLM of nitrate-tolerant pigs decreases sympathetic activity by 70%,[81] and intracerebroventricular injection of SOD-expressing adenoviruses inhibits increases in heart rate, BP, and drinking behavior induced by Ang II in mice.[32] Similarly, iNOS is downregulated in SHR, leading to a decreased inhibitory effect of arginine injection into the RVLM. This downregulation, present in young prehypertensive animals, persists in adults with antihypertensive treatment, suggesting that it may be a cause of the dis-

ease.[84] This view is supported by the fact that adenoviral-mediated overexpression of eNOS in the NTS of SHR decreases superoxide formation and BP.[85]

Therefore, in the brain as in other tissues, the superoxide/NO balance appears to be an important factor in the control of BP. Hypertension may be secondary to excessive central Ang II stimulation, or due to a loss of NOS or antioxidant enzymes.

Conclusion

The evidence presented makes a convincing case that ROS are an intrinsic part of the pathology of hypertension. They contribute to the disease through multiple mechanisms, involving the vasculature, heart, kidneys, and central nervous system. Their effects are amplified by positive feedback loops such as inactivation of MnSOD by peroxynitrite; NOS uncoupling; self-reinforced synthesis of ET-1; and pressure-induced ROS formation. Furthermore, ROS induce vascular remodeling and kidney damage that may lead to chronic hypertension. Antioxidant therapy can curtail the development of hypertension in animal models, but remains controversial in humans. Possible confounding factors in patients include co-existing pathologies and treatments, lack of selection of treatments according to ROS levels, and unproven or insufficient antioxidant effect of vitamin supplements. However, our expanding knowledge regarding the sources, triggering mechanisms, and effects of ROS provides a rationale for preventive monitoring in humans and offers new targets for future antihypertensive therapy.[64,65]

References

1. Guo W, Adachi T, Matsui R, Xu S, Jiang B, Zou MH, Kirber M, Lieberthal W, Cohen RA: Quantitative assessment of tyrosine nitration of manganese superoxide dismutase in angiotensin II-infused rat kidney. Am J Physiol Heart Circ Physiol 2003;285:H1396–1403.
2. Chabrashvili T, Kitiyakara C, Blau J, Karber A, Aslam S, Welch WJ, Wilcox CS: Effects of ANG II type 1 and 2 receptors on oxidative stress, renal NADPH oxidase, and SOD expression. Am J Physiol Regul Integr Comp Physiol 2003;285:R117–R124.
3. Mervaala EM, Cheng ZJ, Tikkanen I, Lapatto R, Nurminen K, Vapaatalo H, Muller DN, Fiebeler A, Ganten U, Ganten D, Luft FC: Endothelial dysfunction and xanthine oxidoreductase activity in rats with human renin and angiotensinogen genes. Hypertension 2001; 37:414–418.
4. Cosentino F, Patton S, d'Uscio LV, Werner ER, Werner-Felmayer G, Moreau P, Malinski T, Luscher TF: Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats. J Clin Invest 1998;101:1530–1537.
5. Lassègue B, Clempus RE: Vascular NAD(P)H oxidases: specific features, expression, and regulation. Am J Physiol Regul Integr Comp Physiol 2003;285:R277–R297.
6. Hoagland KM, Maier KG, Roman RJ: Contributions of 20-HETE to the antihypertensive effects of Tempol in Dahl salt-sensitive rats. Hypertension 2003;41:697–702.
7. Lenda DM, Boegehold MA: Effect of a high-salt diet on oxidant enzyme activity in skeletal muscle microcirculation. Am J Physiol Heart Circ Physiol 2002;282:H395–H402.
8. Li L, Fink GD, Watts SW, Northcott CA, Galligan JJ, Pagano PJ, Chen AF: Endothelin-1 increases vascular superoxide via endothelin(A)-NADPH oxidase pathway in low-renin hypertension. Circulation 2003;107:1053–1058.
9. Li L, Watts SW, Banes AK, Galligan JJ, Fink GD, Chen AF: NADPH oxidase-derived superoxide augments endothelin-1-induced venoconstriction in mineralocorticoid hypertension. Hypertension 2003;42:316–321.
10. Sedeek MH, Llinas MT, Drummond H, Fortepiani L, Abram SR, Alexander BT, Reckelhoff JF, Granger JP: Role of reactive oxygen species in endothelin-induced hypertension. Hypertension 2003;42: 806–810.
11. Li L, Galligan JJ, Fink GD, Chen AF: Vasopressin induces vascular superoxide via endothelin-1 in mineralocorticoid hypertension. Hypertension 2003;41:663–668.
12. 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 2003;111:1201–1209.
13. Zheng JS, Yang XQ, Lookingland KJ, Fink GD, Hesslinger C, Kapatos G, Kovesdi I, Chen AF: Gene transfer of human guanosine 5′-triphosphate cyclohydrolase I restores vascular tetrahydrobiopterin level and endothelial function in low renin hypertension. Circulation 2003;108:1238–1245.
14. Vaziri ND, Dicus M, Ho ND, Boroujerdi-Rad L, Sindhu RK: Oxidative stress and dysregulation of superoxide dismutase and NADPH oxidase in renal insufficiency. Kidney Int 2003;63:179–185.
15. Awe SO, Tsakadze NL, D'Souza SE, Adeagbo AS: Tert-butyl hydroperoxide-mediated vascular responses in DOCA-salt hypertensive rats. Vascul Pharmacol 2003;40:51–57.
16. 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 2003;124:811–817.
17. Maffei A, Poulet R, Vecchione C, Colella S, Fratta L, Frati G, Trimarco V, Trimarco B, Lembo G: Increased basal nitric oxide release despite enhanced free radical production in hypertension. J Hypertens 2002;20:1135–1142.
18. Ulker S, McMaster D, McKeown PP, Bayraktutan U: Impaired activities of antioxidant enzymes elicit endothelial dysfunction in spontaneous hypertensive rats despite enhanced vascular nitric oxide generation. Cardiovasc Res 2003;59:488–500.
19. Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, Schiffrin EL: Spironolactone improves angiotensin-induced vascular changes and oxidative stress. Hypertension 2002;40:504–510.
20. Taddei S, Virdis A, Ghiadoni L, Magagna A, Pasini AF, Garbin U, Cominacini L, Salvetti A: Effect of calcium antagonist or β blockade treatment on nitric oxide-dependent vasodilation and oxidative stress in essential hypertensive patients. J Hypertens 2001;19:1379–1386.
21. Girouard H, Chulak C, Wu L, Lejossec M, de Champlain J: N-acetylcysteine improves nitric oxide and α-adrenergic pathways in mesenteric beds of spontaneously hypertensive rats. Am J Hypertens 2003;16:577–584.
22. Ortiz PA, Garvin JL: Superoxide stimulates NaCl absorption by the thick ascending limb. Am J Physiol Renal Physiol 2002;283:F957–F962.
23. Makino A, Skelton MM, Zou AP, Cowley AW Jr: Increased renal medullary H_2O_2 leads to hypertension. Hypertension 2003;42:25–30.
24. Makino A, Skelton MM, Zou AP, Roman RJ, Cowley AW Jr: Increased renal medullary oxidative stress produces hypertension. Hypertension 2002;39:667–672.
25. Didion SP, Ryan MJ, Didion LA, Fegan PE, Sigmund CD, Faraci FM: Increased superoxide and vascular dysfunction in CuZnSOD-deficient mice. Circ Res 2002;91:938–944.

26. 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 2003;93:622–629.
27. Ganafa AA, Socci RR, Eatman D, Silvestrova N, Abukhalaf IK, Bayorh MA: Acute inhibition of glutathione biosynthesis alters endothelial function and blood pressure in rats. Eur J Pharmacol 2002;454:217–223.
28. Kawada N, Imai E, Karber A, Welch WJ, Wilcox CS: A mouse model of angiotensin II slow pressor response: role of oxidative stress. J Am Soc Nephrol 2002;13:2860–2868.
29. Welch WJ, Wilcox CS: AT1 receptor antagonist combats oxidative stress and restores nitric oxide signaling in the SHR. Kidney Int 2001;59:1257–1263.
30. Chen X, Touyz RM, Park JB, Schiffrin EL: Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. Hypertension 2001;38:606–611.
31. Chu Y, Iida S, Lund DD, Weiss RM, DiBona GF, Watanabe Y, Faraci FM, Heistad DD: Gene transfer of extracellular superoxide dismutase reduces arterial pressure in spontaneously hypertensive rats: role of heparin-binding domain. Circ Res 2003;92:461–468.
32. Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR, Davisson RL: Superoxide mediates the actions of angiotensin II in the central nervous system. Circ Res 2002;91:1038–1045.
33. Yang H, Shi M, VanRemmen H, Chen X, Vijg J, Richardson A, Guo Z: Reduction of pressor response to vasoconstrictor agents by overexpression of catalase in mice. Am J Hypertens 2003;16:1–5.
34. Barton CH, Ni Z, Vaziri ND: Enhanced nitric oxide inactivation in aortic coarctation-induced hypertension. Kidney Int 2001;60:1083–1087.
35. 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 2002;90:1205–1213.
36. Gragasin FS, Xu Y, Arenas IA, Kainth N, Davidge ST: Estrogen reduces angiotensin II-induced nitric oxide synthase and NAD(P)H oxidase expression in endothelial cells. Arterioscler Thromb Vasc Biol 2003;23:38–44.
37. 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 2003;23:981–987.
38. Li JM, Shah AM: Mechanism of endothelial cell NADPH oxidase activation by angiotensin II. Role of the p47phox subunit. J Biol Chem 2003;278:12094–12100.
39. Grote K, Flach I, Luchtefeld M, Akin E, Holland SM, Drexler H, Schieffer B: Mechanical stretch enhances mRNA expression and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species. Circ Res 2003;92:e80–e86.
40. Touyz RM, Schiffrin EL: Increased generation of superoxide by angiotensin II in smooth muscle cells from resistance arteries of hypertensive patients: role of phospholipase D-dependent NAD(P)H oxidase-sensitive pathways. J Hypertens 2001;19:1245–1254.
41. Brennan LA, Steinhorn RH, Wedgwood S, Mata-Greenwood E, Roark EA, Russell JA, Black SM: Increased superoxide generation is associated with pulmonary hypertension in fetal lambs: a role for NADPH oxidase. Circ Res 2003;92:683–691.
42. Moreno MU, San Jose G, Orbe J, Paramo JA, Beloqui O, Diez J, Zalba G: Preliminary characterisation of the promoter of the human p22(phox) gene: identification of a new polymorphism associated with hypertension. FEBS Lett 2003;542:27–31.
43. Ortiz PA, Garvin JL: Cardiovascular and renal control in NOS-deficient mouse models. Am J Physiol Regul Integr Comp Physiol 2003;284:R628–R638.
44. Kuzkaya N, Weissmann N, Harrison DG, Dikalov S: Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. J Biol Chem 2003;278:22546–22554.
45. 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 2001;21:496–502.
46. Mitchell BM, Dorrance AM, Webb RC: GTP cyclohydrolase 1 inhibition attenuates vasodilation and increases blood pressure in rats. Am J Physiol Heart Circ Physiol 2003;285:H2165–H2170.
47. Podjarny E, Benchetrit S, Rathaus M, Pomeranz A, Rashid G, Shapira J, Bernheim J: Effect of tetrahydrobiopterin on blood pressure in rats after subtotal nephrectomy. Nephron Physiol 2003;94:6–9.
48. El Midaoui A, Elimadi A, Wu L, Haddad PS, de Champlain J: Lipoic acid prevents hypertension, hyperglycemia, and the increase in heart mitochondrial superoxide production. Am J Hypertens 2003;16:173–179.
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 2003;285:H2290–H2297.
50. Lariviere R, Thibault G, Schiffrin EL: Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats. Hypertension 1993;21:294–300.
51. Lopez-Ongil S, Saura M, Zaragoza C, Gonzalez-Santiago L, Rodriguez-Puyol M, Lowenstein CJ, Rodriguez-Puyol D: Hydrogen peroxide regulation of bovine endothelin-converting enzyme-1. Free Radic Biol Med 2002;32:406–413.
52. Intengan HD, He G, Schiffrin EL: Effect of vasopressin antagonism on structure and mechanics of small arteries and vascular expression of endothelin-1 in deoxycorticosterone acetate salt hypertensive rats. Hypertension 1998;32:770–777.
53. Rajagopalan S, Laursen JB, Borthayre A, Kurz S, Keiser J, Haleen S, Giaid A, Harrison DG: Role for endothelin-1 in angiotensin II-mediated hypertension. Hypertension 1997;30:29–34.
54. Gonzalez-Pacheco FR, Caramelo C, Castilla MA, Deudero JJ, Arias J, Yague S, Jimenez S, Bragado R, Alvarez-Arroyo MV: Mechanism of vascular smooth muscle cells activation by hydrogen peroxide: role of phospholipase C gamma. Nephrol Dial Transplant 2002;17:392–398.
55. Grover AK, Samson SE, Robinson S, Kwan CY: Effects of peroxynitrite on sarcoplasmic reticulum Ca^{2+} pump in pig coronary artery smooth muscle. Am J Physiol Cell Physiol 2003;284:C294–C301.
56. Kimura C, Cheng W, Hisadome K, Wang YP, Koyama T, Karashima Y, Oike M, Ito Y: Superoxide anion impairs contractility in cultured aortic smooth muscle cells. Am J Physiol Heart Circ Physiol 2002;283:H382–H390.
57. Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, Gutterman DD: Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. Circ Res 2003;92:e31–e40.
58. Lacza Z, Puskar M, Kis B, Perciaccante JV, Miller AW, Busija DW: Hydrogen peroxide acts as an EDHF in the piglet pial vasculature in response to bradykinin. Am J Physiol Heart Circ Physiol 2002;283:H406–H411.
59. Cai H, Li Z, Davis ME, Kanner W, Harrison DG, Dudley SC Jr: Akt-dependent phosphorylation of serine 1179 and mitogen-activated protein kinase kinase/extracellular signal-regulated kinase 1/2 cooperatively mediate activation of the endothelial nitric-oxide synthase by hydrogen peroxide. Mol Pharmacol 2003;63:325–331.

60. Shimizu S, Shiota K, Yamamoto S, Miyasaka Y, Ishii M, Watabe T, Nishida M, Mori Y, Yamamoto T, Kiuchi Y: Hydrogen peroxide stimulates tetrahydrobiopterin synthesis through the induction of GTP-cyclohydrolase I and increases nitric oxide synthase activity in vascular endothelial cells. Free Radic Biol Med 2003;34:1343–1352.
61. Wedgwood S, Black SM: Induction of apoptosis in fetal pulmonary arterial smooth muscle cells by a combined superoxide dismutase/catalase mimetic. Am J Physiol Lung Cell Mol Physiol 2003;285: L305–L312.
62. Saito S, Frank GD, Mifune M, Ohba M, Utsunomiya H, Motley ED, Inagami T, Eguchi S: Ligand-independent trans-activation of the platelet-derived growth factor receptor by reactive oxygen species requires protein kinase C-delta and c-Src. J Biol Chem 2002;277: 44695–44700.
63. Iglarz M, Touyz RM, Amiri F, Lavoie MF, Diep QN, Schiffrin EL: Effect of peroxisome proliferator-activated receptor-alpha and -gamma activators on vascular remodeling in endothelin-dependent hypertension. Arterioscler Thromb Vasc Biol 2003;23:45–51.
64. Galis ZS, Khatri JJ: Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002;90:251–262.
65. Frank GD, Mifune M, Inagami T, Ohba M, Sasaki T, Higashiyama S, Dempsey PJ, Eguchi S: Distinct mechanisms of receptor and nonreceptor tyrosine kinase activation by reactive oxygen species in vascular smooth muscle cells: role of metalloprotease and protein kinase C-delta. Mol Cell Biol 2003;23:1581–1589.
66. Li J, Li PF, Dietz R, von Harsdorf R: Intracellular superoxide induces apoptosis in VSMCs: role of mitochondrial membrane potential, cytochrome C and caspases. Apoptosis 2002;7:511–517.
67. Somova LI, Nadar A, Gregory M, Khan N: Antioxidant status of the hypertrophic heart of Dahl hypertensive rat as a model for evaluation of antioxidants. Methods Find Exp Clin Pharmacol 2001;23:5–12.
68. Csonka C, Pataki T, Kovacs P, Muller SL, Schroeter ML, Tosaki A, Blasig IE: Effects of oxidative stress on the expression of antioxidative defense enzymes in spontaneously hypertensive rat hearts. Free Radic Biol Med 2000;29:612–619.
69. Carlos DM, Goto S, Urata Y, Iida T, Cho S, Niwa M, Tsuji Y, Kondo T: Nicardipine normalizes elevated levels of antioxidant activity in response to xanthine oxidase-induced oxidative stress in hypertensive rat heart. Free Radic Res 1998;29:143–150.
70. Bello-Klein A, Bock PM, Travacio M, Senna SM, Llesuy S, de Bittencourt PI Jr, Irigoyen MC, Bello AA, Kumar D, Singal PK: Myocardial oxidative stress and antioxidants in hypertension as a result of nitric oxide synthase inhibition. Cardiovasc Toxicol 2001; 1:43–50.
71. Husain K, Hazelrigg SR: Oxidative injury due to chronic nitric oxide synthase inhibition in rat: effect of regular exercise on the heart. Biochim Biophys Acta 2002;1587:75–82.
72. Pinelli A, Trivulzio S, Tomasoni L, Bertolini B, Brenna S, Bonacina E, Accinni R: Drugs modifying nitric oxide metabolism affect plasma cholesterol levels, coagulation parameters, blood pressure values and the appearance of plasma myocardial necrosis markers in rabbits: opposite effects of L-NAME and nitroglycerine. Cardiovasc Drugs Ther 2003;17:15–23.
73. Pacca SR, de Azevedo AP, De Oliveira CF, De Luca IM, De Nucci G, Antunes E: Attenuation of hypertension, cardiomyocyte hypertrophy, and myocardial fibrosis by beta-adrenoceptor blockers in rats under long-term blockade of nitric oxide synthesis. J Cardiovasc Pharmacol 2002;39:201–207.
74. Sampaio RC, Tanus-Santos JE, Melo SE, Hyslop S, Franchini KG, Luca IM, Moreno H Jr: Hypertension plus diabetes mimics the cardiomyopathy induced by nitric oxide inhibition in rats. Chest 2002;122:1412–1420.
75. Sand C, Peters SL, Pfaffendorf M, van Zwieten PA: The influence of endogenously generated reactive oxygen species on the inotropic and chronotropic effects of adrenoceptor and ET-receptor stimulation. Naunyn Schmiedebergs Arch Pharmacol 2003;367:635–639.
76. de Andrade Zorzi RL, Meirelles Pereira LM, Mandarim-de-Lacerda CA: Beneficial effect of enalapril in spontaneously hypertensive rats cardiac remodeling with nitric oxide synthesis blockade. J Cell Mol Med 2002;6:599–608.
77. Delbosc S, Cristol JP, Descomps B, Mimran A, Jover B: Simvastatin prevents angiotensin II-induced cardiac alteration and oxidative stress. Hypertension 2002;40:142–147.
78. Millette E, Demeilliers B, Wu R, Laplante MA, El Midaoui A, Moreau P, Lamontagne D, de Champlain J: Comparison of the cardiovascular protection by omapatrilat and lisinopril treatments in DOCA-salt hypertension. J Hypertens 2003;21:125–135.
79. Rugale C, Delbosc S, Cristol JP, Mimran A, Jover B: Sodium restriction prevents cardiac hypertrophy and oxidative stress in angiotensin II hypertension. Am J Physiol Heart Circ Physiol 2003; 284:H1744–H1750.
80. Bell JP, Mosfer SI, Lang D, Donaldson F, Lewis MJ: Vitamin C and quinapril abrogate LVH and endothelial dysfunction in aortic-banded guinea pigs. Am J Physiol Heart Circ Physiol 2001;281: H1704–H1710.
81. Zanzinger J: Mechanisms of action of nitric oxide in the brain stem: role of oxidative stress. Auton Neurosci 2002;98:24–27.
82. Lin HH, Chen CH, Hsieh WK, Chiu TH, Lai CC: Hydrogen peroxide increases the activity of rat sympathetic preganglionic neurons in vivo and in vitro. Neuroscience 2003;121:641–647.
83. Chan P, Liao SS, Hsu CT, Lee YS, Tomlinson B, Kuo JS, Cheng JT: Superoxide dismutase gene expression and activity in the brain of spontaneously hypertensive rats and normotensive rats. Chin Med J (Engl) 1999;112:1119–1124.
84. Chan JY, Wang LL, Chao YM, Chan SH: Downregulation of basal iNOS at the rostral ventrolateral medulla is innate in SHR. Hypertension 2003;41:563–570.
85. Tai MH, Hsiao M, Chan JY, Lo WC, Wang FS, Liu GS, Howng SL, Tseng CJ: Gene delivery of endothelial nitric oxide synthase into nucleus tractus solitarii induces biphasic response in cardiovascular functions of hypertensive rats. Am J Hypertens 2004;17:63–70.