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Oxidative Stress and Hypertensive Diseases

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Introduction

It has become clear that reactive oxygen species (ROS) contribute to the development of hypertension via myriad effects. ROS are essential for normal cell function, however they mediate pathological changes in the brain, the kidney and blood vessels that contribute to the genesis of chronic hypertension. There is also emerging evidence that ROS contribute to immune activation in hypertension. In this review, we will discuss these events and how they coordinate to contribute to hypertension and its consequent end-organ damage.

Reactive oxygen species

Reactive oxygen species (ROS) are formed by oxidation-reduction reactions in which one molecule is reduced by removal of an electron, which is then transferred to a recipient molecule. ROS can be divided into two major groups: free radicals and non-radical derivatives. Free radicals possess an unpaired electron in their outer orbital, which make them highly reactive. These include superoxide ($O_2^{\cdot-}$), the hydroxyl radical ($OH\cdot$), lipid peroxy-radicals ($LOO\cdot$) and alkoxy-radicals ($LO\cdot$). Nitric oxide (NO) is also a free radical, and often referred to as a reactive nitrogen species. Non-radical ROS include hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), hypochlorous acid ($HOCl$) and reactive carbonyls. These do not possess unpaired electrons, and are more stable with a longer half-life but have strong oxidant properties.

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Physiological roles of ROS

Although originally considered toxic by-products of cellular metabolism, ROS are now recognized to have signaling roles that are critical for normal cell function, including proliferation, differentiation, aging, host defense and repair processes. Recent studies show that ROS, including H_2O_2 , may drive pro-survival signaling and protect from the aging process.¹ As a part of innate immunity, ROS not only contribute to host defense via respiratory bursts in phagocytes, but also by signaling chemotaxis of inflammatory cells to sites of infection or injury. Related to this, ROS also participate in tissue repair and remodeling by inducing expression of matrix metalloproteinases (MMPs).² These responses, which are vital for normal cell function, become exaggerated in disease states and promote pathological processes.

Oxidative stress

The term oxidative or oxidant stress traditionally refers to an imbalance between the production of reactive oxygen species and antioxidant defenses. This can lead to an increase in ambient levels of ROS that can damage various cellular components including DNA, proteins and lipids. This traditional definition of oxidant stress has been modified, because it is now clear that such an imbalance might be localized to subcellular compartments such as the mitochondria, the nucleus or localized at the cellular membrane. Localized alterations of ROS production in the mitochondria can affect energy homeostasis, while localized ROS production in the nucleus can affect transcriptional events and epigenetic control. Extracellular ROS can participate in outside in signaling and affect cellular function.

Major ROS molecules

Superoxide radical

Superoxide, produced by 1-electron reduction of molecular oxygen, can act both as an oxidant and as a reductant in biological systems, depending on the redox potential of the molecule with which it is reacting. Superoxide is important as it serves as the progenitor for many other biologically relevant ROS, including hydrogen peroxide (H_2O_2), the hydroxyl radical ($\text{HO}\cdot$) and peroxynitrite (OONO^-), which forms upon reaction of $\text{O}_2^{\cdot-}$ with NO.

Hydrogen Peroxide

Hydrogen peroxide is formed by dismutation of $\text{O}_2^{\cdot-}$, which can occur either spontaneously or can be catalyzed by the superoxide dismutases (SODs). In contrast to $\text{O}_2^{\cdot-}$, H_2O_2 is relatively stable under physiological conditions. Because it is uncharged and lipophilic, H_2O_2 can readily diffuse across membranes and thus can react with targets in organelles and cells apart from where it is formed. In this regard, H_2O_2 has been implicated as a signaling molecule that can, among other actions, promote vasodilatation, activate gene transcription, modify phosphatase activity and activate other sources of ROS. As part of the antioxidant defense mechanisms, catalase and glutathione peroxidase (Gpx) can further reduce H_2O_2 to H_2O . Myeloperoxidase catalyzes the reaction of H_2O_2 with the chloride ion to generate hypochlorous acid (HOCl), which is a strong oxidant with high reactivity. Other

peroxidases use alternate anions to generate other oxidants. Some of these reactions are illustrated in Figure 1.

Hydroxyl radical

Hydroxyl is formed when $O_2^{\cdot-}$ reacts with H_2O_2 in the Haber-Weiss reaction where $O_2^{\cdot-}$ donates one electron to H_2O_2 . Hydrogen peroxide can also accept one electron from the Ferrous cation (Fe^{2+}) in the Fenton reaction to generate OH. and a Ferric cation (Fe^{3+}). The hydroxyl radical is a highly reactive oxidant that can attack a variety of biomolecules including lipids, proteins and DNA.

Peroxynitrite

As mentioned above, $OONO^-$ is the product of the spontaneous reaction between $O_2^{\cdot-}$ and NO. This reaction is essentially diffusion limited, with a rate that has been estimated to be $9 \times 10^9 \text{ mol} \times \text{sec}^{-1}$. At physiological pH, $OONO^-$ exists in the protonated form, HOONO or peroxynitrous acid, which is uncharged and can diffuse across cell membranes. Moreover, HOONO undergoes homolysis to yield hydroxyl, and in fact might serve as a more important source of this radical than the Fenton reaction mentioned above. Like hydroxyl, $OONO^-$ is a very strong oxidant and can react with lipids, DNA and proteins. Peroxynitrite can react with and modify proteins and other cellular structures causing oxidative damage to these macromolecules. In particular, $OONO^-$ modifies protein tyrosine residues to form 3-nitrotyrosine, a biomarker for $OONO^-$ in tissues and blood.

Reactive carbonyls

Carbonyls are highly reactive molecules that contain a carbon atom double bonded to an oxygen atom ($C=O$). These are formed both enzymatically and non-enzymatically from lipids, sugars and proteins, and include reactive aldehydes and advanced glycation end products. These have been extensively reviewed elsewhere,³ but are highly reactive and can modify macromolecules such as proteins and DNA, forming cross-links and altering both structure and function. Reactive carbonyls accumulate with aging, increased oxidative stress and in the setting of various diseases, and likely underlie the pathogenesis of these diseases. An example is isolevuloglandin-modification of lysines, which as noted below, produces protein modifications that lead to immune activation in hypertension.⁴

Sources of ROS

NADPH oxidase

The NADPH oxidases are major sources of ROS in mammalian cells. The NADPH oxidase was first identified in professional phagocytes as a multi-subunit enzyme complex responsible for the oxidative burst used to kill invading micro-organisms. The catalytic subunit of the neutrophil oxidase is also referred to as gp91^{phox}, due to its apparent molecular weight and the fact that it is extensively glycosylated. Subsequently, it was discovered that there are 6 related proteins, which have been named the Nox enzymes, termed Nox 1 to 5 and the two Duox enzymes. These vary in tissue distribution, function and regulatory mechanisms. Nox1 exists in colon, muscle, prostate, uterus and blood vessels and plays important roles in host defense and blood pressure regulation. As noted above,

Nox2 is found in phagocytes, where it is responsible for the oxidative burst. It is also present in endothelial cells, tubular cells of the kidney and in other immune cells. Nox3 is present in fetal tissue and in the inner ear where it is essential for vestibular function. Nox4, expressed in kidneys, vessels and bone, is involved in vasoregulation and erythropoietin synthesis. Nox5, not present in rodents, is a Ca^{2+} -dependent homolog that is activated in response to intracellular Ca^{2+} mobilization in lymph nodes, testes and blood vessels. Nox5 is also expressed in atherosclerotic lesions and seems to accumulate in more complex lesions.⁵ Duox1/2 are distant Nox homologues involved in hormone biosynthesis in the thyroid.

Nox 1 through 4 require the small docking subunit p22^{phox} for function and stability. In addition the various Nox enzymes are activated upon translocation of cytoplasmic subunits. For Nox2, these include p47^{phox}, p67^{phox}, p40^{phox} and the small G protein rac. For Nox1, alternate subunits, termed NoxA1 and NoxO1 can substitute for p47^{phox} and p67^{phox}. Poldip2 associates with Nox4 and positively regulates its activity and cellular functions.⁶ Factors that cause assembly of these cytoplasmic units with the membrane components include inflammatory cytokines, growth factors, mechanical forces and various G protein coupled receptor agonists. Of particular importance to cardiovascular disease, ang II activates the NADPH oxidases via the AT1 receptor and stimulation of a signaling pathway involving c-Src, protein kinase C (PKC), phospholipase D (PLD) and phospholipase A₂ (PLA₂).⁷

The NADPH oxidases utilize a Fe^{2+} containing heme group in their catalytic center as a means of electron transfer. This center should be able to only perform one-electron reductions of oxygen, and thus the major initial product of these enzymes should be $\text{O}_2^{\cdot-}$. Surprisingly, the most measurable product of Nox4 and the Duox enzymes seems to be H_2O_2 . In the case of Nox4, this has been attributed to structural characteristics that retard the release of $\text{O}_2^{\cdot-}$ until it spontaneously dismutates to H_2O_2 .⁸ We have also found that Nox5 seems to predominately release H_2O_2 .⁵

Studies of rodents lacking or overexpressing components of the NADPH oxidase have been most revealing in demonstrating a role of these enzymes in hypertension. Mice lacking p47^{phox}, a cytosolic component essential for activation of Nox2 are protected against ang II and DOCA-salt hypertension.^{9, 10} Overexpression p22^{phox}, the small docking subunit of the Nox enzymes in vascular smooth muscle enhanced the hypertensive response to ang II aging. Mice overexpressing Nox1 develop augmented hypertension when given a chronic infusion of ang II, and mice lacking this enzyme are protected against ang II-induced hypertension.^{11, 12} Recently, Nox4 has been shown to play a role in salt-sensitive hypertension in rats.¹³ The role of Nox4 of modulating renal sodium transport is discussed more completely below. Overexpression of human Nox5 in the podocytes of increases albuminuria and causes a proportional increase in baseline blood pressure.¹⁴

Nitric oxide synthase uncoupling and tetrahydrobiopterin

The nitric oxide synthase (NOS) enzymes are the endogenous sources of NO in mammalian cells. NO has myriad effects on cardiovascular function, including modulation of vascular tone, blood pressure, sympathetic outflow, renal renin release and renal sodium excretion. In

the absence of their critical cofactor tetrahydrobiopterin (BH₄), or their substrate L-arginine, the NOS enzymes become uncoupled, such that they produce O₂^{•-} rather than NO. NOS uncoupling has been documented as a source of ROS in diseases such as hypertension, atherosclerosis, diabetes and following ischemia and reperfusion injury. In several of these diseases, oral supplementation of BH₄ reverses NOS uncoupling, and improves endothelial function. Importantly, oral BH₄ blunts the elevation of blood pressure in angiotensin II- and salt-induced hypertension in animals and has been shown to lower blood pressure in humans with hypertension.^{15–17} There seems to be interplay between overproduction of NO by inflammatory monocytes and uncoupling of the endothelial cell isoform of NOS.¹⁸ Treatment with a tetrahydrobiopterin precursor reduces the pressor response and increase in sympathetic outflow that occurs in response to hand grip in subjects with chronic kidney disease.¹⁹ Moreover, administration of BH₄ also prevents the development of endothelium dysfunction, vascular inflammation and atherosclerosis induced by disturbed flow.^{20, 21} Acute and short-term administration of BH₄ improves endothelial function in humans with rheumatoid arthritis.²² There is also convincing evidence that eNOS uncoupling occurs in humans with obstructive sleep apnea, leading to increased vascular superoxide production and endothelial dysfunction.²³ Thus, reversing NOS uncoupling is an attractive approach to improve endothelium function and prevent the pathogenesis of vascular diseases.

A major cause of NOS uncoupling is oxidation of tetrahydrobiopterin by oxidants such as peroxynitrite. Interestingly, ROS produced by the NADPH oxidase play a role in this process, and mice lacking the NADPH oxidase are protected against tetrahydrobiopterin oxidation in the setting of hypertension.¹⁰

Xanthine Oxidase

Xanthine oxidoreductase (XOR) is another important source of ROS in mammalian cells. XOR exists in two forms, including xanthine dehydrogenase (XDH), and xanthine oxidase (XO). XDH transfers electrons from hypoxanthine and xanthine to NAD⁺ yielding NADH and uric acid, whereas XO transfers electrons to oxygen from these same substrates to generate O₂^{•-} and H₂O₂. The cellular ratio of XO to XDH is therefore critical in modulating ROS production by these enzymes. XDH is converted to XO when a critical cysteine residue is oxidized by peroxynitrite.²⁴ This conversion is also favored in several pathophysiological settings including inflammation,²⁵ hypoxia,²⁶ and radiation exposure,²⁷ and likely contributes to increased ROS production and in these situations. XO has been shown to contribute to experimental hypertension in animal models,^{28–30} and there is accumulating evidence supporting a role of xanthine oxidase in human hypertension.^{31–33} While a recent study found no significant effect of the xanthine oxidase inhibitor allopurinol in blood pressure control in hypertensive humans,³⁴ a prior retrospective analysis of a large database showed that treatment with allopurinol was associated with a small (2.1 mmHg) reduction of systolic blood pressure.³⁵ A carefully controlled randomized trial showed that 4 weeks of treatment of adolescent with allopurinol reduced systolic pressure by 6 mmHg while the placebo group had no change in pressure.³⁵ These effects of allopurinol could be due to lowering of uric acid, which is known to activate the inflammasome, or due to inhibition of xanthine oxidase. In one study, allopurinol markedly lowered blood pressure in obese adolescents with pre-hypertension, while the uricosuric agent probenecid had no effect.³⁶

These findings implicate xanthine oxidase, and potential ROS derived from this enzyme, as having an important role in adolescent pre-hypertension.

Mitochondria

The mitochondria are responsible for the majority of ATP production in the cell. These organelles contain 5 enzyme complexes that comprise the electron transport chain. Electrons are transported sequentially from NADH through complexes I to V, the site of ATP generation. During normal mitochondrial function, this electron transfer from one complex to the next is efficient and there is minimal loss or leak from electron transport, however in various disease states, electron leak is increased and can lead to reduction of oxygen and formation of $O_2^{\cdot-}$ and H_2O_2 . Electron leak can occur at complexes I to IV, but occurs predominantly at complexes I and III due to defects in these complexes. These defects can include changes in their levels, post-translational modifications like glutathionylation or nitration. A recurring paradigm is that oxidative damage to mitochondrial DNA promotes deficiency in components of the electron transport chain, promoting electron leak. An important phenomenon is reverse electron transport, which occurs particularly in complex I in various pathophysiological states. Mitochondrial dysfunction is a major source of cellular ROS production in various pathophysiological states. Importantly, ROS from the NADPH oxidase have been shown to enter the mitochondria and promote electron leak and ROS production from the electron transport chain in hypertension.³⁷ Of note, antioxidants target the mitochondria and have proven effective in both preventing and reversing experimental hypertension.³⁸ Mitochondrial cyclophilin D (CypD) plays a critical role in opening of membrane transition pore, which depolarizes the mitochondria and enhances ROS production by this organelle. Recently it was found that pharmacological inhibition or deletion of CypD in mice prevents ang II induced hypertension.³⁹ Mitochondrial DNA fragments, indicative of mitochondrial damage, are increased in the urine of hypertensive humans and directly correlate with markers of renal injury in these individuals.⁴⁰

Antioxidant defense mechanisms

Antioxidants are molecules that prevent the oxidation of other molecules often by being oxidized themselves. Due to our oxygen rich atmosphere, all living organisms, including bacteria, plants and animals have adapted and developed enzymatic and non-enzymatic defenses against chronic oxidative stress. In mammalian cells, the major intracellular antioxidant enzymes include superoxide dismutase, catalase and glutathione peroxidase. As mentioned above, SOD catalyzes the dismutation of $O_2^{\cdot-}$ to H_2O_2 and molecular O_2 . Catalase and glutathione peroxidase further decompose H_2O_2 into H_2O and O_2 . Glutathione peroxidase requires glutathione as a co-substrate, yielding oxidized glutathione (GSSG) upon reaction. In addition to H_2O_2 , glutathione peroxidase also reduces lipid hydroperoxides to their respective alcohols and thus protects the cell membrane from lipid peroxidation. Glutathione peroxidase is also protective against $OONO^-$, which it efficiently reduces to nitrite.⁴¹ Unlike $O_2^{\cdot-}$ or H_2O_2 , there are no enzymatic antioxidants that scavenge hydroxyl, but a variety of non-enzymatic antioxidants including vitamin C (ascorbic acid), vitamin E (α -tocopherol) and glutathione react with and eliminate this radical.

Mammalian cells have the capacity to modulate their antioxidant levels by transcriptional modulators such as Nrf2 and DJ-1. The former is a transcription factor that binds to the antioxidant response element of many genes, including the glutathione-S transferases, heme oxygenase 1 and glutamate-cysteine ligase which controls glutathione synthesis.⁴² Nrf2 is activated in the setting of oxidative stress, and moves to the nucleus to induce these and other important antioxidant genes. DJ-1 is a recently discovered protein that has antioxidant properties, and translocates to either the nucleus or the mitochondria upon oxidative stress.⁴³ As discussed below, DJ-1 plays a major role in the response of proximal tubular cells to dopamine, and ultimately regulates blood pressure.⁴⁴

Limitations of Antioxidant Therapy

Although basic studies have strongly supported a role of ROS in cell dysfunction and animal models of disease, clinical trials with high dose antioxidants have been disappointing. Numerous large clinical trials have failed to show beneficial effects of either vitamin C or vitamin E supplementation in cancer, cardiovascular disease and neurodegenerative diseases.⁴⁵ A very recent meta-analysis of 50 randomized trials including almost 300,000 patients confirmed the futility of treatment with a variety of antioxidants in cardiovascular disease.⁴⁶ In hypertensive subjects, a few small trials initially showed benefit,^{47,48} but, later large clinical trials, including the SU.VI.MAX study, showed no improvement in blood pressure with antioxidant therapy.^{49, 50} Surprisingly, large doses of beta-carotene, vitamin A and vitamin E have paradoxically worsened cardiovascular outcomes in some studies.^{51, 52} The failure of antioxidants in humans might reflect the low rate constant of vitamins such as E and C with superoxide and related ROS, the inability to target subcellular sites where ROS are formed, and the fact that some ROS have beneficial effects. Prevention of ROS generation by inhibiting specific enzymatic sources might be more efficient than nonspecific antioxidants such as vitamin C and E. In this regard, a recent study showed that a Nox 1 inhibitor is effective in reducing atherosclerosis in mice with experimental diabetes.⁵³ It is also possible that these trials were negative because they did not target patients that actually have oxidative stress. As an example, the effect of vitamin E vs. placebo was studied on cardiovascular outcomes in almost 1500 middle-aged diabetic patients with the haptoglobin 2/2 genotype.⁵⁴ The 2/2 genotype is associated with a reduction of haptoglobin's antioxidant properties and therefore these patients were deemed to be at risk for oxidative stress. Vitamin E reduced all cardiovascular events, myocardial infarction and stroke by 50% in this population. Because of this striking benefit of vitamin E, the study was stopped prematurely. It is also possible that more potent, catalytic agents such as Tempol or targeted agents such as mitoTempol, which scavenges radicals specifically in the mitochondria, would have greater efficacy. This topic has been reviewed recently.⁵⁵

Another drawback to the use of individual antioxidants is that individual agents are unlikely able to replenish deficiencies in multiple endogenous antioxidants that might be encountered in a disease like hypertension or caused by long-standing dietary indiscretion. For example, treatment with vitamin E would not be expected to restore selenium or ascorbate levels. In contrast, consumption of a diet rich in antioxidants might have a beneficial effect. As an example, the dietary approach to stop hypertension (DASH) study showed that a diet high in fruits and vegetables had a striking benefit in control of blood pressure.⁵⁶ Likewise a recent

study showed that grape seed extract, which contains numerous potential nutrients, was also effective in lowering blood pressure in subjects with pre-hypertension.⁵⁷

Potential roles of reactive oxygen species in hypertension

A large body of literature has shown that excessive production of ROS contributes to hypertension and that scavenging of ROS decreases blood pressure. Despite the evidence that oxidative stress contributes to hypertension, the mechanisms involved are not well understood. Hypertension is associated with increased ROS production by multiple organs, including the brain, the vasculature, and the kidney, all of which are likely important. A major problem is that we currently lack a complete understanding of which of these organs or cell types predominate in the genesis of hypertension or if there is important interplay between them that causes this disease. In the following section, we discuss evidence that ROS in the central nervous system (CNS), the kidney, and the vasculature contribute to hypertension and review recent data showing that the adaptive immunity is activated by oxidative events and can contribute to hypertension by interacting with these organs.

Renal oxidative stress and hypertension

There is ample evidence supporting that ROS generated in the kidney and its blood vessels contribute to the development and maintenance of hypertension. Virtually all cells in the kidney, including vessels, glomeruli, podocytes, interstitial fibroblasts, the medullary thick ascending limb (mTAL), the macula densa, the distal tubule, and the collecting duct express components of the NADPH oxidase, and various stimuli have been shown to activate these. Several of the effects of ROS in the kidney are summarized in Fig. 2. For purposes of discussion, we first focus on oxidative events in the renal cortex and then in the medulla.

Several studies have examined the effect of various hypertensive stimuli on the renal cortex and how these are modulated by ROS. The structures that are targets of oxidant stress include the afferent arteriole, the glomerulus, the proximal tubule, and the cortical collecting duct (CCD). As with other vessels, an increase in $O_2^{\cdot-}$ in the afferent arteriole can oxidatively degrade NO, which would enhance afferent arteriolar vasoconstriction and reduce glomerular filtration rate. Indeed, studies in rabbits have shown that ang II-induced hypertension increases expression of the NADPH oxidase subunit p22^{phox} activates the NADPH oxidase, and causes endothelial dysfunction in afferent arterioles.⁵⁸ Studies of isolated afferent arterioles have also shown that $O_2^{\cdot-}$ generated by the NADPH oxidase potentiates intracellular calcium.⁵⁹ ROS are also generated in the afferent arterioles of spontaneous hypertensive rats and in the kidney of animals in response to other vasoconstrictors such as endothelin-1 (ET-1) and thromboxane prostanoids.^{60, 61} A recent study has shown that $O_2^{\cdot-}$ enhances afferent arteriolar myogenic tone, while H_2O_2 inhibits this via actions on voltage-gated potassium channels.⁶² These findings illustrate the complexity of attempting to scavenge all ROS to improve renal perfusion.

Podocyte injury causes proteinuria and is a precursor to glomerulosclerosis. Moreover, proteinuria seems to promote further podocyte injury in a feed-forward fashion. Dahl-salt sensitive rats demonstrate up-regulation of glomerular p22^{phox} and Nox2 and the antioxidant

tempol reduces glomerular sclerosis and proteinuria in these animals.^{63,64} Recent evidence has implicated a role of plasminogen in stimulating expression of Nox2 and Nox4 in podocytes and causing podocyte injury.⁶⁵ Ang II also stimulates mitochondria ROS generation and induces podocyte autophagy.⁶⁶ Oxidative injury impairs the crosstalk between nephrin and caveolin-1 in podocytes, leading to disruption of glomerular filtration barrier.⁶⁷ ROS also mediate mesangial cell proliferation, migration and extracellular matrix deposition, features characteristic of glomerulosclerosis induced by angiotensin II or aldosterone.⁶⁸ Mice lacking p47^{phox} are protected against the development of glomerulosclerosis and albuminuria following either Adriamycin injection or 5/6 nephrectomy, supporting a role of the NADPH oxidase in this common form of glomerular injury.⁶⁹

Components of the NADPH oxidase exist in lipid rafts within epithelial cells of the proximal tubule, where they are maintained in an inactive state. Dopamine-1 (D1) receptor agonists inhibit, whereas disruption of lipid rafts and angiotensin II stimulate the proximal tubule NADPH oxidase function.^{70, 71} ROS from the NADPH oxidase modulate sodium transport via altering Na/K ATPase and Na/H exchange-3 (NHE-3) function on the basal and apical membranes of the proximal tubular cells, respectively.^{72, 73} Deletion of the dopamine D2 receptor (D2R) inhibits ROS production in renal proximal tubular cells and leads to hypertension, supporting a role of ROS in the proximal tubule in blood pressure control.⁴⁴

An important mechanism by which ROS in the cortex modulates sodium reuptake and blood pressure is via tubuloglomerular feedback. This is mediated by an interaction of the macula densa of the thick ascending limb as it makes contact with its own glomerulus in the cortex. Sodium concentration is sensed by the macula densa via its apical Na/K/2Cl co-transporter, which in turn stimulates NO produced by the neuronal nitric oxide synthase. This promotes dilatation of afferent arterioles and increases glomerular filtration.^{74, 75} Increases in O₂^{•-} in macula densa can inactivate NO, leading to afferent arteriolar vasoconstriction and a reduction of glomerular filtration rate.⁷⁶ An elegant study of isolated, single nephrons by Nouri and colleagues showed that *in vivo* RNA silencing of the NADPH oxidase subunit p22^{phox} enhances single tubular glomerular filtration in angiotensin II-treated rats but not in control rats. By either including or excluding the distal tubule, these authors showed that this effect was likely mediated by ROS produced in the macula densa.⁷⁷

The epithelial Na⁺ channel (ENaC) mediates final tubular adjustment of Na⁺ reabsorption in the cortical collecting duct (CCD).⁷⁸ Angiotensin II activates the NADPH oxidase in the CCD, stimulating ENaC activity.⁷⁹ This is likely mediated by aldosterone, the principle regulator of ENaC activity. Aldosterone induces superoxide generation in A6 epithelial cells, which in turn reduces inhibition of ENaC by NO.⁸⁰ A similar pathway modulates ENaC activity in response to insulin, the insulin-like growth factor and the epidermal growth factor.⁸¹

Oxidative stress also modulates sodium reabsorption and blood pressure in the renal medulla. As in the blood vessel, there is a balance between O₂^{•-} and NO produced by epithelial cells of the medullary thick ascending limb (mTAL) and the pericytes of the vasa recta. NO synthase activity is higher in the renal medulla compared than in the cortex, likely

contributing to independent regulation of medullary and cortical perfusion.⁸² Cowley's group has shown that NO released by cells of the mTAL promotes dilation of the adjacent vasa recta, increasing medullary flow. This augments interstitial Starling forces and promotes sodium movement to the tubule and thus natriuresis and diuresis.^{83, 84} Activation of the NADPH oxidase in the medulla has the opposite effect; promoting vasa recta vasoconstriction, sodium movement into the vasa recta, reducing natriuresis and increasing blood pressure. A growing body of evidence indicates that medullary $O_2^{\cdot -}$ affects sodium transport.^{85, 86} Superoxide enhances Na/K/2Cl cotransporter activity via a protein kinase C activation in mTAL preparations.⁸⁷ Infusion of angiotensin II *in vivo* mimics this effect and administration of the $O_2^{\cdot -}$ scavenger tempol prevents this.⁸⁸ Nitric oxide has the opposite effect on NaCl transport in the mTAL. NO generated by eNOS inhibits the Na/K/2Cl cotransporter and NHE-3 in isolated thick ascending limb.⁸⁹ This inhibitory effect on the Na/K/2Cl cotransporter is mediated by phosphodiesterase-mediated degradation of cyclic AMP while NO directly inhibits NHE-3.⁹⁰ Of interest, increased sodium delivery to cells of the mTAL stimulates H_2O_2 production by the mitochondria, and this is linked to vasodilatation of the nearby vasa recta.⁹¹

The role of ROS in control of renal perfusion and sodium handling likely illustrates a normal physiological role of these molecules and show that they are not uniformly deleterious. The kidney must retain sodium and water during times of salt deprivation and in land-dwelling mammals survival would be impossible without this. It is therefore likely that generation of $O_2^{\cdot -}$ and other ROS within the kidney play a crucial role in this important physiological process.

Reactive oxygen species, the central nervous system and hypertension

It is now obvious that the CNS is essential for production and maintenance of most forms of experimental hypertension, principally by enhancing sympathetic efferent outflow. Even the hypertension caused by hormones like angiotensin II and aldosterone, which have myriad systemic effects, is importantly mediated by actions in the central nervous system.^{92, 93} As an illustration of this, the development of many forms of experimental hypertension is prevented by destruction of a region of the forebrain surrounding the anteroventral third cerebral ventricle (AV3V) in rodents.^{94, 95} This region includes the median preoptic eminence, the organum vasculosum of the lateral terminalis, and the preoptic periventricular nucleus (Fig. 3).⁹⁶ Following AV3V lesioning, virtually all of the central actions of angiotensin II, including drinking behavior, vasopressin secretion and increased sympathetic outflow, are diminished or eliminated. These portions of the forebrain are connected to other regions involved in central cardiovascular regulation. Important among these are the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT), which are circumventricular organs (CVO) lacking a blood-brain barrier. Other CVOs include the median eminence and the area postrema. Hormones in the periphery can act on these regions, modulating neuronal input into cardiovascular control centers in the mid- and hindbrain, including the parabrachial nucleus, the nucleus tractus solitarius (NTS) and the rostral ventral lateral medulla (VLM) (see Fig. 3).

There is strong evidence that signaling in these brain centers and their influence on blood pressure is modulated by local production of ROS.⁹⁷ Intracerebroventricular (ICV) injection of an adenovirus encoding SOD attenuates the hypertension caused by either local injection or systemic infusion of angiotensin II.⁹⁸ Zimmerman and colleagues showed that angiotensin II stimulates $O_2^{\cdot-}$ production in cultured neurons and that this increases intracellular calcium.⁹⁹ Peterson et al, using selective siRNA expressing adenoviruses, have shown that Nox2 and Nox4 have different roles in the SFO, such that both are linked to blood pressure regulation, but only Nox2 modulates drinking behavior.¹⁰⁰ Lob et al used Cre-Lox technology to delete *p22^{phox}* in the SFO and showed that this completely abrogated the hypertensive response to long-term infusion of angiotensin II.¹⁰¹

There is also evidence that ROS derived from the NADPH oxidase enhances nerve firing within the hypothalamus. ICV injection of angiotensin II increases NADPH oxidase-mediated $O_2^{\cdot-}$ production not only in the SFO but also in anterior hypothalamic nuclei such as the median preoptic eminence and in the paraventricular nucleus of the hypothalamus.¹⁰² These effects were blocked by the NADPH oxidase inhibitor apocynin as were the hemodynamic effects of centrally administered angiotensin II. Thus, angiotensin II and its effects on the NADPH oxidase seem to coordinate activation of several forebrain centers to promote a hypertensive response.

Signaling between the forebrain and the hindbrain pontomedullary cardiovascular control centers is also important for blood pressure control. The nucleus tractus solitarius (NTS) is such a hindbrain nucleus that receives input from the CVO and relays inhibitory stimuli from baroreceptors. Angiotensin II augments ROS production by the NADPH oxidase in neurons from the NTS, and this in turn activates L-type calcium channel activity and neuronal firing.¹⁰³ NADPH oxidase is increased in the NTS of stroke-prone, spontaneously hypertensive rats.¹⁰⁴ H_2O_2 causes delayed hyperexcitability of NTS neurons via actions on barium sensitive potassium channels. This might be expected to enhance sympathetic outflow *in vivo*.¹⁰⁵

As discussed above, NO can be consumed by $O_2^{\cdot-}$ and thus can act as a radical scavenger. NO produced in response to ang II stimulation of the AT_2 receptor limits the effects of $O_2^{\cdot-}$ in the medial NTS and thus inhibits L-type channel activation.¹⁰⁶ NO has a similar role in the ventral lateral medulla, which lies below the NTS and receives and sends signals to the NTS and thus regulates sympathetic tone.¹⁰⁷ Experimental interventions that increase NO in the rostral VLM lower blood pressure, whereas increase in oxidative stress in this region raises blood pressure.^{108, 109}

ROS modulate baroreflex function, which is commonly abnormal in the setting of chronic hypertension. Studies by Li and colleagues have shown that ROS generated in the carotid bulb of atherosclerotic rabbits reduce carotid sinus nerve responses to elevations of pressure and that this could be mimicked by exogenous administration of ROS and prevented by ROS scavenging.¹¹⁰

Efferent sympathetic renal nerves govern both the vasculature and tubular segments of the kidney. Stimulation of the renal sympathetic nerves promotes afferent arteriolar

vasoconstriction, renin release and increases Na⁺ reabsorption.¹¹¹ Alpha1 adrenergic receptor activation enhances ROS generation and constriction of the afferent arterioles, reducing renal blood flow (RBF) in angiotensin II-infused rabbits.¹¹² In contrast, β 1 adrenergic receptor activation inhibits ROS generation and promotes vasodilation.¹¹³ In keeping with these important roles of renal sympathetic nerves in controlling renal ROS production, renal denervation blunts blood pressure elevation in multiple experimental hypertensive models, including angiotensin II-induced, DOCA-salt and two kidney one clip hypertension as well as SHR and SHRSP rats.¹¹⁴ The role of renal denervation to control human hypertension remains a topic of debate.

A consequence of renal denervation is ablation of the renal afferent nerves. These are mainly located in the renal pelvis and are activated by various physical and chemical stimuli.¹¹⁵ During kidney injury, increased input from these afferent nerves activates central sympathetic nuclei in a ROS-dependent manner.¹¹⁶ For instance, renal denervation prevents release of norepinephrine from the posterior hypothalamic nuclei and blood pressure elevation following kidney injury induced intrarenal phenol injection.¹¹⁷ Intrarenal phenol injection increases NADPH oxidase expression in the central nuclei and this is prevented by intracerebroventricular (ICV) injection of the SOD mimetic tempol or PEG-SOD. Interestingly, a vitamin E-fortified diet blunts the increase of sympathetic nerve activity and hypertension in response to intrarenal phenol injection.¹¹⁸ These data indicate that ROS modulate both efferent and afferent renal nerve activity and therefore promote development of hypertension.

Vascular oxidative stress and hypertension

Elevated vascular ROS promote the development of hypertension in various animal models, including angiotensin II-induced and DOCA-salt hypertension,^{9, 119, 120} Dahl salt-sensitive hypertension and the spontaneous hypertensive rat (SHR).^{121–123} Humans with hypertension have alterations of vascular reactivity. The several mechanisms by which ROS modulate the vasculature are illustrated in figure 4.

The endothelium regulates vascular tone via releasing of endothelium-derived relaxing factors (EDRFs) and endothelium-derived contractile factors (EDCFs). Oxidative stress causes imbalanced production and bioavailability of these molecules leading to endothelial dysfunction. NO is one of the most important mediators of endothelium-dependent relaxation in blood pressure regulation. The major mechanism for alteration of NO bioavailability when O₂^{•−} is increased is that this radical rapidly reacts with NO, as discussed above. Uncoupling of endothelial NOS, due to deprivation of its cofactor tetrahydrobiopterin, results in reduced NO release, increased O₂^{•−} production, impaired endothelium-dependent relaxation and elevated arterial pressure. Similarly, a lack of the eNOS substrate L-arginine also reduces NO bioavailability and impairs NO-induced dilation. L-arginine is the substrate for both eNOS and arginase, and increased arginase activity reduces the local bioavailability of L-arginine.¹²⁴ Interestingly, peroxynitrite and hydrogen peroxide increase the expression and activity of arginase in endothelial cells, potentially contributing to endothelial dysfunction.¹²⁵

Oxidation of membrane fatty acids and in particular arachidonic acid can lead to formation of F₂-isoprostanes, which are present in the blood of patients with oxidative stress (e.g. those with hypercholesterolemia, diabetics, and cigarette smokers). Importantly, plasma F₂-isoprostanes are increased in animals with experimental hypertension and in humans with renovascular hypertension.^{126, 127} These oxidatively modified fatty acids act on prostaglandin H/thromboxane receptors to enhance vasoconstriction.

Superoxide and other ROS can also affect the structure and function of the vascular media. An important consequence of ROS formation is vascular smooth muscle hypertrophy. Eutrophic vascular remodeling leads to an increase in medial thickness at the expense of a reduction in luminal diameter. This reduces the cross-sectional area of the effective vasculature, increasing systemic vascular resistance.¹²⁸ H₂O₂ seems to be a major mediator of the hypertrophic effect of angiotensin II in cultured cells,¹²⁹ and vascular smooth muscle hypertrophy is strikingly increased in mice overexpressing the NADPH oxidase in the vascular smooth muscle.¹³⁰

Vascular ROS also stimulate the production of collagen and fibronectin both in vitro and in vivo,^{131, 132} and removal of vascular ROS prevents these fibrotic changes.^{133, 134} Structural changes such as these in resistance vessels could add to the increased systemic vascular resistance that occurs in established hypertension and, therefore, worsen the disease.^{135, 136} In larger central arteries, this leads to loss of the Windkessel effect, and increases pulse wave velocity. In population studies, increased pulse wave velocity precedes the development of hypertension by several years. There is substantial evidence that ROS contribute to vascular stiffening. Plasma levels of myeloperoxidase and F₂-isoprostanes, markers of oxidation, correlate with arterial stiffening in humans.¹³⁷ Studies in experimental animals have shown that ROS contribute to fibrotic changes in several tissues including blood vessels.^{138, 139}

Endothelium-dependent hyperpolarization represents an NO-independent mechanism of vasodilatation that is particularly important in resistance vessels.¹⁴⁰ The nature of the endothelium-derived hyperpolarizing factor (EDHF) remains a topic of study, but H₂O₂ produced by the mitochondria is clearly one EDHF that acts by activating the Ca⁺⁺-dependent potassium (BK) channel.¹⁴¹ This seems to be an important mechanism of flow mediated vasodilatation in human coronary arterioles. The production of H₂O₂ is mediated by calcium entry into endothelial cells through the TRP vanilloid type 4 (TRPV4) channel in several vascular beds.¹⁴² Another EDHF is likely a cytochrome p450 epoxide metabolite of arachidonic acid, which also opens the TRPV4 channel.^{143, 144}

H₂O₂ also affects the NO-cGMP pathway. H₂O₂ acutely stimulates NO production and over the long term induces expression of the endothelial nitric oxide synthase (NOS3).^{145–147} H₂O₂ also activates protein kinase G (PKG) by inducing cross-linking two alpha subunits of this enzyme via disulfide bond formation, and therefore promotes vasodilation independent of NO.

Reactive oxygen species, inflammation and hypertension

Diverse stimuli common to the hypertensive milieu, including angiotensin II, aldosterone, catecholamines, increased vascular stretch, and endothelin promote ROS production, which then increases expression of proinflammatory molecules that cause rolling, adhesion, and transcytosis of inflammatory cells.^{148, 149} As a result, there is a striking accumulation of inflammatory cells in the vessel and kidney.^{150–152} In keeping with this, there is an increase in plasma markers of inflammation in hypertensive humans.^{153, 154} Although macrophages are commonly considered important in the genesis of cardiovascular disease, increasing evidence has accumulated suggesting that the adaptive immune response and, in particular, T lymphocytes are important in hypertension. Mice and rats lacking the *Rag1* gene are partly protected against the blood pressure elevation and renal damage caused by angiotensin II or salt.^{155–157} Effector T cells enter the kidney and the perivascular fat, where they release cytokines that promote salt retention and vasoconstriction, ultimately promoting hypertension. These cells mediate their effect by producing cytokines that potently affect vascular and renal function, including TNF α , IFN- γ and IL-17A (Figure 5). Mice lacking IL-17A do not sustain angiotensin II-induced hypertension, and treatment with soluble IL17C lowers blood pressure and prevents oxidative stress in experimental pre-eclampsia.^{158, 159} IL17A directly evokes endothelial dysfunction and ROS production in isolated vessels and causes hypertension when infused in mice.¹⁶⁰ Recent data indicate that IL17A modulates expression and function of the sodium hydrogen exchanger 3 and sodium chloride cotransporter in renal tubular epithelial cells, thus promoting sodium reuptake by these cells.¹⁶¹ IL-17A also promotes aortic stiffening and directly stimulates production of collagen by aortic fibroblasts.¹⁶²

Recently, we identified a novel mechanism linking oxidative events and immune activation in hypertension. This involves formation of isolevuloglandins (isoLGs), which are a form of reactive carbonyl produced upon oxidation of fatty acids and phospholipids. These react rapidly with lysines on proteins and we found that hypertension increases formation of isoLG-modified proteins in monocyte-derived dendritic cells of mice and in monocytes of humans. These modified proteins seem immunogenic (Figure 6).⁴ We have found that iso-LG-modified peptides are presented in major histocompatibility complexes and can promote T cell activation. Chronic vascular oxidant stress also seems to enhance formation of these and their accumulation in dendritic cells.¹⁶³

The observation that circulating cells, including T cells and monocytes, are important in the genesis of hypertension might help provide a unifying link between oxidative events in the CNS, the vasculature, and the kidney. These interactions, illustrated in Fig. 6, are dependent on ROS formation in all of these sites. Centrally, in the SFO, and other CVOs, NADPH oxidase activation increases neuronal firing and sympathetic nerve outflow to peripheral lymphoid tissues, leading to T cell activation.¹⁶⁴ In DCs, ROS lead to isoLG formation and modification of self-proteins. ROS in the kidney and vessels initiates signal that cause T cell homing and infiltration.¹⁵⁰ Finally, cytokines released by T cells diffuse to renal and vascular cells, promoting further NADPH oxidase activation, sodium retention, and vasoconstriction, leading to overt hypertension.

Summary

This review has summarized some of the data supporting a role of ROS and oxidant stress in hypertension. There is evidence that hypertensive stimuli, such as high salt and angiotensin II, promote production of ROS in the brain, the kidney, and the vasculature and that each of these sites contributes either to hypertension or to the untoward sequelae of this disease. Although the NADPH oxidase in these various organs is a predominant source, other enzymes likely contribute to ROS production and signaling in these tissues. A major clinical challenge is that the routinely used antioxidants are ineffective in preventing or treating cardiovascular disease and hypertension. This is likely because these drugs are either ineffective or act in a non-targeted fashion, such that they remove not only injurious but also beneficial ROS involved in normal cell signaling. An important and relatively new concept is that inflammatory cells contribute to hypertension in a ROS-dependent fashion. Future studies are needed to understand the interaction of inflammatory cells with the CNS, the kidney, and the vasculature and how this might be interrupted to provide therapeutic benefit.

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Key Points

- Oxidative stress is considered a major mechanism in hypertension.
- Formation of reactive oxygen species contribute to dysfunction in the vasculature, the kidney and the central nervous system.
- Recent evidence supports a role of reactive oxygen species in inflammation in hypertension.

Figure 1.

Sources and formation of reactive oxygen species in mammalian cells that are relevant to hypertension.

Figure 2.
Renal effects of ROS and NO.

Figure 3. Sites in the brain implicated in hypertension. Shown also are documented actions of ROS and NO to modulate central control of blood pressure.

Figure 4.
Mechanisms by which ROS and NO modulate vascular function and structure in hypertension.

Figure 5.
Formation of isolevuloglandins in antigen presenting cells and modification of self-proteins leading to T cell activation.

Figure 6.
Interplay between immune activation, the central nervous system, the vasculature and the kidney in hypertension.