Reactive Oxygen Species, NADPH Oxidases, and Hypertension

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Reactive oxygen species (ROS) produced in the neuronal, renal, and vascular systems not only influence cardiovascular physiology but are also strongly implicated in pathological signaling leading to hypertension. Different sources of ROS have been identified, ranging from xanthine-xanthine oxidase and mitochondria to NADPH oxidase (Nox) enzymes. Of 7 Nox family members, Nox1, Nox2, and Nox4 (and Nox5 in humans) influence the cardiovascular system. Their activation processes and cell and tissue distribution vary widely, adding complexity to understanding their functional roles. Whether these systems act collectively or independently in disease conditions is unclear, but recently feed forward mechanisms have been established between ROS sources. Studies published in Hypertension over the last few years are the focus of this review, and they provide a framework with which to consider the roles of Nox enzymes in neuronal, renal, and vascular hypertensive mechanisms, as well as cardiac remodeling, and their relationships with other ROS-generating systems.

Neuronal ROS in Hypertension

Redox signaling in the central nervous system is well recognized in neuronal control of blood pressure (BP), as well as in response to angiotensin II (Ang II) and aldosterone, which are linked to ROS-dependent hypertension. Recently, new roles for ROS have been described in the hypothalamus and brain stem, nucleus tractus solitarius (NTS), subnucleus organ (SFO), rostral ventrolateral medulla, and area postrema (Figure 1).

Several studies suggest that Noxs are a primary source of superoxide (O$_2^{-}$) in Ang II–induced neuronal activity. In primary neuronal cultures from the hypothalamus and brain stem, losartan, an angiotensin type 1 receptor (AT1R) antagonist; gp91 ds-tat, a peptide inhibitor of Nox2; and Tempol, a superoxide dismutase (SOD) mimic, attenuate Ang II–induced ROS production and reduce neuronal firing rate. Hypothalamic Nox is also implicated in norepinephrine secretion and renal sympathetic nerve activity of phenol-induced renal injury and Dahl salt-sensitive (DHSS) hypertensive rat models. Nox activity and expression of Nox2, as well as its subunits p22phox and p47phox, increase in these animals, and this, as well as BP, is reversed by treatment with Tempol, polyethylene-glycolated SOD, or the nonspecific Nox inhibitor diphenylene iodonium.

Systemic Ang II infusion induces hypertension by increasing O$_2^{-}$ in the SFO, a primary brain sensor for blood-borne Ang II. Lob et al$^4$ reported that selective deletion of SOD3 in the SFO specifically increases Ang II–induced vascular T-cell and leukocyte infiltration in addition to increasing sympathetic modulation of heart rate, BP, and vascular O$_2^{-}$. These observations suggest that ROS in the central nervous system influence peripheral organs in hypertension. Such effects seem to be specific for individual Nox homologues. Peterson et al$^8$ showed that Nox2 and Nox4 mediate Ang II–mediated ROS production in the SFO, but although both are necessary for the vasopressor response to Ang II, only Nox2 participates in the dipsogenic response.

In rats subjected to coronary artery ligation, 4-week intracerebroventricular infusion of the mineralocorticoid receptor (MR) antagonist RU28318 reduces AT1R, p47phox, and Nox2 expression, as well as Nox-dependent ROS, in the paraventricular nucleus (PVN) with a concomitant reduction in plasma norepinephrine levels. This study suggests that cross-talk exists between MR and Ang II ROS-dependent signaling in the PVN (Figure 1).

ROS signaling to hypertension is also implicated in the NTS. Compared with Wistar-Kyoto rats, stroke-prone spontaneously hypertensive rats (SHR) exhibit elevated activity of Rac1, a regulator of Nox1 and Nox2, in the NTS, and adenosine-mediated inhibition of Rac1 or expression of CuZnSOD decreases BP, heart rate, and urinary norepinephrine excretion. In addition, in the dorsomedial NTS, Ang II stimulates Nox activity and modulates Ca$^{2+}$ current, response that is blocked by gp91 ds-tat and apocynin, a Nox subunit assembly inhibitors. Moreover, in dorsomedial NTS neurons of mice lacking Nox2, Ang II fails to elevate ROS or to potentiate the $L$-type Ca$^{2+}$ current.

Short-term infusion of Ang II elevates BP, heart rate, and renal sympathetic nerve activity in parallel with upregulated expression of Nox2, p22phox, p47phox, and p67phox in the rostral ventrolateral medulla, a brain stem site that maintains sympathetic vasomotor tone. In addition, in SHR and Ang II–infused Wistar-Kyoto rats, mitochondrial dysfunction in the rostral ventrolateral medulla and the subsequent produc-
tion of mitochondrial-localized ROS play a critical role in cardiovascular pathology. Coenzyme Q10 treatment restores electron transport capacity and reduces BP and sympathetic neurogenic vasomotor tone. Interestingly, p22phox antisense, MnSOD, and catalase prevent Ang II–dependent ROS generation, suggesting the existence of a feed-forward effect of Nox on mitochondrial function (Figure 1).

Renal ROS in Hypertension

The kidney regulates BP by controlling water and electrolyte balance and secreting hormones, including Ang II. Several studies implicate ROS and, in particular, Nox enzymes, in multiple kidney functions, where dysregulation can contribute to hypertension, and in the end-organ damage that accompanies hypertension (Figure 2). For example, coinfusion of UK14 304, an α2-adrenoceptor agonist, enhances intrarenal Ang II infusion–induced renal vascular resistance, an effect abolished by Tempol, gp91ds-tat, and diphenylene iodonium. In macula densa cells, which sense luminal NaCl and control afferent arteriolar tone in an ROS-dependent manner as part of the tubuloglomerular feedback process, Nox2 is responsible for salt-induced changes in O$_2^-$ generation, and Nox4 regulates basal ROS. The precise mechanism of Nox2 activation is unclear; however, different possibilities include alterations in the intracellular pH and depolarization of the macula densa cells. In human renal proximal tubule cells, the dopamine 1 receptor agonist fenoldopam inhibits, whereas disruption of lipid rafts and Ang II stimulates, Nox2 and Nox4. Moreover, impaired proximal tubule fluid reabsorption in SHRs is reversed by apocynin and p22phox downregulation, and an increase in Nox-dependent ROS abolishes the inhibitory effect of Ang II on Na/K-ATPase, which may contribute to increased sodium reabsorption and hypertension in rats treated with the oxidant L-buthionine sulfoximine.

In the thick ascending limb of the loop of Henle, NO- and O$_2^-$-mediated destruction of NO can be counterregulated by Nox-dependent, O$_2^-$-mediated destruction of NO. Nox-dependent ROS production in the thick ascending limb is observed in streptozotocin-treated rats, SD rats treated with Ang II, and salt-treated DHSS rats. The importance of NO and O$_2^-$ interactions is further strengthened by the observation that coinfusion of L-arginine (to increase NO bioavailability) blunts Ang II–induced hypertension and associated renal damage. Interestingly, cellular stretch and H$^+$ efflux stimulate O$_2^-$ in the medullar thick ascending limb of the loop of Henle.

As noted, Nox is also linked to renal damage. In aldosterone-induced damage to podocytes, which constitute the final glomerular filtration barrier, increased Nox activity and upregulation of Nox2, p22phox, and p47phox were reported. Moreover, upregulation of Nox2 and p22phox in glomeruli is associated with salt-induced podocyte damage in DHSS rats, and inhibition of Nox activity improves podocyte function. Transgenic TG(mRen2)27 (Ren2) rats treated with rosuvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A
reductase inhibitor that effectively reduces Ang II–mediated Nox activity and Nox2, Nox4, Rac, and p22phox expression in podocytes, exhibit reduced periarteriolar fibrosis and podocyte footprocess effacement along with reduced systolic BP, albuminuria, and renal Nox activity. Ang II infusion also causes renal damage by inducing inflammation. In CCR2−/− mice, which lack the receptor for monocyte chemoattractant protein 1, Ang II–induced renal damage characterized by macrophage infiltration and albuminuria is significantly reduced independent of hypertension. Interestingly, in CCR2+/+ mice, Ang II infusion increases Nox2 expression and 3-nitrotyrosine staining, an effect that is significantly less in CCR2−/− mice, indicating a role for Nox-dependent ROS in renal inflammation and damage. Different approaches targeting Nox are beneficial in preventing renal damage. In DHSS rats, kallistatin, a serine protease inhibitor, effectively prevents high-salt–induced Nox-dependent O$_2^-·$ formation and renal damage by improving NO levels and endothelial NO synthase expression. Similarly, kidney-specific expression of heme oxygenase 1 prevents Ang II–dependent hypertension, which could be explained by a possible influence of heme oxygenase 1 end products on Nox function.

Cross-talk between MR and Nox-dependent ROS also exists in renal hypertension. In SHR/cp rats, a model of metabolic syndrome, salt-induced renal damage, and hypertension, Tempol treatment blocks MR expression, elevated proteinuria, and renal abnormalities, suggesting a role for ROS in MR activation. Interestingly, Nox-dependent ROS production is also a consequence of MR activation in DHSS rats. MR inhibition prevents Nox subunit upregulation in both the kidney and left ventricle in uremia, indicating a role for Nox in MR signaling. These observations clearly suggest a feed-forward mechanism between MR and Nox-dependent ROS.

**Vascular ROS in Hypertension**

Superoxide induces vascular dysfunction in hypertension by its well-described interaction with NO. New animal studies support this concept, showing that high-salt intake, along with L-buthionine sulfoximine treatment, causes vascular dysfunction by reducing NO levels and endothelial NO synthase activity in rats. Similarly, Ang II–induced ROS cause vascular contractions in Wistar-Kyoto mesenteric arterioles that are reversed by atorvastatin treatment, possibly via inhibition of Nox1–induced ROS. A link to human disease was established by Lavi et al., who showed that local oxidative stress reduces NO bioavailability in humans with coronary endothelial dysfunction.

The importance of effective antioxidant systems in the vasculature to limit excessive O$_2^-·$ and prevent hypertension is only beginning to be appreciated. In SOD3 knockout animals, Ang II infusion increases O$_2^-·$ and reduces endothelium-dependent vasorelaxation and further supporting a role for SOD3 in vascular protection. Moreover, deletion of glutathione peroxidase 1, an enzyme that metabolizes H$_2$O$_2$ to water, augments Ang II–induced impairment of endothelium-dependent vasorelaxation. Conversely, overexpression of human thioredoxin 2, a mitochondrial-specific antioxidant enzyme, attenuates Ang II induction of O$_2^-·$, H$_2$O$_2$, and mitochondrial O$_2^-·$ and significantly reduces Nox2, p47phox, and Rac expression.

Much of the work linking ROS to hypertension was performed in animal models with elevated or altered Ang II; however, recent studies have implicated MR-induced ROS as...
well (Figure 3). Savoia et al.\textsuperscript{44} showed that the MR blocker eplerenone significantly reduces arterial wall stiffness, medial collagen:elastin ratio, and circulating inflammatory mediators in hypertensive patients. Another MR blocker, spironolactone, protects Ren2 rats from vascular apoptosis and structural injury via Nox-dependent ROS inhibition.\textsuperscript{45} In contrast, in endothelial cells, aldosterone treatment increases ROS generation by translocating p47phox to the membrane from the cytosol.\textsuperscript{46} Eplerenone or knockdown of p47phox reverses the reduced NO levels and the reduction in endothelial NO synthase Ser 1177 phosphorylation.\textsuperscript{47} Likewise, eplerenone pretreatment ameliorates aortic endothelial dysfunction observed 1 week after myocardial infarction in rats, in part because of normalization of NO bioavailability by reduction in p22phox expression and aortic ROS generation and restoration of endothelial NO synthase phosphorylation.\textsuperscript{48}

An additional mechanism linking Ang II to elevated ROS is Nox-mediated activation of mitochondrial ROS production (Figure 3). Similar to the central nervous system, Ang II–induced Nox activation increases mitochondrial ROS production in aortic endothelial cells.\textsuperscript{49} Interestingly, the mitochondrial targeted antioxidant MitoQ10 reduces systolic BP and improves NO bioavailability in thoracic aorta of SHRs.\textsuperscript{49} These studies suggest the existence of a feed-forward loop between 2 important ROS sources within the same cells.

Recently, a role for T cells in Ang II–induced hypertension was proposed.\textsuperscript{50} Ang II induces T-cell activity, proinflammatory cytokine production, and infiltration in perivascular fat. AT1R expression in immune cells has been shown to be involved in Ang II–induced hypertension.\textsuperscript{51} As noted, deletion of SOD3 in the circumventricular organ can increase T-cell activation, leading to increased Ang II–induced vascular \( \text{O}_2^- \) production and vascular inflammation because of T-cell and leukocyte infiltration, and suggesting that a feed-forward loop exists between organ systems.\textsuperscript{4}

**Cardiac ROS in Hypertension**

One of the deleterious effects of hypertension is hypertrophy or thickening of the heart muscle. Similar to the central nervous, renal and vascular systems, different ROS sources participate in cardiac hypertrophy and remodeling.\textsuperscript{52} and different antioxidant systems play an important role in reducing hypertrophic conditions. For example, glutathione peroxidase 1 prevents cardiac hypertrophy in Ang II–dependent hypertension.\textsuperscript{53} Carbon monoxide, one of the end products of heme oxygenase 1, inhibits Ang II–induced left ventricular hypertrophy by reducing the expression of p47phox, p67phox, and ROS generation.\textsuperscript{54} Interestingly, it was also reported that inhibition of mitochondrial ROS by MitoQ treatment reduces hypertrophy in stroke-prone SHRs.\textsuperscript{55}

Cross-talk between Ang II and MR is also observed in cardiac hypertrophy and remodeling. In transgenic mice with conditional, cardiomyocyte-restricted overexpression of the human MR, Ang II infusion causes a greater increase in left ventricle mass/body weight than in wild-type mice.\textsuperscript{55} These effects are associated with increased expression of hypertrophic markers (collagen and fibronectin) and Nox2.\textsuperscript{55} Similarly, in uremic rats, spironolactone attenuates left ventricular hypertrophy, cardiac \( \text{O}_2^- \) production, and Nox2, Nox4, and p47phox expressions, which indicate a direct role for MR in cardiac hypertrophy.\textsuperscript{36} Thus, similar to other organs, there exists an interplay between the MR and Ang II systems in cardiac hypertrophy.

**Summary**

One concept that emerges from the recent *Hypertension* articles reviewed here is the existence of ROS-induced feed-forward loops in the cardiovascular system. This notion adds more complexity to cardiovascular ROS signaling, but at the same time helps to improve our understanding of the multiple roles of ROS in cardiovascular pathology. There appear to be 3 different loops: (1) cross-activation of different receptors; (2) activation of 1 ROS source by another; and (3) cross-talk between organ systems. More specifically, a feed-forward loop exists between Ang II and MR systems. Acti-
vation of 1 system, Ang II for example, can lead to indirect activation of the other (eg, MR), and vice versa. Thus, specific receptor blockers of AT1R or MR prevent the subsequent activation of the MR or AT1R, respectively. With respect to ROS-induced ROS generation, a feed-forward loop occurs between Nox and mitochondria in both the neuronal and vascular systems. Last but not least, stimulation of ROS signals in one organ can trigger ROS generation in other organs, as when the central alteration of $O_2^-$ levels induces vascular inflammation.

Uncovering the role of Nox in hypertension is complicated by the fact that many Nox enzymes also play important roles in normal physiology. Dissecting these roles is limited by the lack of availability of good antibodies, sufficient genetic models for individual Nox homologues, and specific Nox inhibitors. Moreover, recent studies from Touyz’s group showed that, in the presence of chronic upregulation of the renin-angiotensin system, deletion of either Nox1 or Nox2 does not prevent development of hypertension. In the face of only modest beneficial effects of antioxidants on human hypertension, the data summarized here suggest that targeting specific Nox enzymes may be beneficial more to suppress end-organ damage than to regulate BP, per se. Nonetheless, these recent studies strongly support a role for ROS in the pathogenesis of hypertension.

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References
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