Role of mitochondrial oxidative stress in hypertension

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Dikalov, S. I., Ungvari, Z. Role of mitochondrial oxidative stress in hypertension. Am J Physiol Heart Circ Physiol 305: H1417–H1427, 2013. First published September 11, 2013; doi:10.1152/ajpheart.00089.2013.—Based on mosaic theory, hypertension is a multifactorial disorder that develops because of genetic, environmental, anatomical, adaptive neural, endocrine, humoral, and hemodynamic factors. It has been recently proposed that oxidative stress may contribute to all of these factors and production of reactive oxygen species (ROS) play an important role in the development of hypertension. Previous studies focusing on the role of vascular NADPH oxidases provided strong support of this concept. Although mitochondria represent one of the most significant sources of cellular ROS generation, the regulation of mitochondrial ROS generation in the cardiovascular system and its pathophysiological role in hypertension are much less understood. In this review, the role of mitochondrial oxidative stress in the pathophysiology of hypertension and cross talk between angiotensin II signaling, pathways involved in mechanotransduction, NADPH oxidases, and mitochondria-derived ROS are considered. The possible benefits of therapeutic strategies that have the potential to attenuate mitochondrial oxidative stress for the prevention/treatment of hypertension are also discussed.

hypertension; mitochondria; oxidative stress; superoxide; antioxidant

Introduction

In 1967, Dr. Irvine H. Page proposed the mosaic theory of multiple causes of hypertension (103). The theory states that hypertension is a result of perturbation of interdigitating regulatory mechanisms acting on the variety of tissues, all of which control blood pressure. This includes alterations in neural, endocrine, and immune systems and increased vascular shear stress and stretch due to increased hemodynamics, genetic factors, and maladaptive changes (Fig. 1). Recently, Dr. David Harrison pointed out that a common molecular and cellular event manifested in various organs underlie many features of the mosaic theory (59), namely, enhanced production of reactive oxygen species (ROS; Fig. 1).

In the past two decades, a number of preclinical studies have been published implicating enhanced production of reactive oxygen and nitrogen species (ROS/RNS) in the development and progression of hypertension (Fig. 2) (25, 61, 145). Increased vascular oxidative stress is also thought to play a key role in the pathophysiological consequences of hypertension (including vascular remodeling, inflammation, endothelial dysfunction, atherosclerosis, vascular cognitive impairment, stroke, and aorta aneurysm formation). Accordingly, animal studies show that attenuation of cellular oxidative stress by overexpression of superoxide dismutase (SOD) or treatment with antioxidants scavenging superoxide attenuate hypertension, whereas depletion of SOD expression exacerbates hypertension (46). Similarly, attenuation of cellular oxidative stress by molecular or pharmacological methods was shown to confer multifaceted cardiac and vascular protective effects, preventing/delaying the development of complications of hypertension in animal models (140). Duffy et al. (42) demonstrated a beneficial effect of vitamin C on blood pressure. Yet, no large studies have confirmed this effect. Recent clinical studies on the role of antioxidant supplements in hypertension failed to show any effect of low-dose antioxidants or yielded mixed results (27). A number of clinical trials showed that the routinely used antioxidants per se are ineffective in preventing or treating cardiovascular diseases and hypertension (50). Yet, many antihypertensive drugs were shown to inhibit ROS production and reduce vascular oxidative stress (60).

According to the model using a system biology approach, recently proposed by Dr. Harrison based on the mosaic theory of hypertension (59), ROS production represents an important common pathway (a “node”, as depicted in Fig. 1) in vascular...
remodeling, vasoconstriction, endocrine dysregulation, anatomical maladaptation, neural, and emotional factors that lead to development of hypertension. To identify molecular mechanisms of oxidative stress that can be targeted pharmacologically for treatment of hypertension, it is, therefore, important to investigate separately the different sources of vascular ROS production. In general, oxidative stress is a result of an imbalance between ROS production and activity of cellular antioxidant system. Major sources of vascular ROS production include NADPH oxidases, xanthine oxidase, uncoupled nitric oxide synthase, and mitochondria (33). In recent years, a number of studies have been published on the role of NADPH oxidases, xanthine oxidase, and uncoupled nitric oxide synthase in hypertension-related cardiovascular pathologies [reviewed recently (138)]. Although mitochondria represent one of the most significant sources of cellular ROS generation (mitochondrial ROS production can reach up to 2% of the electron flow), the regulation of mitochondrial ROS generation and its pathophysiological role in hypertension are much less understood. In this review, the role of mitochondrial oxidative stress in the pathophysiology of hypertension and cross talk between angiotensin II signaling, pathways involved in mechanotransduction, NADPH oxidases, and mitochondria-derived ROS are considered. The possible benefits of therapeutic strategies that have the potential to attenuate mitochondrial oxidative stress for the prevention/treatment of hypertension are also discussed.

**Dysregulation of Mitochondrial ROS Production in the Cardiovascular System in Hypertension**

Mitochondria, in addition to generating most of the cell’s supply of ATP by oxidative phosphorylation, are also involved in regulation of apoptosis, maintenance of cellular redox status, and ROS production (33). ATP synthesis is based on electron transfer through the mitochondrial respiratory chain (Fig. 3). Electrons can be supplied by either NADH at complex I or by succinate at complex II. Ubiquinone (QH2) mediates electron transfer to complex III, which in turn reduces complex IV. Complex IV couples oxygen reduction to water and the proton pump, transporting protons (H+) from the matrix to the intermembrane space (19). The resultant proton motive force across the inner mitochondrial membrane is used by complex V for ATP synthesis. Since mitochondria is a major source of cellular superoxide (O2·−), it has been previously suggested that electrons “leak” from electron transport chain to O2 producing O2·− (11, 55). It was called a nonenzymatic reaction where O2 interacts with the ubisemiquinone radical of the respiratory chain leading to a one electron reduction of O2. In the past decade, it has been shown that free ubiquinone does not represent a significant source of mitochondrial O2·− but complex I (33) and complex III (123, 126) are the main sources of mitochondrial O2·− (Fig. 3). Other sources of mitochondrial O2·− may include α-ketoglutarate dehydrogenase, pyruvate dehydrogenase (124), glyceral 3-phosphate dehydrogenase, and fatty acid β-oxidation (12). A new concept therefore is emerging that production of mitochondrial ROS is a highly regulated enzymatic process, and it is a part of mitochondrial physiological function (54). Complex I can produce O2·− both in the forward and reverse electron transfer (Fig. 3). It has been shown that succinate-dependent reverse electron transfer is
much more efficient in O$_2^\cdot$ production compared with NADH-dependent forward electron transfer (104). Reverse electron transport is physiologically regulated by several mechanisms including: 1) control of succinate dehydrogenase activity in the citric acid cycle (106), 2) fatty acids (107), 3) and mitochondrial membrane potential (99). Complex I mediated production of mitochondrial O$_2^\cdot$ has been implicated in various pathophysiological processes, including ischemic preconditioning (15) and in aging. Recent findings demonstrate increased production of O$_2^\cdot$ in the mitochondrial matrix by complex I by reverse electron flow from complex II in the setting of hypertension (97) (Fig. 3). O$_2^\cdot$ produced by complex I mainly on the matrix side is rapidly dismutated to H$_2$O$_2$ by mitochondrial SOD2 (16, 56). H$_2$O$_2$ is a neutral molecule and will easily leave mitochondria regardless of mitochondrial energization. Complex III produces O$_2^\cdot$ both in mitochondrial matrix and intermembrane space (Fig. 3) under condition of hypoxia and complex IV inhibition (94). It is thought to be important in oxygen sensing and redox-dependent cell signaling (54).

There is increasing evidence that hypertension is associated with an increased mitochondria-derived production of ROS in various animal models, including angiotensin II-infused mice (36, 37, 40). Mitochondria also contribute to increased vascular ROS production in mesenteric resistance arteries and the aorta from DOCA-salt rats (136). There are other mechanisms by which mitochondria may affect vascular function in hypertension. For example, deficiency of mitochondrial aldehyde dehydrogenase, an enzyme that detoxifies aldehydes to carboxylic acids, is known to increase oxidative stress. Interestingly, recent studies suggest that mitochondrial aldehyde dehydrogenase attenuates vascular contractions in angiotensin-II treated hypertensive mice (22).

In addition to the role of mitochondria in the peripheral vasculature in hypertension, there is increasing evidence that mitochondria-derived ROS play an important role in the central regulation of systemic cardiovascular function as well (17, 96). Recent data suggest that in the brain activation of the renin-angiotensin system elicits intraneuronal signaling, which involves an increased production of mitochondrial O$_2^\cdot$ (17), modulating ion channel activity and increasing neuronal firing (144). The aforementioned mitochondrial redox signaling pathways are thought to play a key role in central regulation of blood pressure. Accordingly, overexpression of MnSOD in the brain effectively abolishes the central angiotensin II-induced pressor response and decreases blood pressure in rodent models of hypertension (18, 149).

The critical role of mitochondrial ROS in hypertension-induced cardiomyopathy in angiotensin II-infused rodents is suggested by the findings that mice overexpressing catalase targeted to mitochondria (mCat), but not mice overexpressing peroxisomal catalase, are resistant to hypertension-induced cardiac hypertrophy, fibrosis, and diastolic dysfunction (29). Future studies should explore whether mCat mice are also resistant to hypertension-induced pathological alterations in the vasculature as well.

Mitochondria are not only the major source of O$_2^\cdot$ and H$_2$O$_2$ in vascular cells (7, 84) but also represent a targets of cellular ROS (7). Mitochondrial membranes, proteins, and mitochondrial DNA seem particularly sensitive to oxidative damage (8, 143). Oxidative macromolecular damage in the mitochondria in vascular cells is known to adversely affect mitochondrial function (8). ROS posttranslationally modify mitochondrial proteins leading to their inactivation, as in the case of SOD2 and aconitase, or alter their function as occurs with cytochrome c (14, 21, 87). Superoxide reacts with 4Fe-4S clusters of complex I, complex II, and aconitase, resulting in release of Fe$^{3+}$ and altered protein function (45). It has been shown that oxidative damage to complex I and complex II, presumably at the level of 4Fe-4S clusters, increases mitochondrial O$_2^\cdot$ production. Interestingly, a decrease in complex II activity due to oxidative modification increases its O$_2^\cdot$ production by three- to fourfold (105). Oxidative mitochondrial DNA damage may affect the synthesis of components of the respiratory chain, which in turn can further increase ROS production, initiating a vicious cycle. Interestingly, mutations in mitochondrial DNA also associate with increased risk for hypertension (43, 114, 128).
Potential Role of Mitochondrial Antioxidant Systems in Attenuating Hypertension-Induced Oxidative Stress in the Cardiovascular System

Mitochondrial antioxidant systems play an important role in protecting mitochondria and attenuating vascular oxidative stress. SOD2 and glutathione peroxidase are major scavengers of mitochondrial O$_2^-$ and H$_2$O$_2$ (45, 139). SOD2 plays an important role in regulation of redox-sensitive signaling pathways and control mitochondrial O$_2^-$ (98). By inhibiting the reaction of O$_2^-$ with Fe-FeS clusters, this enzyme prevents inactivation of aconitate, complex I and complex II (110). SOD2 is inactivated by peroxynitrite (112), and its activity is reduced with age (142). Expression of SOD2 is regulated in a redox-dependent manner (121). SOD2 overexpression attenuates H$_2$O$_2$-induced apoptosis (115), decreases lipid peroxidation, and reduces the age-related decline in mitochondrial ATP (72). Multiple lines of evidence suggest that impaired function of mitochondrial antioxidant systems is causally linked to the pathogenesis of hypertension. Depletion of mitochondrial SOD2 predisposes mice to both age-related and salt-induced hypertension (116). Earlier reports have found that hypertension and cardiac hypertrophy were associated with reduced expression of SOD1 and SOD2 in spontaneously hypertensive rats compared with Wistar-Kyoto rats (70). Furthermore, increased SOD2 expression in intracerebroventricular region of mice fed a high-salt diet downregulates the UCP2 gene and protein expression in stroke-resistant rats (32). These data suggest that UCP2 plays a key role in attenuation of mitochondrial ROS production (139). Overexpression of SOD2 and thioredoxin 2 showed that this mitochondrial antioxidant system plays a key role in attenuation of mitochondrial ROS production in the aorta, endothelial protection, and regulation of blood pressure in mice with angiotensin II-induced hypertension (139). Overexpression of thioredoxin 2 was also shown to inhibit cardiac hypertrophy and cardiac oxidative stress in mice with chronic angiotensin II infusion (139). The aforementioned studies suggest that imbalance between ROS production and mitochondrial antioxidants contribute to the pathogenesis of hypertension and the development of various vascular pathologies associated with hypertension. There are studies extant showing that mice overexpressing peroxiredoxin 3, the mitochondria-specific peroxidase linked to thioredoxin 2, exhibit improved survival under conditions of increased mitochondrial oxidative stress (88), but the role of peroxiredoxin 3 in hypertension remains elusive. It has been recently suggested that oxidative stress is a result of ROS-induced ROS release due to feed-forward regulation of ROS sources (33, 150). Indeed, production of mitochondrial ROS is redox dependent, and overexpression of mitochondrial SOD2 and thioredoxin 2 simultaneously reduces both production of mitochondrial and cytoplasmic ROS (45, 139). Mitochondrial ATP-sensitive K$^+$ channel (mitoK$_{ATP}$) and PKC$\varepsilon$ are potential targets of mitochondrial and cellular ROS, because they are ROS sensitive (23, 111, 147). Importantly, stimulation of mitoK$_{ATP}$ and PKC$\varepsilon$ has been shown to induce production of mitochondrial ROS (3, 23), representing a potential mechanism of ROS-induced mitochondrial ROS release.

Increased O$_2^-$ production activates series of feedback protective mechanisms including increased antioxidant expression by NF-E2-related factor 2 (NRF2) activation (74) or stimulation of mitochondrial uncoupling protein 2 (UCP2) (85). UCP2 dissipates the mitochondrial proton gradient that contributes to O$_2^-$ formation by reverse electron flow from complex II to complex I. It has been shown that UCP2 has a compensatory role to offset the hypertensive effects of the high-salt diet and partially offset salt-induced vascular dysfunction (86). Interestingly, angiotensin receptor blockade in Otsuka Long-Evans Tokushima fatty mice reduces blood pressure and recovers hepatic UCP2 expression (91). This may suggest that protective role of UCP2 is impaired in hypertension. Indeed, high-salt diet downregulates the UCP2 gene and protein expression in kidneys of stroke-prone spontaneously hypertensive rats, but not of stroke-resistant rats (32). These data suggest that UCP2 is an important protein to prevent oxidative stress in the settings of hypertension.

Cross Talk Between Mitochondrial ROS and NADPH Oxidases: Role in the Pathophysiology of Hypertension

In the past decade an important role of NADPH oxidases in hormone and cytokine responses have been found (79). ROS production by NADPH oxidases has been implicated both in physiological and pathological conditions. For example, NADPH oxidases regulates endothelial nitric oxide synthase activity (81) but overexpression of NADPH oxidases contributes to development of diabetes (118) and hypertension (38). We have found that activation of vascular NADPH oxidases increases production of mitochondrial O$_2^-$ (37, 40). For example, stimulation of endothelial cells with angiotensin II increases mitochondrial O$_2^-$, and depletion of p23$^{\text{phox}}$, a docking subunit of NADPH oxidase complex, abolishes this effect (40). This can be mediated by Nox1 or Nox2 isoforms that are required for angiotensin II-stimulated O$_2^-$ (34). Nox1 is expressed in smooth muscle and other vascular cells, whereas Nox2 is present in endothelial and phagocytic cells (79). Nox4 is constitutively expressed and active in vascular smooth muscle and endothelial cells (2, 63) and is responsible for the basal production of H$_2$O$_2$, whereas increased Nox5 Ca$^{2+}$-dependent H$_2$O$_2$ production in human endothelial cells has been implicated in cardiovascular diseases (53). Angiotensin II increases activity of Nox1 and/or Nox2 (80) via angiotensin II type 1 receptor-dependent activation of PKC and c-Src pathways (82). It is important to note that activation of c-Src is redox sensitive and stimulated by H$_2$O$_2$ (135), which appears to represent a feed-forward mechanism whereby H$_2$O$_2$-mediated activation of c-Src amplifies NADPH oxidase activity.
oxidase activity. In addition to Nox1 and Nox2, there is increasing evidence that Nox4 also plays an important role in the pathophysiology of hypertension. Importantly, Nox4 is localized to the mitochondrial membrane and its activation increases mitochondrial oxidative stress (28). The expression of Nox4 is reported to be increased in spontaneously hypertensive rats (13). Moreover, angiotensin II induces expression of Nox4 in smooth muscle cells (13) and in cardiac myocytes (28). A recent study also reported that mitochondrial-located Nox4 is a major source of pressure overload-induced oxidative stress in the heart (77). In addition of direct production of $O_2^-$ by Nox4 it is also possible that ROS produced by Nox4 alter mitochondrial electron transports chain exacerbating ROS production from this source as well. The fact that mitochondria-targeted catalase overexpression but not cytoplasmic catalase attenuates hypertension-induced cardiac hypertrophy in angiotensin II-infused mice emphasize the key role of ROS amplification within mitochondria (29).

The cross talk between mitochondria and NADPH oxidases is bidirectional. Mitochondria apparently regulate both expression (45) and activity of NADPH oxidases (35). Partial depolarization reduces $Ca^{2+}$ uptake by mitochondrial uniporter and increases $Ca^{2+}$-dependent activation of phagocytic NADPH oxidase (35), whereas depletion of SOD2 results in increase of basal and stimulated vascular NADPH oxidase activity (37). These data show that mitochondrial ROS provide feed-forward regulation of NADPH oxidases which is likely mediated by activation of redox sensitive c-Src with mitochondrial H$_2$O$_2$ (Fig. 4). Recently, it has been reported that functional angiotensin II type 2 receptors are present on mitochondrial inner membranes and that binding of angiotensin II to these receptors activate mitochondrial nitric oxide production (1). Mitochondria-derived nitric oxide has been shown to regulate oxygen consumption by inhibiting the electron transport chain (44, 109). It is tempting to speculate that in hypertension, when mitochondria-derived $O_2^-$ production is also increased, this mechanism would contribute to the increased generation of peroxynitrite in mitochondria, promoting nitrosative damage to mitochondrial proteins. Manganese SOD (SOD2) is a key mitochondrial antioxidant enzyme that is inactivated by reactant stress in the vasculature. Mitochondria may also scavenge cellular ROS and thereby represent an important checkpoint down regulating production of cellular ROS. ROS production by NADPH oxidases was reduced by SOD2 overexpression (37), by overexpression of mitochondrial thioredoxin 2 (139), by treatment with mitochondria targeted SOD mimetic mitoTEMPO (37) or supplementation with mitoK$_{ATP}$ blocker 5-hydroxydecanoic acid (40). Taken together, these data suggest that mitochondria can attenuate or amplify production of cellular ROS. Since mitochondrial ROS have been proposed as second messengers for regulations provided by many hormones and cytokines such as angiotensin II, VEGF, TNF-$\alpha$, and IL-1 (20, 37, 119), we suggest that mitochondrial dysfunction (7) may result in dysregulation of cellular redox status and overproduction of ROS leading to the development vicious cycle of oxidative stress (Fig. 4).

**Potential Role of Hemodynamic Forces in Regulation of Mitochondrial ROS Production**

Several line of evidence suggests that changes in the hemodynamic environment associated with hypertension, either directly or indirectly, can activate vascular ROS production (131). A primary role for high intraluminal pressure in the upregulation of vascular ROS generation is supported by the findings that in aortic banded rats (in which only blood vessels proximal to the coarctation are exposed to high pressure), regional increases in blood pressure result in selective increases in vascular $O_2^-$ production (132). Furthermore, exposure of isolated arteries to high pressure in vitro was shown to increase vascular ROS production (131), eliciting endothelial dysfunction (67). Short-term increases in blood pressure in vivo also impair endothelial function and promote oxidative stress (30, 47, 75, 137). It is likely that in the aforementioned experiments wall tension-dependent cellular stretch is the primary mechanical stimulus for increased vascular ROS production, because exposure of isolated arterial rings to in vitro stretching was also reported to activate NADPH oxidase-mediated $O_2^-$ generation (101), mimicking the effects of high pressure. Previous studies also demonstrate that in cultured

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**Fig. 4. Proposed role of redox-dependent cross talk between mitochondria and NADPH oxidases (NOXs) in vascular dysfunction in hypertension.** CVD, cardiovascular disease; NO, nitric oxide; ONOO$^-$, peroxynitrite; eNOS, endothelial NO synthase; GPx, glutathione peroxidase; Trx2, thioredoxin 2; mCat, catalase targeted to mitochondria; mK$_{ATP}$, ATP-sensitive K$^+$ channel; $\Delta \Psi_m$, mitochondrial transmembrane potential.
endothelial and smooth muscle cells subjected to in vitro stretching production of ROS also significantly increases (51, 64, 65). The available evidence suggests that the prooxidative effects of high pressure itself and the downstream effects angiotensin II signaling are synergistic. In addition to the established role of NADPH oxidases in mechanosensitive ROS production (131), there is also evidence suggesting that high pressure (similar to angiotensin II) can also increase ROS production by the mitochondria. An elegant study by Ichimura et al. (69) demonstrated that elevation of pulmonary capillary pressure increases the amplitude of cytosolic Ca$^{2+}$ oscillations in endothelial cells, which trigger increases in the amplitude of mitochondrial Ca$^{2+}$ oscillations. The aforementioned study provided evidence that pressure-induced mitochondrial Ca$^{2+}$ signals promote mitochondrial ROS production in endothelial cells, which likely play a role in proinflammatory signaling pathways (69). Recently we found that exposure of isolated mouse middle cerebral arteries to high pressure in vitro also promote mitochondria-derived ROS generation (Fig. 5), although further studies are needed to elucidate the underlying signaling pathways. Taken together, the aforementioned studies raise the possibility that mechanosensitive mitochondrial ROS production exacerbates vascular oxidative stress in hypertension (37). Importantly, aging was reported to exacerbate high pressure-induced vascular ROS production (71). There is also evidence suggesting that aging renders cardiac mitochondria more susceptible to hypertension-induced dysfunction (5), although a detailed characterization of aging and hypertension on mitochondrial ROS generation in cardiac myocytes has yet to be performed. Because vascular aging is associated with increased propensity for mitochondrial ROS generation (134), abnormal mitochondrial ROS detoxification, and impaired resistance to mitochondrial oxidative stressors (130), future studies should investigate the interaction among aging, hypertension, and mitochondrial ROS production and its role in increased vulnerability of the elderly to complications of hypertension.

In addition to pressure, other modalities of blood flow, such as shear stress, also regulate vascular ROS production. During hypertension, the hemodynamic energy dissipation increases due to changes in wall shear stress along the arterial tree. Hypertension is also associated with increases in retrograde and oscillatory shear in large arteries (102). Recent clinical studies in patients with uncomplicated hypertension demonstrate flow reversal in the descending aorta (62). Moreover, hypertension is often associated with aortic regurgitation, which also leads to flow reversal in the thoracic aorta. It is likely that the aforementioned changes in the hemodynamic microenvironment in hypertension affect ROS production in vascular cells. Shear stress was demonstrated to promote the release of H$_2$O$_2$ in human coronary arterioles (isolated from hearts of cardiac patients) (90). In cultured endothelial cells, oscillatory shear stress is also a potent stimulus of ROS production (31, 58, 68, 89). The available data suggest that mitochondria is an important source of shear stress-mediated endothelial ROS production both in intact vessels (84) and cultured endothelial cells (127). In addition, there are studies in vivo showing that shear stress can also increase endothelial ROS production via stimulation of NADPH oxidases (68). Recent studies suggest that shear stress promotes mitochondrial ROS generation via a pathway that involves both NADPH oxidase and JNK activation (127), suggesting that a cross talk exists between the two pathways. Laminar shear stress-induced nitric oxide synthesis and simultaneous increase in mitochondrial O$_2^{•-}$ generation were reported to lead to mitochondrial peroxynitrite formation and suppression of respiration in endothelial cells (73). Interestingly, oscillatory shear stress was shown to upregulate Nox2 and Nox1 while decreasing Nox4 expression in human endothelial cells (122). Although it has been proposed that acute administration of H$_2$O$_2$ elicits vasodilation (141), it is likely that chronic mechanosensitive increases in vascular H$_2$O$_2$ production predominantly have signaling roles (e.g., activation of NF-κB, MAPKs), regulating cell proliferation and structural remodeling, and/or contribute to oxidative vascular injury. Importantly, recent data suggest that shear stress-induced mitochondria-derived release of H$_2$O$_2$ contributes to shear stress-mediated Nrf2 activation in endothelial cells as well (57). Taken together, some of the effects exerted by changes in hemodynamic forces on vascular endothelial and smooth muscle cells are similar to those induced by humoral factors associated with hypertension (including angiotensin II), e.g., upregulation of mitochondria-derived ROS production and the signaling pathways leading to NADPH oxidase activation. Future studies on the effects of mechanical forces on signal transduction regulating mitochondrial function, mitochondrial biogenesis, and mitochondrial gene expression will provide insights into the molecular mechanisms by which hemodynamic factors and humoral factors interact in hypertension to regulate vascular physiology and pathophysiology.

**Mitochondria-Targeted Antioxidants: Role in Pharmacological Treatment and Prevention of Hypertension**

Recent preclinical studies by the Dikalov laboratory (37) show that SOD2 overexpression attenuates hypertension. These studies provided important proof of concept for the development of pharmacological strategies to attenuate mitochondrial oxidative stress for the prevention/treatment of hypertension. Importantly, supplementation with a mitochondria-targeted
SOD mimetic mitoTEMP also significantly attenuates angiotensin II-induced hypertension in mice (37). Infusion of mitoTEMPO in mice after onset of both angiotensin II- and DOCA-salt induced hypertension resulted in a significant decrease (by ~25 mmHg) of blood pressure (37). MitoTempo treatment also inhibited vascular oxidative stress and completely restored endothelial-dependent relaxation (37). In 2009, it has been reported that 8-wk treatment of spontaneously hypertensive rats with mitochondria-targeted antioxidant MitoQ significantly reduced systolic blood pressure by 25 mmHg (49). MitoQ supplementation, however, may have unwanted side effects, including promotion of inflammation (39, 93, 100). A recent study reported that treatment with rotenone (as an effort to inhibit production of mitochondrial ROS by inhibiting complex I) attenuates DOCA-salt induced hypertension (146). Rotenone, however, causes neurodegeneration (105), and it is not appropriate for treatment of humans. Treatment of angiotensin II-infused mice with the mitochondrial-targeted antioxidant Szeto-Schiller (SS) compound SS-31 was reported to reduce oxidative stress, improve cardiac function, and attenuate cardiac hypertrophy, despite the absence of blood pressure-lowering effect (28). In that regard it should be noted that the mechanism of action of various mitochondria-targeted antioxidants is different, for example, SS-31 is able to scavenge H$_2$O$_2$ and peroxynitrite (125, 148), which may explain their differential effect on blood pressure.

Recent findings demonstrate that in the cardiovascular system of healthy animals in response to increased production of ROS, adaptive mechanisms are invoked that involve induction of Nrf2-driven antioxidant defense mechanisms (129, 130, 133). Nrf2 is an evolutionarily highly conserved redox sensitive transcription factor that upregulates the expression of numerous antioxidant systems (e.g., enzymes involved in glutathione synthesis). This homeostatic response serves to attenuate mitochondrial and cytoplasmic oxidative stress and limit the damage caused by the increased production of ROS. In recent years, Nrf2 has emerged as a potential target for pharmacological attenuation of mitochondrial oxidative stress in the cardiovascular system (129). Recent studies report that treatment of hypertensive rodents with naturally occurring Nrf2-activating compounds (10, 48), lower blood pressure, restore endothelial function, and decrease vascular oxidative stress. Collectively, the aforementioned studies demonstrate that mitochondrial ROS production is important for the development of hypertension and that antioxidant strategies targeting mitochondria ROS confer multifaceted therapeutic benefits in hypertension.

**Perspectives**

A large body of evidence supports an important role for mitochondrial oxidative stress in the development of hypertension, vascular pathologies, and cardiac dysfunction, which characterize the hypertensive disease. We are at the beginning of an exciting phase of research on understanding the genetic and epigenetic mechanisms underlying increased individual susceptibility for mitochondrial alterations and development of hypertension. Within the vascular wall mitochondria-derived ROS are generated, both in endothelial and vascular smooth muscle cells, and both function as signaling molecules, regulating many aspects of cellular function and contribute to oxidative macromolecular damage. Importantly, a consensus should be reached whether contribution of mitochondria-derived ROS to cardiovascular pathologies occurs primarily via increased macromolecular damage or other mechanisms by which mitochondria-derived ROS and signaling affect the cellular function play an equally important role. The role of neurohormonal factors, including the interplay between angiotensin II signaling and mechanosensitive signaling pathways, in hypertension-related mitochondrial alterations needs to be further elucidated. In hypertension, excess generation of ROS cannot be counterbalanced by endogenous mitochondrial protective antioxidant mechanisms, leading to a state of increased mitochondrial oxidative stress. Despite the fact that contribution of mitochondrial ROS in pathophysiology of hypertension is well documented, we still do not know the precise mechanism of upregulation and the specific sources of mitochondrial ROS. This information will be critical for the development of treatment of mitochondrial oxidative stress. Further studies are warranted to determine whether upregulation of endogenous antioxidant defense mechanisms (e.g., by induction of Nrf2-regulated pathways) is a viable option to attenuate mitochondrial oxidative stress for the prevention/treatment of hypertension. The available evidence, suggesting that pharmacological treatment with mitochondria-targeted antioxidants confers cardiovascular protective effects in animal models of hypertension, looks particularly promising. Further experimental and clinical studies examining the interaction between mitochondria-targeted antioxidants and inhibitors of the renin-angiotensin system in hypertension are necessary. We think that these future studies will discover new pharmacological targets and will advance the development of new therapies of hypertension and possibly other oxidative stress-related conditions.

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**

Z.I.U. and S.I.D. prepared figures; Z.I.U. and S.I.D. drafted manuscript; Z.I.U. and S.I.D. edited and revised manuscript; Z.I.U. and S.I.D. approved final version of manuscript.

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Review


