# Contribution of oxidative stress to endothelial dysfunction in hypertension

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Endothelial dysfunction is the hallmark of hypertension, which is a multifactorial disorder. In the cardiovascular system reactive oxygen species play a pivotal role in controlling the endothelial function and vascular tone. Physiologically, the endothelium-derived relaxing factors (EDRFs) and endothelium-derived contractile factors (EDCFs) that have functions on the vascular smooth muscle cells. The relaxation induced by the EDRFs nitric oxide (NO), prostacyclin, and the endothelium-derived hyperpolarization factor (EDHF) could be impaired in hypertension. The impaired ability of endothelial cells to release NO along with enhanced EDCFs production has been described to contribute to the endothelium dysfunction, which appears to lead to several cardiovascular diseases. The present review discusses the role of oxidative stress, vascular endothelium, and vascular tone control by EDRFs, mainly NO, and EDCFs in different models of experimental hypertension.

**Keywords: oxidative stress, endothelial dysfunction, hypertension, vascular relaxation, vascular contraction, NO availability**

Hypertension is a multifactorial disorder that involves many mechanisms leading to risk factors for cardiovascular diseases. Endothelial dysfunction is defined as the imbalance between the production and bioavailability of endothelium-derived relaxing factors (EDRFs) and endothelium-derived contractile factors (EDCFs), associated with increased bioavailability of oxygen reactive species (ROS) and decreased antioxidant capacity characterized as oxidative stress. In this review we will discuss the involvement of oxidative stress and vascular endothelium as well as the importance of vascular tone control, relaxation, and contraction in hypertension.

NO is an important mediator released by endothelial cells. It is produced by NO synthases (NOS), which convert L-arginine and molecular oxygen to L-citrulline and NO, using such co-factors as tetrahydrobiopterin ($BH_4$), flavin-adenine-dinucleotide, flavin-mononucleotide, and nicotinamide-adenine-dinucleotide-phosphate (Thomas et al., 2008). The activity of NOS is regulated by substrate, cofactor availability, and electron transfer rate. The regulating factors such as arginine (Gornik and Creager, 2004) and $BH_4$ (Bevers et al., 2006) can be affected by ROS that can lead to dysfunctional eNOS. As summarized in the **Figure 1**, in pathological states involving oxidative stress such as hypertension NOS could be uncoupled (Schulz et al., 2008). L-arginine is the substrate for both enzymes, NOS and arginase (Tousoulis et al., 2002). Zhang et al. (2004) showed that the activity of arginase in the endothelial cells of coronary arterioles is increased in hypertension, which impairs the NO-mediated dilation. Similarly, as reported by Chandra et al. (2012) peroxynitrite ($ONOO^-$) and hydrogen peroxide ($H_2O_2$) increase arginase activity/expression in the endothelial cells. This should lead to NOS uncoupling with reduced NO production and augmented superoxide anion ($O_2^-$) production. As shown by Romero et al. (2008), increased arginase activity in diabetes contributes to vascular endothelium dysfunction by decreasing L-arginine availability to NOS.

Endothelial dysfunction culminates in impaired endothelium-dependent relaxation due to decreased vascular NO bioavailability caused by ROS consumption. The result is $ONOO^-$ formation, lower NOS protein expression, or lack of substrate or co-factor for NOS (Crimi et al., 2007). The eNOS phosphorylation state can alter its activity; i.e., Akt-dependent phosphorylation at $Ser^{1177}$ (human) or $Ser^{1179}$ (bovine) activates eNOS (Fulton et al., 1999), while phosphorylation at $Thr^{495}$ (human) or $Thr^{497}$ (bovine) decreases its activation (Bouloumié et al., 1997). $H_2O_2$ initially raises eNOS $Ser^{1179}$ phosphorylation and activity, in parallel with transient Akt activation (Hu et al., 2008).

*In vivo* measurements of NO and $H_2O_2$ in the mesenteric arteries of spontaneously hypertensive rats (SHR) revealed higher baseline NO and $H_2O_2$ concentrations than normotensive rats (Zhou et al., 2008). It is known that in resistance arteries more than in conduit vessels, EDHF is an important control of vascular tone. $H_2O_2$ has been shown to be a component of EDHF in several vascular beds (Meurer et al., 2005; Shimokawa, 2010; Prysyazhna et al., 2012).

Peroxynitrite can also activate eNOS by increasing basal and agonist-stimulated $Ser^{1179}$ phosphorylation, although it reduces NO bioavailability and elevates $O_2^-$ production (Zou et al., 2002a). eNOS exposure to oxidants like $ONOO^-$ causes increased enzymatic uncoupling and $O_2^-$ generation in diabetes that contributes to endothelial cell oxidant stress (Zou et al., 2002b). Increased formation of $ONOO^-$ can inhibit prostacyclin synthase (PGIS) (Wu and Liou, 2005) and impairs $K^+$ channel activation (Gutterman et al., 2005).
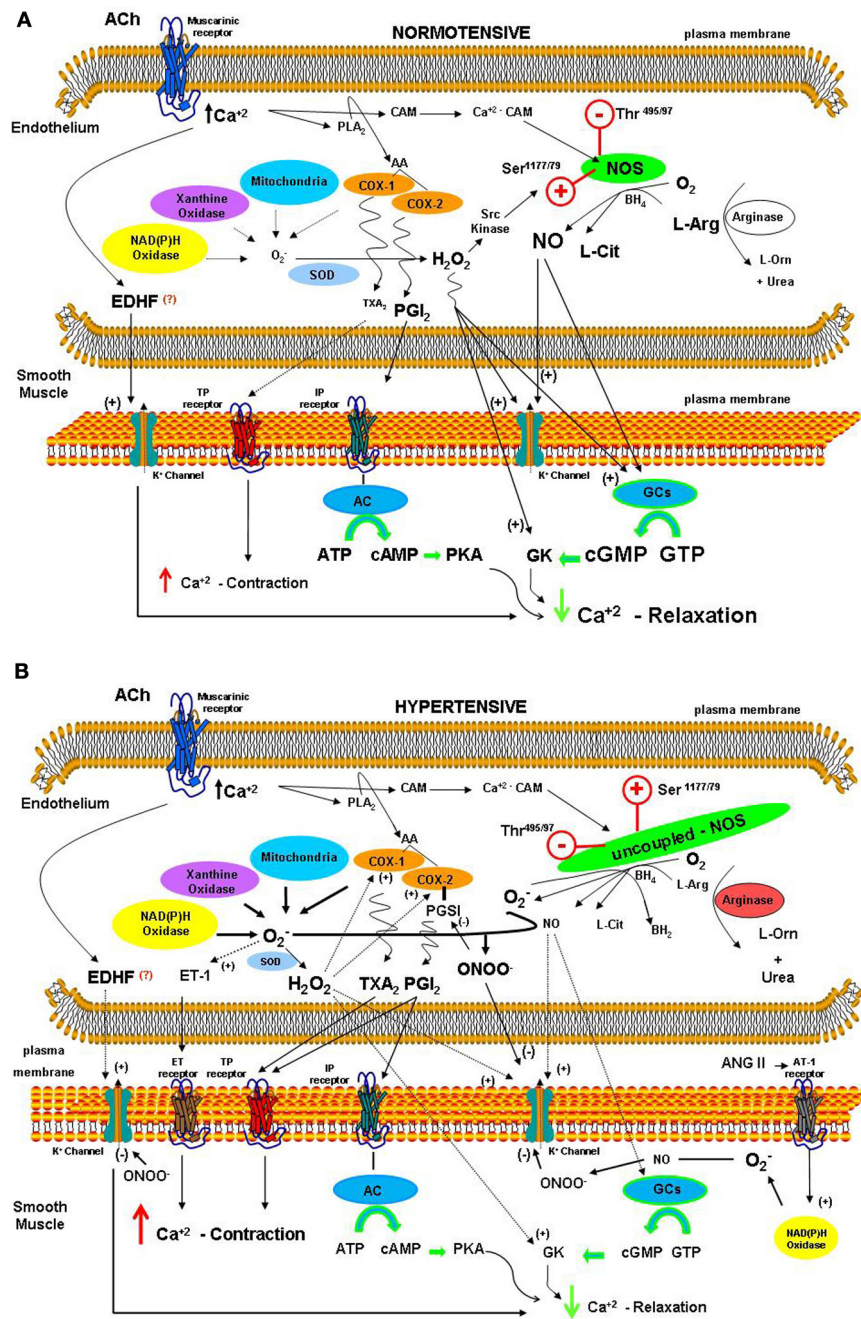
**FIGURE 1 | Sources of reactive oxygen species (ROS) and proposed mechanisms for their contribution to EDRFs and EDCFs releasing involved in the control of vascular tone in isolated vessels from normotensive (A) and hypertensive (B) animals.**

Increased ROS bioavailability, decreased antioxidant capacity, or both occur in many models of hypertension such as SHR (Suzuki et al., 1995), Dahl salt-sensitive (Swei et al., 1997), AngII-infused rats (Laursen et al., 1997), renal hypertensive 2K-1C (Rodrigues et al., 2008), and human hypertension (Vaziri, 2004). In endothelial cells, the ROS producers are NADPH oxidase (Rajagopalan et al., 1996), xanthine oxidase (Phan et al., 1989), uncoupled NOS (Satoh et al., 2005), cyclooxygenase (COX) (Tang

et al., 2007), and mitochondria (Callera et al., 2006). The DOCA-salt model present augmented oxidative stress caused by increased NADPH oxidase activity, which accounts for enhanced $O_2^-$ production (Beswick et al., 2001). In 2K-1C rats, the increased vascular $O_2^-$ is secondary to a protein kinase C (PKC)-mediated activation of NADPH oxidase (Heitzer et al., 1999). However, eNOS activity is reduced by phosphorylation of the $Thr^{495}$ residue in the $Ca^{2+}$/CaM binding domain by PKC (Mount et al., 2007).

Mimicking of Thr[495] dephosphorylation results in eNOS uncoupling and $O_2^-$ production rather than NO generation (Lin et al., 2003). However, whether the Thr[495] eNOS phosphorylation site is more phosphorylated in hypertension or contains uncoupled eNOS remains unknown.

We have investigated the vascular mechanisms involved in the vasorelaxation induced by NO donors that present potential capacity to replenish vascular NO upon reduced NO bioavailability. Most of the studies using NO donors are performed on endothelium-denuded arteries to avoid interference of endogenously produced NO (Bonaventura et al., 2004; Pereira et al., 2011). Impaired 2K-1C rat aorta relaxation is endothelium-dependent (Callera et al., 2004) or endothelium-independent (Bonaventura et al., 2005). Vitamin-C normalized the impaired relaxation induced by a NO donor in 2K-1C rat aorta that shows the increased ROS production in the vascular smooth muscle cells (Rodrigues et al., 2008). Interestingly, the endothelium can contribute to the vasorelaxation induced by sodium nitroprusside (SNP) via NOS activation (Bonaventura et al., 2008). The endothelium negatively modulates the vasorelaxation induced by the complex (TERPY) in the rat aorta. $BH_4$ supplementation reverses the effect of uncoupled NOS induced by TERPY (Bonaventura et al., 2009).

The altered function of endothelial cells leads to enhanced contraction (Endemann and Schiffrin, 2004). The EDCFs released under different stimuli include ET-1 (Taddei et al., 2003), some prostanoids, and ROS (Tang and Vanhoutte, 2009). ET-1 activates $ET_A$ and $ET_B$ receptors. $ET_A$ receptors are expressed on smooth muscle cells and promote contraction. $ET_B$ receptors are located on endothelial and smooth muscle cells, with opposite effects. Smooth muscle $ET_B$ activation evokes contraction, whereas endothelial $ET_B$ activation induces relaxation (Taddei et al., 2003). The imbalance in the expression of receptors or increased ET-1 production can contribute to hypertension. Hypertension associated with elevated levels of AngII leads to high vascular ET-1 production (Dohi et al., 1992) as well as ROS originated from NADPH oxidase (Touyz et al., 2002). Both factors are related to larger contractility in hypertensive rat resistance arteries.

The SHR aorta exhibits a characteristic endothelial dysfunction that is not due to decreased EDRF release, but it is the result of simultaneous EDCF release. Indomethacin, a non-selective COX inhibitor, restores the blunted relaxation in SHR aorta to the level of normotensive (Lüscher and Vanhoutte, 1986), which suggests that this EDCF must be a product of the COX. Endothelium-dependent contraction is reported in the rat aorta, mesenteric and femoral arteries, and cerebral arterioles. It occurs in healthy animals, but EDCF release is exacerbated by hypertension. Selective COX-1 inhibitors abolish endothelium-dependent contraction in SHR aorta, while selective COX-2 inhibitors only display modest responses (Tang and Vanhoutte, 2009).

Endoperoxides, $PGI_2$, $TXA_2$, and ROS are proposed as COX-derived EDCFs. Increased endothelial $[Ca^{2+}]_i$ is required to evoke EDCF-mediated responses. Dysfunction in $Ca^{2+}$ handling within the endothelium is important for the exacerbation of endothelium-dependent contractions in SHR aorta (Tang et al., 2007).

Independent of the genesis of hypertension, specific ROS such as $H_2O_2$ modify the vascular activity of NOS and COX in concentration-dependent way (Cai et al., 2003; Gil-Longo and González-Vásquez, 2005). In hypertension, ROS are involved in augmented EDCFs and diminished EDRFs release. In the L-NAME (Qu et al., 2010) and SHR (Félétou et al., 2009) models there is increased COX-derived production of contractile prostanoids. Physiologically, $PGI_2$ evokes vasorelaxation, whereas in aging animals or SHR it induces contraction (Vanhoutte, 2011).

Inhibitors of COX (Taddei et al., 1997), NADPH oxidase (Costa et al., 2009), and xanthine oxidase (Ellis et al., 1998) or antioxidant agents such as Vitamin-C (Nishi et al., 2010) seem to diminish ROS production and EDCFs generation.

In conclusion, the data presented in this work suggest that decreased NO availability along with enhanced EDCFs production contribute to the endothelium dysfunction and impaired vascular relaxation in hypertension (**Figure 1**). Considering the enormous progress in the area in the last years, this work addresses the function of oxidative stress on the pathogenesis of hypertension.

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