Contribution of oxidative stress to endothelial dysfunction in hypertension

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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 (BH4), 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 BH4 (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, NO and arginase (Tousoulis et al., 2002). Zhang et al. (2004) showed that the activity of arginase in the endothelial cells of coronary arteries is increased in hypertension, which impairs the NO-mediated dilation. Similarly, as reported by Chandra et al. (2012) peroxynitrite (ONOO⁻) and hydrogen peroxide (H₂O₂) increase arginase activity/expression in the endothelial cells. This should lead to NOS uncoupling with reduced NO production and augmented superoxide anion (O₂⁻) production. As shown by Romero et al. (2008), increased arginase activity in diabetes contributes to vascular endothelial 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 Ser1177 (human) or Ser1179 (bovine) activates eNOS (Fulton et al., 1999), while phosphorylation at Thr495 (human) or Thr497 (bovine) decreases its activation (Bouloumié et al., 1997). H₂O₂ initially raises eNOS Ser1179 phosphorylation and activity, in parallel with transient Akt activation (Hu et al., 2008).

In vivo measurements of NO and H₂O₂ in the mesenteric arteries of spontaneously hypertensive rats (SHR) revealed higher baseline NO and H₂O₂ 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. H₂O₂ 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 Ser1179 phosphorylation, although it reduces NO bioavailability and elevates O₂⁻ production (Zou et al., 2002a). eNOS exposure to oxidants like ONOO⁻ causes increased enzymatic uncoupling and O₂⁻ 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\textsuperscript{495} dephosphorylation results in eNOS uncoupling and O\textsubscript{2}•\textsuperscript{-} production rather than NO generation (Lin et al., 2003). However, whether the Thr\textsuperscript{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 endothelial-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\textsubscript{4} supplementation reverses the effect of uncoupled NO 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\textsubscript{A} and ET\textsubscript{B} receptors. ET\textsubscript{A} receptors are expressed on smooth muscle cells and promote contraction. ET\textsubscript{B} receptors are located on endothelial and smooth muscle cells, with opposite effects. Smooth muscle ET\textsubscript{B} activation evokes contraction, whereas endothelial ET\textsubscript{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 originating from NADPH oxidase (Touyz et al., 2001). 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, PG\textsubscript{II}\textsubscript{2}, TXA\textsubscript{2}, and ROS are proposed as COX-derived EDCFs. Increased endothelial [Ca\textsuperscript{2+}] \textsubscript{i} is required to evoke EDCF-mediated responses. Dysfunction in Ca\textsuperscript{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\textsubscript{2}O\textsubscript{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 (Féletou et al., 2009) models there is increased COX-derived production of contractile prostanoids. Physiologically, PG\textsubscript{II}\textsubscript{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.

**REFERENCES**

Beswick, R. A., Dorrance, A. M., and Romulo, R. C. L. (2001). NADH/NADPH oxidase and enhanced superoxide production in the mineralocorticoid hypertensive rat. Hypertension 38, 1107–1111.

Bevers, L. M., Braam, B., Post, J. A., van Zonneveld, A. J., Rabelink, T. J., Koomans, H. A., et al. (2006). Tetrahydrobiopterin, but not L-arginine, decreases NO synthase uncoupling in cells expressing high levels of endothelial NO synthase. Hypertension 47, 87–94.

Bonaventura, D., Lunardi, C. N., Rodrigues, G. J., Neto, M. A., and Bendhack, L. M. (2008). A novel mechanism of vascular relaxation induced by sodium nitroprusside in the isolated rat aorta. Nitric Oxide 18, 287–295.

Bonaventura, D., Lunardi, C. N., Rodrigues, G. J., Neto, M. A., Vercesi, J. A., de Lima, R. G., et al. (2009). Endothelium negatively modulates the vascular relaxation induced by nitric oxide donor, due to uncoupling NO synthase. J. Inorg. Biochem. 103, 1366–1374.

Bonaventura, D., Oliveira, F. S., da Silva, R. S., and Bendhack, L. M. (2004). A macrocyclic nitrosyl ruthenium complex is a NO donor that induces rat aorta relaxation. Nitric Oxide 10, 83–91.

Bouloumié, A., Bauersachs, J., Linz, W., Schöllens, B. A., Wiemer, G., Fleming, L., et al. (1997). Endothelial dysfunction coincides with an enhanced nitric oxide synthase expression and superoxide anion production. Hypertension 30, 934–941.

Cai, H., Li, Z., Davis, M. E., Kanner, W., Harrison, D. G., and Dudley, S. C. Jr. (2003). Akt-dependent phosphorylation of serine\textsuperscript{1177} and mitogen-activated protein kinase kinase/extracellular signal-regulated kinase 1/2 cooperatively mediate activation of the endothelial nitric-oxide synthase by hydrogen peroxide. Mol. Pharmacol. 63, 325–331.

Callera, G. E., Tostes, R. C., Yogi, A., Montezano, A. C. I., and Touyz, R. M. (2006). Endothelin-1-induced oxidative stress in DOCA-salt hypertension involves NADPH-oxidase-independent mechanisms. Clin. Sci. 110, 243–253.

Callera, G. E., Yogi, A., Tostes, R. C., Rossoni, L. V., and Bendhack, L. M. (2004). Ca\textsuperscript{2+}−activated K\textsuperscript{+} channels underlying the impaired acetylcholine-induced vasodilation in 2K-1C hypertensive rats.

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Renovascular hypertension: evidence for an involvement of protein kinase C. *Kidney Int.** 55, 252–260.

Hu, Z., Chen, J., Wei, Q., and Xia, Y. (2008). Bidirectional actions of hydrogen peroxide on endothelial nitric-oxide synthase phosphorylation and function – co-commitment and interplay of Akt and AMPK. *J. Biol. Chem.* 283, 25256–25263.

Laursen, J. B., Rajagopalan, S., Galis, Z., Tarpey, M., Freeman, B. A., and Harrison, D. G. (1997). Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* **95**, 588–593.

Lin, M. I., Fulton, D., Babbitt, R., Fleming, I., Busse, R., Pritchard, K. A. Jr., et al. (2003). Phosphorylation of threonine997 in endothelial nitric-oxide synthase coordinates the coupling of L-arginine metabolism to efficient nitric oxide production. *J. Biol. Chem.* 278, 44719–44726.

Lüscher, T. F., and Vanhoutte, P. M. (1986). Endothelium-dependent contractions to acetycholine in the aorta of the spontaneously hypertensive rat. *Hypertension* **8**, 344–348.

Meurer, S., Pioch, S., Gross, S., and Müller-Esterl, W. (2005). Reactive oxygen species induce tyrosine phosphorylation of and Src kinase recruitment to NO-sensitive guanylyl cyclase. *J. Biol. Chem.* **280**, 33149–33156.

Mount, P. F., Kemp, B. E., and Power, D. A. (2007). Regulation of endothelial and myocardial NO synthesis by multi-site eNOS phosphorylation. *J. Mol. Cell. Cardiol.* **42**, 271–279.

Nishi, D., Oliveira-Sales, E. B., Bergamaschi, C. T., Oliveira, T. G. C., Boim, M. A., and Campos, R. R. (2010). Chronic antioxidant treatment improves arterial renal-vascular hypertension and oxidative stress markers in the kidney in Wistar rats. *Am. J. Hypertens.* **23**, 473–480.

Pereira, A. C., Ford, P. C., da Silva, R. S., and Bendhack, L. M. (2011). Ruthenium-nitrite complex as a pro-drug releases NO in a tissue and enzyme-dependent way. *Nitric Oxide* **24**, 192–198.

Phan, S. H., Gannon, D. E., Varani, J., Ryan, U. S., and Ward, P. A. (1989). Xanthine oxidase activity in rat pulmonary artery endothelial cells and its alteration by activated neutrophils. *Am. J. Pathol.* **134**, 1201–1211.

Pryszczyna, O., Rudyk, O., and Eaton, P. (2012). Single atom substitution in mouse protein kinase G eliminates oxidant sensing to cause hypertension. *Nat. Med.* **18**, 286–290.

Qu, C., Leung, S. W. S., Vanhoutte, P. M., and Man, R. Y. K. (2010). Chronic inhibition of nitric-oxide synthase potentiates endothelium-dependent contractions in the rat aorta by augmenting the expression of cyclooxygenase-2. *J. Pharmacol. Exp. Ther.** 334, 373–380.

Rajagopalan, S., Kurz, S., Münnel, T., Tarpey, M., Freeman, B. A., Griending, K. K., et al. (1996). Angiotensin II mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation: contribution to alterations of vasomotor tone. *J. Clin. Invest.* **97**, 1916–1923.

Rodrigues, G. J., Lunardi, C. N., Lima, R. G., Santos, C. X., Laurindo, F. R. M., da Silva, R. S., et al. (2008). Vitamin C improves the effect of a new nitric oxide donor on the vascular smooth muscle from renal hypertensive rats. *Nitric Oxide* **18**, 176–183.

Romero, M. J., Platt, D. H., Tawfik, H. E., Labazi, M., El-Remessy, A. B., Bartoli, M., et al. (2008). Diabetes-induced coronary vascular dysfunction involves increased arginine availability. *Circ. Res.** 102, 95–102.

Sato, M., Fujimoto, S., Haruna, Y., Arakawa, S., Horike, H., Komai, N., et al. (2005). NAD(P)H oxidase and uncoupled nitric oxide synthase are major sources of glomerular superoxide in rats with experimental diabetic nephropathy. *Am. J. Physiol.* **288**, F1144–F1152.

Schulz, E., Jansen, T., Wenzel, P., Daiber, A., and Münnel, T. (2008). Nitric oxide, tetrahydrobiopterin, oxidative stress and endothelial dysfunction in hypertension. *Antioxid. Redox Signal.* **10**, 1115–1126.

Shimokawa, H. (2010). Hydrogen peroxide as an endothelium-derived hyperpolarizing factor. *Pflugers Arch.* **459**, 915–922.

Suzuki, H., Swei, A., Zweifach, B. W., and Schmid-Schönbein, G. W. (1995). In vivo evidence for microvascular oxidative stress in spontaneously hypertensive rats. *Microcirculation* **2**, 1627–1633.

Swei, A., Lacy, F., DeLano, F. A., and Schmid-Schönbein, G. W. (1997). Oxidative stress in the Dahl hypertensive rat. *Hypertension* **30**, 1627–1633.

Taddei, S., Ghiadoni, L., Virdis, A., Versari, D., and Salvetti, A. (2003). Mechanisms of endothelial dysfunction: clinical significance and preventive non-pharmacological therapeutic strategies. *Curr. Pharm. Des.* **9**, 2385–2402.

Taddei, S., Virdis, A., Ghiadoni, L., Magagna, A., and Salvetti, A. (1997). Cyclooxygenase inhibition restores nitric oxide activity in essential hypertension. *Hypertension* **29**, S274–S279.

Tang, E. H., Leung, F. P., Huang, Y., Félotou, M., Man, R. Y., and Vanhoutte, P. M. (2007). Calcium and reactive oxygen species increase in endothelial cells in response to releasers of endothelium-derived contracting factor. *Br. J. Pharmacol.* **151**, 15–23.

Tang, E. H. C., and Vanhoutte, P. M. (2009). Prostanoid and reactive oxygen species: team players in endothelium-dependent contractions. *Pharmacol. Ther.* **122**, 140–149.

Thomas, D. D., Ridnour, L. A., Izenberg, J. S., Flores-Santana, W., Switzer, C. H., Donzelli, S., et al. (2008). The chemical biology of nitric oxide: implications in cellular signaling. *Free Radic. Biol. Med.* **45**, 18–31.

Tousoulis, D., Antoniades, C., Tentolouris, C., Goumas, G., Stefanidis, C., and Tountouzas, P. (2002). L-arginine in cardiovascular disease: dream or reality? *Vasc. Med.* **7**, 203–211.

Toouzy, R. M., Chen, X., Tabet, E., Yao, G., He, G., Quinn, M. T., et al. (2002). Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H oxidase in smooth muscle cells from human resistance arteries: regulation by angiotensin II. *Circ. Res.* **90**, 1205–1212.

Vanhoutte, P. M. (2011). Endothelium-dependent contractions in hypertension: when prostacyclin becomes ugly. *Hypertension* **57**, 526–531.

Vaziri, N. D. (2004). Roles of oxidative stress and antioxidant therapy in chronic kidney disease and hypertension. *Curr. Opin. Nephrol. Hypertens.* **13**, 93–99.

Wu, K. K., and Liu, J. (2005). Cellular and molecular biology of prostacyclin synthesis. *Biochem. Biophys. Res. Commun.* **338**, 45–52.

Zhang, C., Hein, T. W., Wang, W., Miller, M. W., Fossum, T. W., McDonald, M. M., et al. (2004). Upregulation of vascular arginase in hypertension decreases nitric oxide-mediated dilation of coronary arteries. *Hypertension* **44**, 935–943.

Zhou, X., Bohlen, H. G., Miller, S. J., and Unthank, J. L. (2008).
NAD(P)H oxidase-derived peroxide mediates elevated basal and impaired flow-induced NO production in SHR mesenteric arteries in vivo. Am. J. Physiol. 295, H1008–H1016.

Zou, M. H., Hou, X. Y., Shi, C. M., Nagata, D., Walsh, K., and Cohen, R. A. (2002a). Modulation by peroxynitrite of Akt- and AMP-activated kinase-dependent Ser1179 phosphorylation of endothelial nitric oxide synthase. J. Biol. Chem. 36, 32552–32557.

Zou, M. H., Shi, C., and Cohen, R. A. (2002b). Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J. Clin. Invest. 109, 817–826.

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