Oxidase Interactions in Cardiovascular Disease

37

Ji Youn Youn, Kin Lung Siu, Qiang Li, David G. Harrison, and Hua Cai

Contents

Major Oxidases in the Cardiovascular System 850
Nox Family Oxidases 851
Mitochondria 854
Uncoupled eNOS 855
Xanthine Oxidase 856
Communication of Oxidases in Cardiovascular System 857
Interaction Between Nox and eNOS Uncoupling 857
Interaction Between Nox and Mitochondria 858
Interaction Between eNOS Uncoupling and Mitochondria 859
Interaction Between XO and Mitochondria 859
Oxidases and Cardiovascular Diseases 859
Hypertension 860
Diabetic Cardiovascular Complications 861
Atherosclerosis 862
Cardiac Diseases 863
Summary and Conclusions 864
References 865

Abstract

Oxidative stress has been implicated in cardiovascular diseases such as hypertension, atherosclerosis, diabetic vascular complications, ischemic heart disease, and heart failure. Reactive oxygen species (ROS) in cardiovascular system are

J.Y. Youn • K.L. Siu • Q. Li • H. Cai (✉)
Cardiovascular Research Laboratories, Divisions of Molecular Medicine and Cardiology, Cardiovascular Research Laboratories, Departments of Anesthesiology and Medicine, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA
e-mail: hcai@mednet.ucla.edu

D.G. Harrison
Division of Clinical Pharmacology, Department of Medicine, Vanderbilt University, Nashville, TN, USA

I. Laher (ed.), *Systems Biology of Free Radicals and Antioxidants*,
DOI 10.1007/978-3-642-30018-9_43,

generated from various enzymatic systems such as NADPH oxidase (NOX), uncoupled nitric oxide synthase (NOS), mitochondria and xanthine oxidase. Interactions among these systems have been intensively investigated for their roles in cardiovascular pathogenesis. The current review discusses up-to-date findings regarding pathophysiologies of each oxidase system, complex crosstalks among different oxidase components, and consequences of these crosstalks in mediating cardiovascular pathogenesis. Better understanding of these mechanisms may promote novel therapeutic strategies to prevent or treat cardiovascular diseases.

Keywords
Cardiovascular disease • eNOS uncoupling • Interaction • Mitochondria • NADPH oxidase • Xanthine oxidase

Major Oxidases in the Cardiovascular System

Accumulating evidence indicates that the major enzymatic sources of reactive oxygen species (ROS) in the cardiovascular system are the NADPH oxidases, uncoupled endothelial nitric oxide synthase (eNOS), the mitochondria and xanthine oxidase (XO). While the NADPH oxidases are generally thought to only produce ROS, (Lambeth 2004) they also generate an electrical potential across cell membranes which likely has important roles in cellular and organelle functions (Ahluwalia 2008). Low levels of ROS produced by the NADPH oxidase have been implicated in physiological processes such as cell proliferation, migration, differentiation, and cytoskeleton organization (Griendling et al. 1994; Lassegue and Griendling 2010; Petry et al. 2006; Gupte et al. 2009; Peshavariya et al. 2009). Excessive production of ROS from activated NADPH oxidases, however, contributes to cardiovascular pathology. Of note, ROS from one source are able to trigger ROS production by activating other enzyme systems. For example, ROS produced from the NADPH oxidases induce oxidative inactivation and deficiency of tetrahydrobiopterin (H_4B), an essential cofactor for eNOS. This leads to a condition referred to as NOS uncoupling in which the NO synthases produce superoxide rather than nitric oxide ($NO^{\bullet}$) (Landmesser et al. 2003; Vasquez-Vivar et al. 1998; Gao et al. 2009; Chalupsky and Cai 2005; Oak and Cai 2007; Youn et al. 2012a). In addition, NADPH oxidase-derived ROS can stimulate conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XO) by oxidation of sulfhydryl residues (Nishino 1994). ROS produced by the NADPH oxidase can oxidize components of membrane permeability transition pore (mPTP) (Ago et al. 2004) and cause opening of the redox-sensitive channel, mitoK_{ATP}, (Costa et al. 2006) both contributing to mitochondrial uncoupling and ROS production (Costa et al. 2006). These different amplification mechanisms are illustrated in Fig. 37.1. Indeed, the Nox enzymes have emerged as the primary oxidase responsible for oxidative stress in vascular diseases such hypertension (Peterson et al. 2009; Harrison et al. 2007), hypercholestelomia,

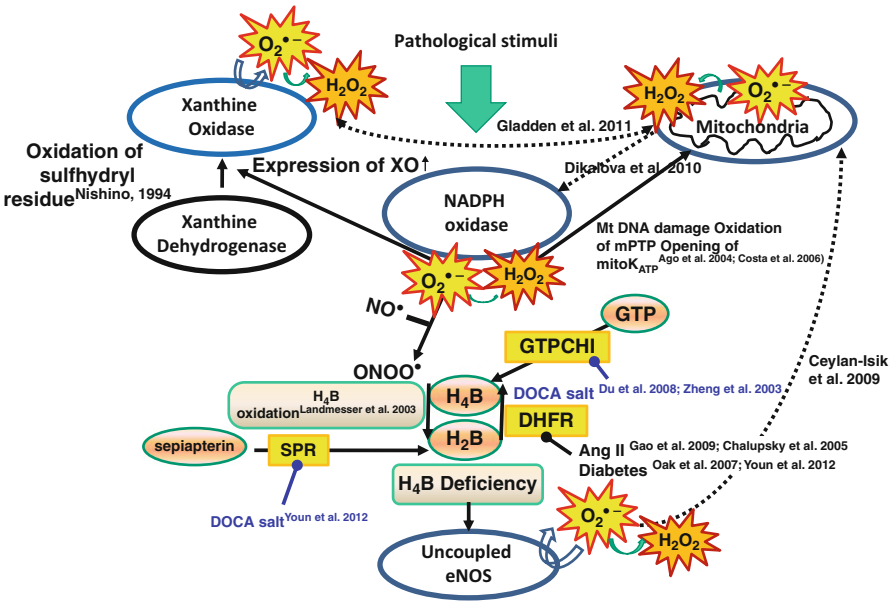

Fig. 37.1 Oxidase interactions among NADPH oxidase, xanthine oxidase, mitochondria, and uncoupled eNOS

(Loffredo et al. 2012) atherosclerosis (Cai and Harrison 2000; Cai et al. 2002; Cai et al. 2003a; Cai 2005), diabetic vascular complications (Oak and Cai 2007; Liu et al. 2007; San Martin et al. 2007) as well as cardiac diseases such as heart failure (Ide et al. 2000), atrial fibrillation (Kim et al. 2005), and myocardial infarction (Krijnen et al. 2003).

Nox Family Oxidases

The Nox proteins represent the catalytic subunit of the NADPH oxidases, which are membrane-bound enzyme complexes present in many mammalian cells. The originally characterized of these was Nox2, also known as gp91^{phox}, which exists in phagocytic cells and mediates killing of invading microorganism by generating a superoxide burst (Bedard and Krause 2007). A brief historical overview of identification and characterization of Nox and Nox isoforms is shown in Figs. 37.2 and 37.3. Seven Nox isoforms (Nox1-5 and DUOX1-2) exist. In addition to the Nox catalytic subunit, the complex also requires regulatory subunits including the membrane-bound subunit p22^{phox} and cytosolic regulatory subunits such as Nox organizer subunits (p47^{phox} and NoxO1), activator subunits (p67^{phox} and NoxA1), two DUOX maturation factors (DUOXA1, DUOXA2), and the small GTP-binding proteins Rac 1/2.

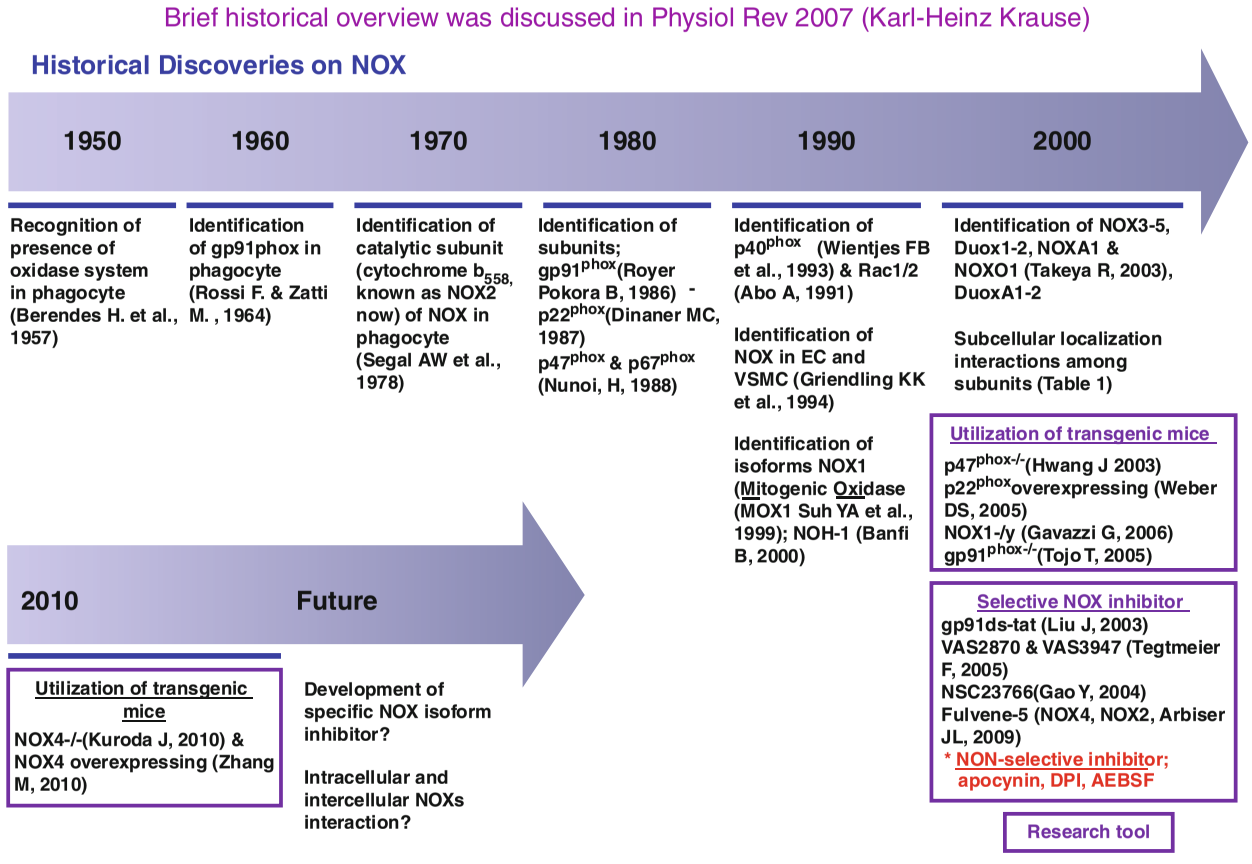

Fig. 37.2 History of discovery and characterization of NOX oxidase family

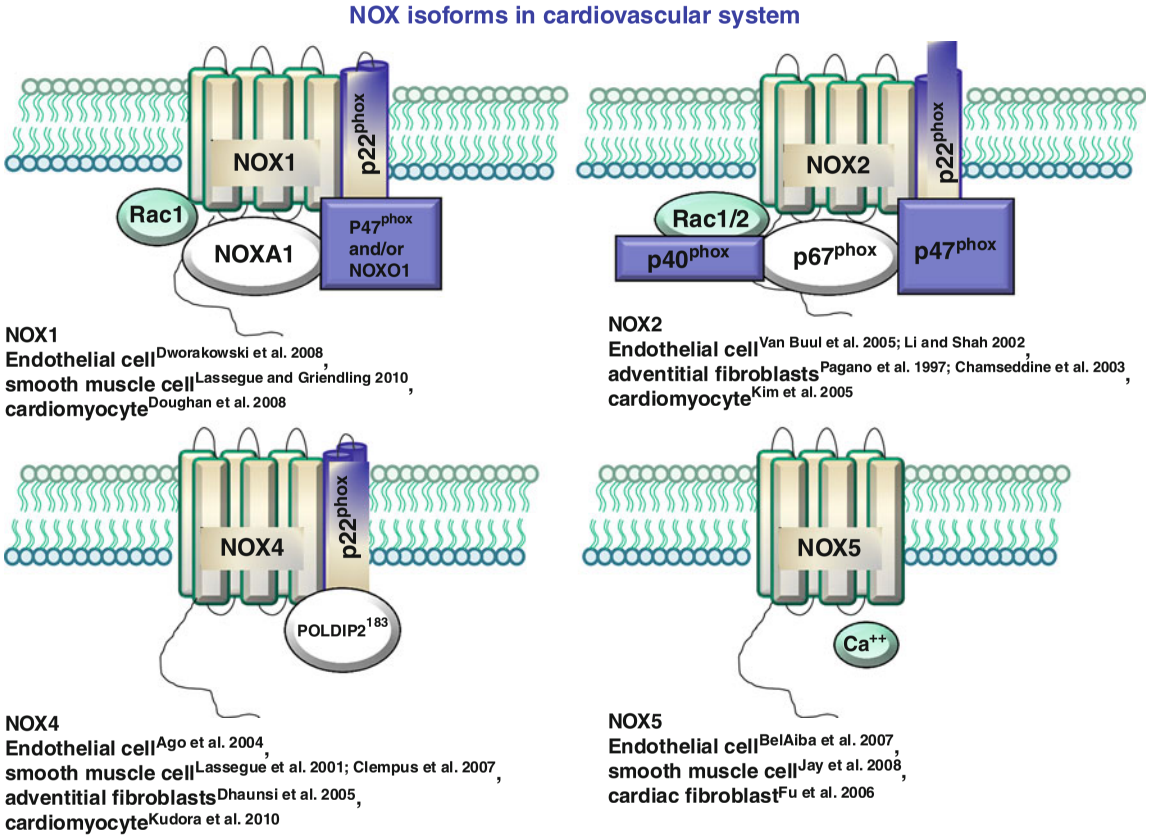

Fig. 37.3 Composition and cell-specific expression of NADPH oxidase isoforms found in the cardiovascular system

In phagocytic cells, assembly of these subunits is required for enzymatic activation of the NADPH oxidases. Nox2, for example, exists in a membrane complex with $p22^{phox}$, referred to as cytochrome b558. The $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ subunits exist in the cytoplasm in resting cells (Van Buul et al. 2005; Wang et al. 2001). Upon activation, Rac1 assembles with the membrane components prior to sequential assembly of other cytosolic components. The other cytoplasmic subunits also translocate to the membrane and interact with $p22^{phox}$ after phosphorylation of $p47^{phox}$ (Groemping et al. 2003). This leads to a functional oxidase that transfers electrons from NADPH to reduce molecular oxygen to form superoxide. Unlike phagocytic Nox2, which is inactive in the resting state, the vascular NADPH oxidases have basal activity in unstimulated state as a pool of them exist in a preassembled form at cytoskeleton (Li and Shah 2002; Sorescu et al. 2001). Mice deficient in Nox2 have modestly reduced blood pressure (Van Buul et al. 2005; Wang et al. 2001). In leukocytes, Rac2 rather than Rac1 interacts with $p67^{phox}$ at the membrane upon stimulation. As summarized in Table 37.1, each Nox isoform has distinct functions, different subunit compositions for assembly, tissue distribution, and intracellular localizations. Moreover, Nox1, 2, 4, and 5 are expressed in cardiovascular cells. Nox5, which is calcium responsive, is absent in rodents but is present in larger animal models or humans.

Mitochondria

The major biological function of the mitochondria is ATP synthesis via oxidative phosphorylation, based on the transfer of electrons through the mitochondrial respiratory chain (Cadenas and Davies 2000). In short, electrons are first supplied into the chain through either NADH at complex I or succinate at complex II, then transferred to complex III via ubiquinone, and subsequently to complex IV, where oxygen is reduced to water and protons are transferred via the proton pump, creating the proton motive force for ATP synthesis (Chance and Williams 1955). The transfer of electrons in the electron transport chain can also leak to oxygen molecules (Han et al. 2003; Boveris 1984), resulting in formation of superoxide that can be rapidly dismutated to H_2O_2 (Cadenas et al. 1977; Han et al. 2001). Under basal conditions, the level of mitochondrial ROS "leakage" is low. However, increased mitochondrial ROS production occurs in endothelial cells during hypoxia (Powell and Jackson 2003), hyperglycemia, and/or diabetes (Giardino et al. 1996), which has been attributed to an increase in the mitochondrial proton gradient (Gokce et al. 2001). In the heart, increased mitochondrial ROS production has been observed during heart failure (Ide et al. 1999), as well as ischemia-reperfusion injury (Arroyo et al. 1987; Bolli et al. 1988; Garlick et al. 1987; Zweier et al. 1987). One of the major processes responsible for increased mitochondrial ROS production is opening of the mitochondrial membrane permeability transition pore (mPTP). While transient opening of mPTP could be protective (Kutala et al. 2007), chronic opening of mPTP is associated with ROS-induced ROS release (Zorov et al. 2000), and implicated in myocardial cell death during reperfusion after ischemia (Crompton 1999; Kutala et al. 2007).

Table 37.1 Nox isoforms in cardiovascular system

Isoform	Distribution	Subcellular localization	Required subunits for activation	Physiological functions
Nox1	Vascular smooth muscle cell (Lassegue and Griendling 2010), endothelial cell (Dworakowski et al. 2008), colon epithelial cell (Szanto et al. 2005), mast cells (Manea et al. 2005), cardiomyocyte (Zhang et al. 2012; Spallarossa et al. 2006)	Plasma membrane (Hanna et al. 2004), caveolae (Hilenski et al. 2004), endosomes (Miller et al. 2007)	$p22^{phox}$, $p47^{phox}$, or Noxo1, Noxa 1, $p40^{phox}$, Rac1 (Banfi et al. 2003; Cheng and Lambeth 2004)	Vascular inflammation (San Martin et al. 2007), migration (Suh et al. 1999), and extracellular matrix production (Schroder et al. 2007)
Nox2	Endothelial cell (Van Buul et al. 2005; Li and Shah 2002), cardiomyocytes (Kim et al. 2005), fibroblasts (Pagano et al. 1997; Chamseddine et al. 2003), neutrophil	Plasma membrane (Petry et al. 2006), perinuclear (Li and Shah 2002; Sipkens et al. 2011), nuclear pore (Hahn et al. 2011), endosome (Li et al. 2008), ER (Petry et al. 2006)	$p22^{phox}$, $p47^{phox}$ (phosphorylation for activation), $p67^{phox}$, $p40^{phox}$, Rac1 or Rac2 (leukocyte only)	Inflammation (Hwang et al. 2003), endothelial cell proliferation & migration (Anilkumar et al. 2008), cardiac hypertrophy and remodeling (Tojo et al. 2005)
Nox4	Fibroblasts (Dhaunsi et al. 2005), vascular smooth muscle cells (Hilenski et al. 2004; Clempus et al. 2007; Lassegue et al. 2001), endothelial cells (Ago et al. 2004; Van Buul et al. 2005; Wingler et al. 2001; Xu et al. 2009), cardiomyocyte (Kudora et al. 2010)	Plasma membrane, ER (Van Buul et al. 2005; Martyn et al. 2006; Pedruzzi et al. 2004), mitochondria (Pedruzzi et al. 2004; Santos et al. 2009), nucleus (Kuroda et al. 2005), and focal adhesions (Hilenski et al. 2004; Clempus et al. 2007; Lyle et al. 2009)	$p22^{phox}$, Poldip2 (Lyle et al. 2009), and Tks5 (Diaz et al. 2009)	Angiogenesis (Craige et al. 2011; Schroder et al. 2012), regulation of focal adhesions and cytoskeletal remodeling (Lyle et al. 2009)
Nox5	Endothelial cell (BelAiba et al. 2007), vascular smooth muscle cells (Jay et al. 2008), cardiac fibroblasts (Fu et al. 2006)	Plasma membrane (Hahn et al. 2012), cytoplasm (Hahn et al. 2012), ER (BelAiba et al. 2007)	N/A	Growth (Montezano et al. 2010)

Uncoupled eNOS

Under physiological conditions, eNOS catalyzes the transfer of electrons from NADPH to the flavins in the reductase domain of one monomer and upon calcium/calmodulin binding to a heme group in the oxygenase domain of another monomer. Oxygen is then reduced and incorporated into one of the terminal

guanidino groups of L-arginine to generate NO and L-citrulline (Wever et al. 1997). When the NOS cofactor tetrahydrobiopterin (H_4B) is either absent or oxidized, electrons are transferred to oxygen to generate superoxide rather than to L-arginine to form NO, leading to a condition known as NOS uncoupling (Wever et al. 1997). NO has multifaceted functions that include relaxation of vascular smooth muscle, inhibition of inflammatory protein expression, and attenuation of platelet and monocyte activation, all critical for vascular health. Activity of eNOS is thus regulated by the bioavailability of its substrate L-Arginine (Xia et al. 1996; Druhan et al. 2008) and its essential cofactor H_4B (Vasquez-Vivar et al. 1998). Under pathophysiological conditions, it is rare for L-arginine to become deficient, unless there is augmented degradation as occurs with excessive arginase activation (Johnson et al. 2005; Romero et al. 2008; Ming et al. 2004; Ryoo et al. 2006).

The eNOS cofactor H_4B is synthesized de novo from GTP by a series of enzymatic steps involving GTP cyclohydrolase-1 (GTPCH-1), 6-pyruvoyl tetrahydropterin synthase (PTPS), and sepiapterin reductase (SPR) (Crabtree and Channon 2011; Thony et al. 2000). Once oxidized to dihydropterin (H_2B), H_4B can be regenerated to H_4B by dihydrofolate reductase (DHFR). The stable exogenous precursor sepiapterin can be converted to H_2B by SPR, and then to H_4B by DHFR. These latter pathways are called salvage pathways (Thony et al. 2000; Werner-Felmayer et al. 2002). eNOS uncoupling due to H_4B deficiency has been implicated in various cardiovascular disorders including atherosclerosis (Takaya et al. 2007), hypertension (Landmesser et al. 2003), diabetes (Oak and Cai 2007; Hink et al. 2001), and heart failure (Takimoto et al. 2005). Ang II treatment can cause deficiency in endothelial DHFR, which in turn mediates eNOS uncoupling in cultured endothelial cells, hypertensive mice, and type 1 diabetic mice (Gao et al. 2009; Chalupsky and Cai 2005; Oak and Cai 2007). Oscillatory shear stress induction of eNOS uncoupling however is attributed to loss of GTPCH-1 activity (Widder et al. 2007; Li et al. 2011). GTPCH-1 overexpression restores H_4B levels and eNOS function in the deoxycorticosterone acetate (DOCA)-salt hypertensive mice, a low renin model of hypertension (Du et al. 2008; Zheng et al. 2003). SPR deficiency has also been observed in DOCA-salt hypertension, making sepiapterin an ineffective agent to restore H_4B levels and NOS function in this particular model of hypertension (Youn et al. 2012b). These data seem to indicate that regulation of different H_4B metabolic enzymes is responsible for eNOS uncoupling in various disease states. More recently, a causal role of uncoupled eNOS has been established in the formation of abdominal aortic aneurysms, in which endothelium-specific DHFR deficiency occurs (Gao et al. 2012).

Xanthine Oxidase

Xanthine oxidase (XO) is one of the two isoforms xanthine oxidoreductase (XOR), the other being xanthine dehydrogenase (XDH) (Hille and Nishino 1995; Stirpe and Della 1969). These isoforms are involved in the last two reactions of the purine degradation pathway, namely, conversion of hypoxanthine to xanthine and the

oxidation of the latter to uric acid (Hille and Nishino 1995; Christen et al. 2001; Nagler et al. 2002). While both forms of XOR catalyze these reactions of purine metabolism, they utilize different electron acceptors. XO can only use oxygen, while XD can use both oxygen and NAD+, but has a greater affinity for NAD+ (Waud and Rajagopalan 1976; Harrison 2002; Meneshian and Bulkley 2002). Hence, in addition to its importance in purine catabolism, XOR is an important generator of oxidative stress. Ironically, uric acid is a potent antioxidant (Becker 1993), making XOR important in both causing oxidative stress and in generating protective antioxidants. Experimental evidence also shows that XO is involved in many different cardiovascular conditions. XOR has also been shown to play an important role in hypertension. For example, inhibition of XOR either by oxypurinol (Nakazono et al. 1991) or by tungsten (Suzuki et al. 1998; Swei et al. 1999) successfully lowers blood pressure in hypertensive rats. Uric acid levels have also been linked with increased blood pressure (Mazzali et al. 2001). Heart failure is another condition where XOR plays a role. Patients with heart failure have increased endothelial XOR activity and reduced SOD activity (Landmesser and Drexler 2002; Cappola et al. 2001). A clinical trial involving the inhibition of XOR using allopurinol (Gavin and Struthers 2005) and the phase II clinical trial by Cardioma Pharma Corp in which oxypurinol was employed have failed to show benefit, casting doubts on the importance of XOR in human heart failure. ROS derived from XOR are increased during ischemia reperfusion (I/R) injury of the heart (Brown et al. 1988; Ferdinandy et al. 1999). Further, inhibition of XOR using allopurinol attenuates I/R damage in animal models (Guan et al. 2003; Gimpel et al. 1995). However, similar to heart failure, multiple clinical trials examining inhibition of XO via allopurinol failed to improve cardiac function (Coghlan et al. 1994; Taggart et al. 1994; Coetzee et al. 1996), with one study actually showing an increase in myocardial infarct extension after allopurinol (Parmley et al. 1992). Taken together, these data show that while XOR is important in experimental animal models, it does not seem to play a central role in the pathogenesis of human cardiovascular disease.

Communication of Oxidases in Cardiovascular System

Interaction Between Nox and eNOS Uncoupling

Nox enzyme activation is associated with clinical risk factors for cardiovascular diseases such as hypercholesterolemia (Sorescu et al. 2002; Guzik et al. 2000; Sheehan et al. 2011), hypertension (Peterson et al. 2009; Harrison et al. 2007), and diabetes (Oak and Cai 2007; Inoguchi et al. 2003) in experimental animals and humans (Lassegue and Griendling 2010; Cai et al. 2003a; Bedard and Krause 2007; Guzik et al. 2000). Interactions between the NADPH oxidases and eNOS uncoupling are well established in hypertension and diabetes (Landmesser et al. 2003). Ang II is a potent activator of the NADPH oxidases. In endothelial cells, Ang II activation of the NADPH oxidases leads to H_2O_2-mediated downregulation of

DHFR, which in turn results in eNOS uncoupling due to H_4B depletion (Chalupsky and Cai 2005). Indeed, DHFR restoration by folic acid supplementation or DHFR overexpression in Ang II-dependent hypertension improved endothelial function along with restored $NO^{\bullet}$ bioavailability (Gao et al. 2009). In type 1 diabetes, eNOS uncoupling occurs following Ang II-dependent Nox activation (Oak and Cai 2007), in particular, activation of Nox1 (Youn et al. 2012a). Nox1-deficient diabetic mice have preserved endothelial function due to recoupling of eNOS (Youn et al. 2012a). In DOCA-salt hypertension, $p47^{phox}$ deficiency prevents H_4B oxidation and NOS uncoupling, indicating NADPH-oxidase-dependent eNOS uncoupling in this low renin model of hypertension. Oscillatory shear stress (OSS) introduction by partial carotid ligation in apoE null mice, which is associated with to $p47^{phox}$-dependent endothelial dysfunction and atherosclerosis (Nam et al. 2009), induces eNOS uncoupling that is due to loss of GTPCH-1 activity (Hattori et al. 2003). The NADPH oxidases are activated in endothelial cells in response to thromboxane A2 receptor activation, via a PKC-ζ-dependent mechanism, subsequently leading to eNOS uncoupling (Zhang et al. 2012). Collectively, NADPH oxidase activation in response to various pathological stimuli causes NOS uncoupling, ROS-dependent oxidation of H_4B, and ROS-induced deficiencies in H_4B metabolic enzymes.

Interaction Between Nox and Mitochondria

Crosstalk between the mitochondria and the NADPH oxidases has been reported in endothelial cells in response to Ang II, which induces mitochondrial dysfunction via protein kinase C–mediated activation of Nox (Doughan et al. 2008). The authors of this study proposed that Ang II activates Nox, which in turn increases mitochondrial ROS production by modulating mitoK_{ATP} channel opening (Doughan et al. 2008; Pain et al. 2000). In this study, L-NAME prevented Ang II induction of mitochondrial dysfunction, indicating that eNOS uncoupling might also lie upstream of mitochondrial ROS production, although the effect of H_4B administration was not examined (Doughan et al. 2008). Administration of apocynin or $p22^{phox}$ knockdown by RNA interference attenuated Ang II-induced mitochondrial ROS production, as measured by DCF fluorescence (Doughan et al. 2008). Furthermore, stimulation of the mitoK_{ATP} channel opening using diazoxide in smooth muscle cells caused Nox activation, mimicking the effect of Ang II (Kimura et al. 2005). Intriguingly, treatment with 5-HD, a specific inhibitor of the mitoK_{ATP} channel, decreased superoxide production and restored bioavailable $NO^{\bullet}$ in endothelial cells treated with angiotensin II, indicating that mitochondrial ROS are acting in a feed-forward mechanism (Kimura et al. 2005). In another study, these authors showed that suppression of mitochondrial ROS using mitochondrial superoxide dismutase (SOD2) overexpression or mitoTEMPO attenuates Nox activity (Dikalova et al. 2010). Hypoxia drives mitochondrial ROS production in pulmonary artery smooth muscle cells (Waypa et al. 2002; Waypa et al. 2006). Further studies using the novel redox-sensitive ratiometric fluorescent protein sensor (RoGFP) demonstrated that hypoxia increases H_2O_2

production in the mitochondrial intermembrane space (Waypa et al. 2010). H_2O_2 can then diffuse to the cytoplasm and activate NADPH oxidase production of ROS and increase calcium via a PKC-dependent pathway in pulmonary artery smooth muscle (Rathore et al. 2008). Conversely, it seems that hypoxia acutely activates the NADPH oxidase, leading to sustained production of mitochondrial ROS (Weidemann and Johnson 2008). Taken together, the NADPH oxidases and mitochondria seem to activate one another in several pathological conditions.

Interaction Between eNOS Uncoupling and Mitochondria

Ceylan-Isik et al. demonstrated that crosstalk exists between eNOS uncoupling and mitochondria production of ROS (Ceylan-Isik et al. 2009). Treatment with the GTPCH-1 inhibitor DAHP depletes H_4B and induces NOS uncoupling in the heart, and this is associated with impaired mitochondrial function in cardiac cells. Cardiac overexpression of the antioxidant metallothionein attenuates mitochondrial dysfunction. These studies provide evidence that ROS-derived NOS uncoupling leads to mitochondrial dysfunction (Ceylan-Isik et al. 2009). Conversely, supplementation of coenzyme Q10, which facilitates electron transport from complexes I and II to complex III and reduces mitochondrial ROS production, recouples eNOS and improves endothelial function in diabetes (Watts et al. 2002) and atherosclerosis (Chew and Watts 2004).

Interaction Between XO and Mitochondria

Ischemia induces ROS in cremaster muscle arterioles and this seems to be due to XO and complex III of the mitochondria (Baudry et al. 2008). A recent study by Gladden et al. showed XO and mitochondria both contribute to ROS production in the volume overloaded heart (Gladden et al. 2011). The mitochondrial inhibitor MitoQ prevented stretch-induced XO activation while the XO inhibitor allopurinol suppressed mitochondrial respiration, suggesting a crosstalk between XO and mitochondria. In this study, the authors found that volume overload increases cardiac XO activity by 300 % and decreases mitochondrial state-3 respiration, which depends on ADP as a substrate. Treatment with allopurinol improved state-3 respiration. It thus seems that XO-derived superoxide damages the mitochondria, leading to generation of additional superoxide.

Oxidases and Cardiovascular Diseases

As described above, a complex relationship exists among the different ROS-generating systems in pathophysiological conditions. In this section, we will examine the roles of ROS-generating oxidases in several cardiovascular diseases.

Hypertension

The earliest study linking ROS and hypertension showed that intravenous injection of a membrane-targeted superoxide dismutase acutely lowers blood pressure in spontaneously hypertensive rats (Nakazono et al. 1991). These authors also showed that oxypurinol lowered blood pressure in the SHR. Subsequent studies showed that angiotensin II and DOCA-salt hypertension increases vascular superoxide production, seemingly by activation of the NADPH oxidase. Increased ROS has been observed in many different animal models of hypertension, including hypertension induced by Ang II (Rajagopalan et al. 1996; Fukui et al. 1997; Mollnau et al. 2002; Dikalova et al. 2005; Murdoch et al. 2011), DOCA-salt (Landmesser et al. 2003; Beswick et al. 2001; Wu et al. 2001), L-NAME (Bauersachs et al. 1998; Usui et al. 1999), renal artery clipping (Jung et al. 2004; Heitzer et al. 1999; Wang et al. 2007), and in genetic models of hypertension (Gao et al. 2012; Kobori and Nishiyama 2004; Wingler et al. 2001; Zalba et al. 2000). Mice deficient in either $p47^{phox}$ or Nox1 have blunted hypertension and reduced vascular superoxide production in response to angiotensin II (Gavazzi et al. 2006; Matsuno et al. 2005; Landmesser et al. 2002), while Nox1-overexpressing animals exhibit enhanced hypertension and vascular ROS production (Dikalova et al. 2005). Similarly, Nox2 is upregulated in many models of hypertension (Wang et al. 2001; Dikalova et al. 2010; Datla and Griendling 2010; Cifuentes et al. 2000). In a study using mice with endothelial-specific overexpression of Nox2, basal BP is unchanged but Ang II infusion causes a greater impairment in endothelium-dependent vasodilatation, vascular remodeling, and a greater increase in blood pressure than observed in wild type controls (Murdoch et al. 2011). Nox2-deficient mice have reduced blood pressure at baseline and following 6 days of angiotensin II infusion (Wang et al. 2001). Nox2 has also been shown to play a role in the central control of hypertension (Peterson et al. 2009; Zimmerman et al. 2004).

The role of Nox4 in hypertension is more controversial. Several studies have found increased vascular expression of Nox4 in hypertension (Wingler et al. 2001; Akasaki et al. 2006), (Mollnau et al. 2002). Interestingly, Nox4 largely releases H_2O_2 rather than superoxide. H_2O_2 can promote vasodilatation, in part by eliciting hyperpolarization of the vascular smooth muscle and by enhancing eNOS expression and activity (Cai et al. 2003b; Miura et al. 2003; Thomas et al. 2002). In keeping with this, targeted overexpression of Nox4 to the endothelium slightly enhances H_2O_2-dependent vasodilation, and slightly lowers blood pressure in mice (Ray et al. 2011).

The NADPH oxidases play a major role in modulating sympathetic outflow from the brain, and in particular neuronal firing within the subfornical organ of the forebrain. This and other circumventricular organs lack a fully developed blood-brain barrier and are responsive to circulating stimuli such as salt and angiotensin II. Nox2 and Nox4 in the subfornical organ are both required for hypertensive and dipsogenic responses to intracerebroventricular (ICV) injections of Ang II (Peterson et al. 2009). Overexpression of the small G-protein Rac1 in the subfornical organ enhances the acute pressor, bradycardic, and dipsogenic effects

of ICV angiotensin II, while a dominant negative form of Rac1 has the opposite effect (Zimmerman et al. 2004). Recently, it has been shown that selective deletion of the NADPH oxidase subunit $p22^{phox}$ from the subfornical organ prevents the long-term hypertensive response to angiotensin II (Lob et al. 2013).

The ROS produced by various oxidants in hypertension do not act in isolation, but interact with each other and with other oxidant systems. As described above, H_2O_2 from the NADPH oxidase downregulates the tetrahydrobiopterin salvage pathway, leading to NOS uncoupling. eNOS uncoupling also occurs in other models of hypertension such as DOCA-salt hypertension (Landmesser et al. 2003). Another study showed that DOCA-salt hypertension is associated with deficiency of GTPCH-1, and that overexpression of GTPCH-1 partially recouples eNOS and improves endothelial dysfunction (Du et al. 2008). In another study, SPR was found to be deficient in DOCA-salt hypertension, so that the H_4B precursor sepiapterin is ineffective in recoupling eNOS. In contrast, co-treatment with the NADPH oxidase inhibitor apocynin and with H_4B effectively restored eNOS function (Youn et al. 2012b). Thus, these data demonstrate that there is the interplay between Nox and eNOS uncoupling in different models of hypertension including Ang II and DOCA-salt dependent.

Evidence also suggests that the mitochondria and NADPH oxidases interact in hypertension. Upregulation of thioredoxin-2, a potent mitochondrial antioxidant, attenuates Ang II-induced hypertension and mitochondrial ROS levels and eliminates the elevation of vascular ROS and the expression of Nox subunits (Nox2, Rac2, p47phox) (Widder et al. 2009). Treatment with the mitochondrial-targeted antioxidant mitoTempol, or overexpression of the mitochondrial superoxide scavenger SOD2, attenuates the increase in blood pressure, mitochondrial ROS production, and Nox activity in response to chronic angiotensin II infusion. These effects are not observed when TEMPOL, which is not targeted to the mitochondria, is used (Dikalova et al. 2010). Taken together, these data not only show the importance of the mitochondria in the pathogenesis of hypertension in experimental models, but also suggests that ROS from the NADPH oxidases induce mitochondrial ROS production.

Diabetic Cardiovascular Complications

Cardiovascular complications are the leading cause of death in patients with diabetes (Macfarlane et al. 2007). Increased vascular production of ROS has been proposed to trigger multiple downstream signaling events that promote endothelial dysfunction in diabetes. Many enzymatic sources, including the mitochondrial electron transport chain, the NADPH oxidases, xanthine oxidase, and uncoupled eNOS, contribute to ROS formation in diabetes. Nonenzymatic sources such as glucose auto-oxidation and AGE formation can contribute as well.

NADPH oxidase activation has been reported in both type I and II diabetes. In particular, upregulation of Nox1 occurs mice with type 1 diabetes induced by streptozotocin (STZ). Nox1, in turn, mediates eNOS uncoupling and impairment of

vessel relaxation in these animals (Youn et al. 2012a). These authors also showed that eNOS uncoupling is attenuated in $p47^{phox}$ and Nox1 null mice (Youn et al. 2012a). Knockdown of Nox1 and Noxo1 by RNA interference in vivo also prevents eNOS uncoupling in diabetes, indicating an upstream role of Noxo1 and $p47^{phox}$ in diabetic eNOS uncoupling (Gao et al. 2009). In contrast, inhibition of mitochondrial complexes does not affect diabetic eNOS uncoupling, indicating that the mitochondria are not upstream system of eNOS uncoupling even though an important role of mitochondria in diabetic endothelium has been previously documented (Nishikawa et al. 2000). Of note, DHFR overexpression and folic acid supplementation improve endothelium-dependent vasodilatation. In the Goto-Kakizaki (GK) rat, a model of non-obese type II diabetes, Nox-derived ROS induce H_4B deficiency and eNOS uncoupling in aorta; however, no information is available regarding which Nox isoform is involved or whether H_4B enzymatic pathways are deficient (Bitar et al. 2005). In the obese db/db mice, Nox1 expression is upregulated and superoxide and peroxynitrite production is increased. In addition, augmented Nox2 expression and mitochondrial dysfunction are observed in the left ventricle, all of which are attenuated by a NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC) (Mariappan et al. 2010). Given that NF-κB is activated by Ang II and PKC, these findings suggest that Ang II-dependent NADPH oxidase activation induces oxidative stress and NF-κB activation, which affects expression of Nox1 and Nox2, mitochondrial ROS production, and subsequently cardiac dysfunction due to oxidant amplification (Mariappan et al. 2010). In keeping with a role of Nox1 in diabetes, a recent study showed that atherosclerotic lesion formation is dramatically enhanced when $ApoE^{-/-}$ mice are made diabetic by STZ injection (Gray et al. 2012). This enhancement of atherosclerosis is prevented by crossing the $ApoE^{-/-}$ mice with mice lacking Nox1 or by treatment with a novel Nox1 inhibitor GKT13783. Taken together, these data show that the NADPH oxidase, and in particular Nox1, plays a role in diabetes and that Nox1-derived ROS likely contribute to NOS uncoupling and activation of pro-inflammatory signaling events.

A major consequence of diabetes is small vessel disease, which leads to blindness, neurologic dysfunction, myocardial ischemia, and kidney failure. Several studies have implicated the NADPH oxidases in microvascular dysfunction in animal models of diabetes (Mayhan et al. 2006; Su et al. 2008; Park et al. 2011). Superoxide generation by the mitochondria is increased in the retina of rats with STZ-induced diabetes, and this promotes retinal endothelial cells and pericytes (Kowluru and Abbas 2003). In this model, uncoupling of NOS has contributed to coronary arteriolar dysfunction, which likely contributes to myocardial ischemia and dysfunction (Bagi and Koller 2003).

Atherosclerosis

The NADPH oxidases have been considered a major source for vascular oxidative stress in human atherosclerosis (Sorescu et al. 2002; Guzik et al. 2000; Azumi et al. 1999)

and animal models (Sheehan et al. 2011). In atherosclerotic human coronary arteries, abundant expression of Nox2, Nox4, and Nox5 occurs, likely due to macrophage infiltration and smooth muscle cell accumulation (Sorescu et al. 2002; Guzik et al. 2008). The presence of Nox5 in advanced lesions leads to calcium-dependent production of H_2O_2, and might explain one benefit of calcium channel inhibitors (Guzik et al. 2008). In ApoE$^{-/-}$ mice, deletion of Nox1 reduces superoxide production, lesion size, and macrophage infiltration (Sheehan et al. 2011), indicating a critical role of Nox1 in atherosclerosis. As discussed below, either Nox1 deficiency or treatment with a Nox1 inhibitor decreases lesions in ApoE$^{-/-}$ mice with diabetes (Gray et al. 2012) Deletion of the cytoplasmic subunit p47^{phox} also reduces aneurysm formation in ApoE$^{-/-}$ mice (Barry-Lane et al. 2001; Thomas et al. 2006). Nox2 is abundantly expressed in macrophages, and levels of the Nox2/p22^{phox} mRNAs in atherosclerotic lesions positively correlate with the presence of these cells in human atherosclerotic lesions (Sorescu et al. 2002). In endothelial cells, Nox2 activity is necessary for induction of the pro-inflammatory transcription factor NF-κB. This and other redox-dependent signals promote upregulation of inflammatory cytokines, such as TNF-α, and of adhesion molecules including VCAM-1, E-selectin, and ICAM-1, all of which are involved in vascular inflammation and atherogenesis (Gertzberg et al. 2004; Chen et al. 2003). The role of Nox2 in atherosclerosis is however controversial. Some studies have shown a reduction in atherosclerosis when Nox2-deficient mice have been crossed with ApoE$^{-/-}$ mice, (Judkins et al. 2010) while others have not (Kirk et al. 2000). Moreover, endothelial-targeted Nox2 overexpression in ApoE$^{-/-}$ mice does not alter atherosclerosis progression even though Nox2 seems to increase vascular superoxide production and macrophage recruitment possibly via activation of endothelial cells (Douglas et al. 2012). Thus, it is clear that Nox1 and Nox2 have distinct roles in atherosclerosis.

Of note, H_4B deficiency and NOS uncoupling activity have been found in ApoE$^{-/-}$ mice. Thus, hypercholesterolemia seems to activate the NADPH oxidases and increase Nox-derived ROS to uncouple eNOS (Alp et al. 2004). Mitochondrial SOD (SOD2) deficiency also increases atherosclerosis in ApoE$^{-/-}$ mice, implicating a role of mitochondrial ROS in atherosclerosis (Ohashi et al. 2006). Taken together, this evidence seems to demonstrate that excessive ROS production from Nox, mitochondria, and uncoupled NOS is involved in the development of atherosclerosis.

Cardiac Diseases

Chronic heart failure is the result of maladaptive changes to stressors such as myocardial infarction, valvular heart disease, and hypertension (Cohn et al. 2000). Studies have suggested that Nox4 is expressed in the mitochondria of cardiomyocytes (Ago et al. 2004), although this could be due to contamination of mitochondrial preparations used for these analyses. Nox4 also plays a role in superoxide generation by the cardiac mitochondria in setting of pressure overload

(Kuroda et al. 2010). NOS uncoupling has also been shown to play an important role in heart failure (Takimoto and Kass 2007). Given that oxidation of the NOS cofactor H_4B leads to its uncoupling, and that the activities of both the mitochondria (Sawyer and Colucci 2000) and NADPH oxidases (Heymes et al. 2003) are increased in heart failure, it is very likely that the three systems interact with each other during this pathophysiological condition.

ROS have also been implicated to play important roles in reperfusion injury following myocardial ischemia (Maxwell and Lip 1997; Eltzschig and Collard 2004). Extensive research has focused upon the mitochondria, which plays a key role in mediating ischemia-reperfusion (I/R) injury via the opening of the mitochondrial mPTP and the release of pro-apoptotic factors such as cytochrome c (Choi et al. 2009; Loor et al. 2011; Perrelli et al. 2011). Interestingly, experimental evidence also shows that there is crosstalk between the different ROS generators during these events. In one study of acute ischemia in the rat heart, it was shown that inhibition of AT1 receptors, which have been shown to increase Nox activity, reduced mitochondrial dysfunction (Monteiro et al. 2005). A link between NADPH oxidase and mitochondrial-dependent cardiomyocyte apoptosis has been defined in a study using $p47^{phox}$-deficient mice (Erickson et al. 2008), where cardiomyocytes from these mice were resistant to CaMKII-dependent apoptosis. Taken together, these data show that while the mitochondria play a major role in the myocardial damage in cardiac I/R, other ROS-generating systems such as the NADPH oxidases and uncoupled eNOS may also play a role via interactions with each other.

Summary and Conclusions

In the cardiovascular system, ROS are generated from various enzymatic sources such as Nox, mitochondria, XO, and uncoupled eNOS. As discussed in detail here, complex interactions among these enzymes contribute to oxidative stress by forming a vicious cycle of ROS-induced ROS generation. Moreover, the NADPH oxidases seem to have a major role in oxidase interactions, and often function as initiating enzymes that lead to oxidant amplification by other enzymes in hypertension, diabetes, atherosclerosis, and cardiac diseases. Even though it is evident that NADPH oxidase activation induces eNOS uncoupling, the underlying mechanisms vary and depend on pathological stimuli (i.e., GTPCH-1 deficiency in DOCA-salt hypertension versus DHFR deficiency in Ang II-induced hypertension). These observations have made it potentially possible to develop disease-specific novel therapies. Although interactions between mitochondria and the NADPH oxidase have been suggested in hypertension and cardiac diseases, direct evidence on interactions among mitochondria, Nox, and uncoupled eNOS is limited. Also, the signaling pathways involved in the interactions among oxidases remain incompletely understood. Future studies in these fields may provide novel therapeutic approaches for prevention and treatment of cardiovascular diseases.

References

Ago T, Kitazono T, Ooboshi H, Iyama T, Han YH, Takada J, Wakisaka M, Ibayashi S, Utsumi H, Iida M (2004) Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase. Circulation 109:227–233

Ahluwalia J (2008) Characterisation of electron currents generated by the human neutrophil NADPH oxidase. Biochem Biophys Res Commun 368:656–661

Akasaki T, Ohya Y, Kuroda J, Eto K, Abe I, Sumimoto H, Iida M (2006) Increased expression of gp91phox homologues of NAD(P)H oxidase in the aortic media during chronic hypertension: involvement of the renin-angiotensin system. Hypertens Res 29:813–820

Alp NJ, McAteer MA, Khoo J, Choudhury RP, Channon KM (2004) Increased endothelial tetrahydrobiopterin synthesis by targeted transgenic GTP-cyclohydrolase I overexpression reduces endothelial dysfunction and atherosclerosis in ApoE-knockout mice. Arterioscler Thromb Vasc Biol 24:445–450

Anilkumar N, Weber R, Zhang M, Brewer A, Shah AM (2008) Nox4 and nox2 NADPH oxidases mediate distinct cellular redox signaling responses to agonist stimulation. Arterioscler Thromb Vasc Biol 28:1347–1354

Arroyo CM, Kramer JH, Dickens BF, Weglicki WB (1987) Identification of free radicals in myocardial ischemia/reperfusion by spin trapping with nitrone DMPO. FEBS Lett 221:101–104

Azumi H, Inoue N, Takeshita S, Rikitake Y, Kawashima S, Hayashi Y, Itoh H, Yokoyama M (1999) Expression of NADH/NADPH oxidase p22phox in human coronary arteries. Circulation 100:1494–1498

Bagi Z, Koller A (2003) Lack of nitric oxide mediation of flow-dependent arteriolar dilation in type I diabetes is restored by sepiapterin. J Vasc Res 40:47–57

Banfi B, Clark RA, Steger K, Krause KH (2003) Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. J Biol Chem 278:3510–3513

Barry-Lane PA, Patterson C, van der Merwe M, Hu Z, Holland SM, Yeh ET, Runge MS (2001) p47phox is required for atherosclerotic lesion progression in ApoE(-/-) mice. J Clin Invest 108:1513–1522

Baudry N, Laemmel E, Vicaut E (2008) In vivo reactive oxygen species production induced by ischemia in muscle arterioles of mice: involvement of xanthine oxidase and mitochondria. Am J Physiol Heart Circ Physiol 294:H821–H828

Bauersachs J, Bouloumie A, Fraccarollo D, Hu K, Busse R, Ertl G (1998) Hydralazine prevents endothelial dysfunction, but not the increase in superoxide production in nitric oxide-deficient hypertension. Eur J Pharmacol 362:77–81

Becker BF (1993) Towards the physiological function of uric acid. Free Radic Biol Med 14:615–631

Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87:245–313

BelAiba RS, Djordjevic T, Petry A, Diemer K, Bonello S, Banfi B, Hess J, Pogrebniak A, Bickel C, Gorlach A (2007) NOX5 variants are functionally active in endothelial cells. Free Radic Biol Med 42:446–459

Beswick RA, Dorrance AM, Leite R, Webb RC (2001) NADH/NADPH oxidase and enhanced superoxide production in the mineralocorticoid hypertensive rat. Hypertension 38:1107–1111

Bitar MS, Wahid S, Mustafa S, Al-Saleh E, Dhaunsi GS, Al-Mulla F (2005) Nitric oxide dynamics and endothelial dysfunction in type II model of genetic diabetes. Eur J Pharmacol 511:53–64

Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB (1988) Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl *N*-tert-butyl nitrone. J Clin Invest 82:476–485

Boveris A (1984) Determination of the production of superoxide radicals and hydrogen peroxide in mitochondria. Methods Enzymol 105:429–435

Brown JM, Terada LS, Grosso MA, Whitmann GJ, Velasco SE, Patt A, Harken AH, Repine JE (1988) Xanthine oxidase produces hydrogen peroxide which contributes to reperfusion injury of ischemic, isolated, perfused rat hearts. J Clin Invest 81:1297–1301

Cadenas E, Davies KJ (2000) Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 29:222–230

Cadenas E, Boveris A, Ragan CI, Stoppani AO (1977) Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. Arch Biochem Biophys 180:248–257

Cai H (2005) Hydrogen peroxide regulation of endothelial function: origins, mechanisms, and consequences. Cardiovasc Res 68:26–36

Cai H, Harrison DG (2000) Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 87:840–844

Cai H, Li Z, Goette A, Mera F, Honeycutt C, Feterik K, Wilcox JN, Dudley SC Jr, Harrison DG, Langberg JJ (2002) Downregulation of endocardial nitric oxide synthase expression and nitric oxide production in atrial fibrillation: potential mechanisms for atrial thrombosis and stroke. Circulation 106:2854–2858

Cai H, Griendling KK, Harrison DG (2003a) The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci 24:471–478

Cai H, Li Z, Davis ME, Kanner W, Harrison DG, Dudley SC Jr (2003b) Akt-dependent phosphorylation of serine 1179 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

Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, Marban E, Hare JM (2001) Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation 104:2407–2411

Ceylan-Isik AF, Guo KK, Carlson EC, Privratsky JR, Liao SJ, Cai L, Chen AF, Ren J (2009) Metallothionein abrogates GTP cyclohydrolase I inhibition-induced cardiac contractile and morphological defects: role of mitochondrial biogenesis. Hypertension 53:1023–1031

Chalupsky K, Cai H (2005) Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. Proc Natl Acad Sci USA 102:9056–9061

Chamseddine AH, Miller FJ Jr (2003) gp91^{phox} contributes to NADPH oxidase activity in aortic fibroblasts but not smooth muscle cells. Am J Physiol Heart Circ Physiol 285:H2284–H2289

Chance B, Williams GR (1955) Respiratory enzymes in oxidative phosphorylation. III. The steady state. J Biol Chem 217:409–427

Chen XL, Zhang Q, Zhao R, Ding X, Tummala PE, Medford RM (2003) Rac1 and superoxide are required for the expression of cell adhesion molecules induced by tumor necrosis factor-alpha in endothelial cells. J Pharmacol Exp Ther 305:573–580

Cheng G, Lambeth JD (2004) NOXO1, regulation of lipid binding, localization, and activation of Nox1 by the Phox homology (PX) domain. J Biol Chem 279:4737–4742

Chew GT, Watts GF (2004) Coenzyme Q10 and diabetic endotheliopathy: oxidative stress and the "recoupling hypothesis". QJM 97:537–548

Choi K, Kim J, Kim GW, Choi C (2009) Oxidative stress-induced necrotic cell death via mitochondira-dependent burst of reactive oxygen species. Curr Neurovasc Res 6:213–222

Christen S, Bifrare YD, Siegenthaler C, Leib SL, Tauber MG (2001) Marked elevation in cortical urate and xanthine oxidoreductase activity in experimental bacterial meningitis. Brain Res 900:244–251

Cifuentes ME, Rey FE, Carretero OA, Pagano PJ (2000) Upregulation of p67(phox) and gp91 (phox) in aortas from angiotensin II-infused mice. Am J Physiol Heart Circ Physiol 279: H2234–H2240

Clempus RE, Sorescu D, Dikalova AE, Pounkova L, Jo P, Sorescu GP, Schmidt HH, Lassegue B, Griendling KK (2007) Nox4 is required for maintenance of the differentiated vascular smooth muscle cell phenotype. Arterioscler Thromb Vasc Biol 27:42–48

Coetzee A, Roussouw G, Macgregor L (1996) Failure of allopurinol to improve left ventricular stroke work after cardiopulmonary bypass surgery. J Cardiothorac Vasc Anesth 10:627–633

Coghlan JG, Flitter WD, Clutton SM, Panda R, Daly R, Wright G, Ilsley CD, Slater TF (1994) Allopurinol pretreatment improves postoperative recovery and reduces lipid peroxidation in patients undergoing coronary artery bypass grafting. J Thorac Cardiovasc Surg 107:248–256

Cohn JN, Ferrari R, Sharpe N (2000) Cardiac remodeling–concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an international forum on cardiac remodeling. J Am Coll Cardiol 35:569–582

Costa AD, Quinlan CL, Andrukhiv A, West IC, Jaburek M, Garlid KD (2006) The direct physiological effects of mitoK(ATP) opening on heart mitochondria. Am J Physiol Heart Circ Physiol 290:H406–H415

Crabtree MJ, Channon KM (2011) Synthesis and recycling of tetrahydrobiopterin in endothelial function and vascular disease. Nitric Oxide 25:81–88

Craige SM, Chen K, Pei Y, Li C, Huang X, Chen C, Shibata R, Sato K, Walsh K, Keaney JF Jr (2011) NADPH oxidase 4 promotes endothelial angiogenesis through endothelial nitric oxide synthase activation. Circulation 124:731–740

Crompton M (1999) The mitochondrial permeability transition pore and its role in cell death. Biochem J 341(Pt 2):233–249

Datla SR, Griendling KK (2010) Reactive oxygen species, NADPH oxidases, and hypertension. Hypertension 56:325–330

Dhaunsi GS, Kaur J, Alsaeid K, Turner RB, Bitar MS (2005) Very long chain fatty acids activate NADPH oxidase in human dermal fibroblasts. Cell Biochem Funct 23:65–68

Diaz B, Shani G, Pass I, Anderson D, Quintavalle M, Courtneidge SA (2009) Tks5-dependent, nox-mediated generation of reactive oxygen species is necessary for invadopodia formation. Sci Signal 2:ra53

Dikalova A, Clempus R, Lassegue B, Cheng G, McCoy J, Dikalov S, San Martin A, Lyle A, Weber DS, Weiss D, Taylor WR, Schmidt HH, Owens GK, Lambeth JD, Griendling KK (2005) Nox1 overexpression potentiates angiotensin II-induced hypertension and vascular smooth muscle hypertrophy in transgenic mice. Circulation 112:2668–2676

Dikalova AE, Bikineyeva AT, Budzyn K, Nazarewicz RR, McCann L, Lewis W, Harrison DG, Dikalov SI (2010) Therapeutic targeting of mitochondrial superoxide in hypertension. Circ Res 107:106–116

Doughan AK, Harrison DG, Dikalov SI (2008) Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. Circ Res 102:488–496

Douglas G, Bendall JK, Crabtree MJ, Tatham AL, Carter EE, Hale AB, Channon KM (2012) Endothelial-specific Nox2 overexpression increases vascular superoxide and macrophage recruitment in ApoE(-)/(-) mice. Cardiovasc Res 94:20–29

Druhan LJ, Forbes SP, Pope AJ, Chen CA, Zweier JL, Cardounel AJ (2008) Regulation of eNOS-derived superoxide by endogenous methylarginines. Biochemistry 47:7256–7263

Du YH, Guan YY, Alp NJ, Channon KM, Chen AF (2008) Endothelium-specific GTP cyclohydrolase I overexpression attenuates blood pressure progression in salt-sensitive low-renin hypertension. Circulation 117:1045–1054

Dworakowski R, Alom-Ruiz SP, Shah AM (2008) NADPH oxidase-derived reactive oxygen species in the regulation of endothelial phenotype. Pharmacol Rep 60:21–28

Eltzschig HK, Collard CD (2004) Vascular ischaemia and reperfusion injury. Br Med Bull 70:71–86

Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, Zimmerman MC, Zimmerman K, Ham AJ, Weiss RM, Spitz DR, Shea MA, Colbran RJ, Mohler PJ, Anderson ME (2008) A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell 133:462–474

Ferdinandy P, Panas D, Schulz R (1999) Peroxynitrite contributes to spontaneous loss of cardiac efficiency in isolated working rat hearts. Am J Physiol 276:H1861–H1867

Fu X, Beer DG, Behar J, Wands J, Lambeth D, Cao W (2006) cAMP-response element-binding protein mediates acid-induced NADPH oxidase NOX5-S expression in Barrett esophageal adenocarcinoma cells. J Biol Chem 281:20368–20382

Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers QT, Taylor WR, Harrison DG, de Leon H, Wilcox JN, Griendling KK (1997) p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. Circ Res 80:45–51

Gao L, Chalupsky K, Stefani E, Cai H (2009) Mechanistic insights into folic acid-dependent vascular protection: dihydrofolate reductase (DHFR)-mediated reduction in oxidant stress in endothelial cells and angiotensin II-infused mice: a novel HPLC-based fluorescent assay for DHFR activity. J Mol Cell Cardiol 47:752–760

Gao L, Siu KL, Chalupsky K, Nguyen A, Chen P, Weintraub NL, Galis Z, Cai H (2012) Role of uncoupled endothelial nitric oxide synthase in abdominal aortic aneurysm formation: treatment with folic acid. Hypertension 59:158–166

Garlick PB, Davies MJ, Hearse DJ, Slater TF (1987) Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy. Circ Res 61:757–760

Gavazzi G, Banfi B, Deffert C, Fiette L, Schappi M, Herrmann F, Krause KH (2006) Decreased blood pressure in NOX1-deficient mice. FEBS Lett 580:497–504

Gavin AD, Struthers AD (2005) Allopurinol reduces B-type natriuretic peptide concentrations and haemoglobin but does not alter exercise capacity in chronic heart failure. Heart 91:749–753

Gertzberg N, Neumann P, Rizzo V, Johnson A (2004) NAD(P)H oxidase mediates the endothelial barrier dysfunction induced by TNF-alpha. Am J Physiol Lung Cell Mol Physiol 286:L37–L48

Giardino I, Edelstein D, Brownlee M (1996) BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. J Clin Invest 97:1422–1428

Gimpel JA, Lahpor JR, van der Molen AJ, Damen J, Hitchcock JF (1995) Reduction of reperfusion injury of human myocardium by allopurinol: a clinical study. Free Radic Biol Med 19:251–255

Gladden JD, Zelickson BR, Wei CC, Ulasova E, Zheng J, Ahmed MI, Chen Y, Bamman M, Ballinger S, Darley-Usmar V, Dell'Italia LJ (2011) Novel insights into interactions between mitochondria and xanthine oxidase in acute cardiac volume overload. Free Radic Biol Med 51:1975–1984

Gokce N, Holbrook M, Duffy SJ, Demissie S, Cupples LA, Biegelsen E, Keaney JF Jr, Loscalzo J, Vita JA (2001) Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. Hypertension 38:1349–1354

Gray SP, Di Marco E, Okabe J, Szyndralewiez C, Heitz F, Montezano AC, de Haan JB, Koulis C, El-Osta A, Andrews KL, Chin-Dusting JP, Touyz RM, Wingler K, Cooper ME, Schmidt HH, Jandeleit-Dahm KA (2012) NADPH oxidase 1 plays a key role in diabetes mellitus-accelerated atherosclerosis. Circulation 127:1888–1902

Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW (1994) Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 74:1141–1148

Groemping Y, Lapouge K, Smerdon SJ, Rittinger K (2003) Molecular basis of phosphorylation-induced activation of the NADPH oxidase. Cell 113:343–355

Guan W, Osanai T, Kamada T, Hanada H, Ishizaka H, Onodera H, Iwasa A, Fujita N, Kudo S, Ohkubo T, Okumura K (2003) Effect of allopurinol pretreatment on free radical generation after primary coronary angioplasty for acute myocardial infarction. J Cardiovasc Pharmacol 41:699–705

Gupte SA, Kaminski PM, George S, Kouznestova L, Olson SC, Mathew R, Hintze TH, Wolin MS (2009) Peroxide generation by p47phox-Src activation of Nox2 has a key role in protein kinase C-induced arterial smooth muscle contraction. Am J Physiol Heart Circ Physiol 296: H1048–H1057

Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM (2000) Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. Circ Res 86:E85–E90

Guzik TJ, Chen W, Gongora MC, Guzik B, Lob HE, Mangalat D, Hoch N, Dikalov S, Rudzinski P, Kapelak B, Sadowski J, Harrison DG (2008) Calcium-dependent NOX5 nicotinamide adenine

dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. J Am Coll Cardiol 52:1803–1809

Hahn NE, Meischl C, Wijnker PJ, Musters RJ, Fornerod M, Janssen HW, Paulus WJ, van Rossum AC, Niessen HW, Krijnen PA (2011) NOX2, p22phox and p47phox are targeted to the nuclear pore complex in ischemic cardiomyocytes colocalizing with local reactive oxygen species. Cell Physiol Biochem 27:471–478

Hahn NE, Meischl C, Kawahara T, Musters RJ, Verhoef VM, van der Velden J, Vonk AB, Paulus WJ, van Rossum AC, Niessen HW, Krijnen PA (2012) NOX5 expression is increased in intramyocardial blood vessels and cardiomyocytes after acute myocardial infarction in humans. Am J Pathol 180:2222–2229

Han D, Williams E, Cadenas E (2001) Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. Biochem J 353:411–416

Han D, Antunes F, Canali R, Rettori D, Cadenas E (2003) Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. J Biol Chem 278:5557–5563

Hanna IR, Hilenski LL, Dikalova A, Taniyama Y, Dikalov S, Lyle A, Quinn MT, Lassegue B, Griendling KK (2004) Functional association of nox1 with p22phox in vascular smooth muscle cells. Free Radic Biol Med 37:1542–1549

Harrison R (2002) Structure and function of xanthine oxidoreductase: where are we now? Free Radic Biol Med 33:774–797

Harrison DG, Gongora MC, Guzik TJ, Widder J (2007) Oxidative stress and hypertension. J Am Soc Hypertens 1:30–44

Hattori Y, Nakanishi N, Akimoto K, Yoshida M, Kasai K (2003) HMG-CoA reductase inhibitor increases GTP cyclohydrolase I mRNA and tetrahydrobiopterin in vascular endothelial cells. Arterioscler Thromb Vasc Biol 23:176–182

Heitzer T, Wenzel U, Hink U, Krollner D, Skatchkov M, Stahl RA, MacHarzina R, Brasen JH, Meinertz T, Munzel T (1999) Increased NAD(P)H oxidase-mediated superoxide production in renovascular hypertension: evidence for an involvement of protein kinase C. Kidney Int 55:252–260

Heymes C, Bendall JK, Ratajczak P, Cave AC, Samuel JL, Hasenfuss G, Shah AM (2003) Increased myocardial NADPH oxidase activity in human heart failure. J Am Coll Cardiol 41:2164–2171

Hilenski LL, Clempus RE, Quinn MT, Lambeth JD, Griendling KK (2004) Distinct subcellular localizations of Nox1 and Nox4 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 24:677–683

Hille R, Nishino T (1995) Flavoprotein structure and mechanism. 4. Xanthine oxidase and xanthine dehydrogenase. Faseb J 9:995–1003

Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl RA, Warnholtz A, Meinertz T, Griendling K, Harrison DG, Forstermann U, Munzel T (2001) Mechanisms underlying endothelial dysfunction in diabetes mellitus. Circ Res 88:E14–E22

Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, Dikalov S, Giddens DP, Griendling KK, Harrison DG, Jo H (2003) Oscillatory shear stress stimulates endothelial production of O2- from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion. J Biol Chem 278:47291–47298. Epub 42003 Sep 47294

Ide T, Tsutsui H, Kinugawa S, Utsumi H, Kang D, Hattori N, Uchida K, Arimura K, Egashira K, Takeshita A (1999) Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. Circ Res 85:357–363

Ide T, Tsutsui H, Kinugawa S, Suematsu N, Hayashidani S, Ichikawa K, Utsumi H, Machida Y, Egashira K, Takeshita A (2000) Direct evidence for increased hydroxyl radicals originating from superoxide in the failing myocardium. Circ Res 86:152–157

Inoguchi T, Sonta T, Tsubouchi H, Etoh T, Kakimoto M, Sonoda N, Sato N, Sekiguchi N, Kobayashi K, Sumimoto H, Utsumi H, Nawata H (2003) Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: role of vascular NAD(P)H oxidase. J Am Soc Nephrol 14:S227–S232

Jay DB, Papaharalambus CA, Seidel-Rogol B, Dikalova AE, Lassegue B, Griendling KK (2008) Nox5 mediates PDGF-induced proliferation in human aortic smooth muscle cells. Free Radic Biol Med 45:329–335

Johnson FK, Johnson RA, Peyton KJ, Durante W (2005) Arginase inhibition restores arteriolar endothelial function in Dahl rats with salt-induced hypertension. Am J Physiol Regul Integr Comp Physiol 288:R1057–R1062

Judkins CP, Diep H, Broughton BR, Mast AE, Hooker EU, Miller AA, Selemidis S, Dusting GJ, Sobey CG, Drummond GR (2010) Direct evidence of a role for Nox2 in superoxide production, reduced nitric oxide bioavailability, and early atherosclerotic plaque formation in ApoE-/- mice. Am J Physiol Heart Circ Physiol 298:H24–H32

Jung O, Schreiber JG, Geiger H, Pedrazzini T, Busse R, Brandes RP (2004) gp91phox-containing NADPH oxidase mediates endothelial dysfunction in renovascular hypertension. Circulation 109:1795–1801

Kim YM, Guzik TJ, Zhang YH, Zhang MH, Kattach H, Ratnatunga C, Pillai R, Channon KM, Casadei B (2005) A myocardial Nox2 containing NAD(P)H oxidase contributes to oxidative stress in human atrial fibrillation. Circ Res 97:629–636

Kimura S, Zhang GX, Nishiyama A, Shokoji T, Yao L, Fan YY, Rahman M, Suzuki T, Maeta H, Abe Y (2005) Role of NAD(P)H oxidase- and mitochondria-derived reactive oxygen species in cardioprotection of ischemic reperfusion injury by angiotensin II. Hypertension 45:860–866

Kirk EA, Dinauer MC, Rosen H, Chait A, Heinecke JW, LeBoeuf RC (2000) Impaired superoxide production due to a deficiency in phagocyte NADPH oxidase fails to inhibit atherosclerosis in mice. Arterioscler Thromb Vasc Biol 20:1529–1535

Kobori H, Nishiyama A (2004) Effects of tempol on renal angiotensinogen production in Dahl salt-sensitive rats. Biochem Biophys Res Commun 315:746–750

Kowluru RA, Abbas SN (2003) Diabetes-induced mitochondrial dysfunction in the retina. Invest Ophthalmol Vis Sci 44:5327–5334

Krijnen PA, Meischl C, Hack CE, Meijer CJ, Visser CA, Roos D, Niessen HW (2003) Increased Nox2 expression in human cardiomyocytes after acute myocardial infarction. J Clin Pathol 56:194–199

Kuroda J, Nakagawa K, Yamasaki T, Nakamura K, Takeya R, Kuribayashi F, Imajoh-Ohmi S, Igarashi K, Shibata Y, Sueishi K, Sumimoto H (2005) The superoxide-producing NAD(P)H oxidase Nox4 in the nucleus of human vascular endothelial cells. Genes Cells 10:1139–1151

Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, Sadoshima J (2010) NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. Proc Natl Acad Sci USA 107:15565–15570

Kutala VK, Khan M, Angelos MG, Kuppusamy P (2007) Role of oxygen in postischemic myocardial injury. Antioxid Redox Signal 9:1193–1206

Lambeth JD (2004) NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4:181–189

Landmesser U, Drexler H (2002) Allopurinol and endothelial function in heart failure: future or fantasy? Circulation 106:173–175

Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, Holland SM, Harrison DG (2002) Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. Hypertension 40:511–515

Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG (2003) Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 111:1201–1209

Lassegue B, Griendling KK (2010) NADPH oxidases: functions and pathologies in the vasculature. Arterioscler Thromb Vasc Biol 30:653–661

Lassegue B, Sorescu D, Szocs K, Yin Q, Akers M, Zhang Y, Grant SL, Lambeth JD, Griendling KK (2001) Novel gp91(phox) homologues in vascular smooth muscle cells : nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. Circ Res 88:888–894

Li JM, Shah AM (2002) Intracellular localization and preassembly of the NADPH oxidase complex in cultured endothelial cells. J Biol Chem 277:19952–19960
Li Q, Zhang Y, Marden JJ, Banfi B, Engelhardt JF (2008) Endosomal NADPH oxidase regulates c-Src activation following hypoxia/reoxygenation injury. Biochem J 411:531–541
Li L, Chen W, Rezvan A, Jo H, Harrison DG (2011) Tetrahydrobiopterin deficiency and nitric oxide synthase uncoupling contribute to atherosclerosis induced by disturbed flow. Arterioscler Thromb Vasc Biol 31:1547–1554
Liu S, Ma X, Gong M, Shi L, Lincoln T, Wang S (2007) Glucose down-regulation of cGMP-dependent protein kinase I expression in vascular smooth muscle cells involves NAD(P)H oxidase-derived reactive oxygen species. Free Radic Biol Med 42:852–863
Lob HE, Schultz D, Marvar PJ, Davisson RL, Harrison DG (2013) Role of the NADPH oxidases in the subfornical organ in angiotensin II-induced hypertension. Hypertension 61(2):382–7
Loffredo L, Martino F, Carnevale R, Pignatelli P, Catasca E, Perri L, Calabrese CM, Palumbo MM, Baratta F, Del Ben M, Angelico F, Violi F (2012) Obesity and hypercholesterolemia are associated with NOX2 generated oxidative stress and arterial dysfunction. J Pediatr 161(6):1004–1009
Loor G, Kondapalli J, Iwase H, Chandel NS, Waypa GB, Guzy RD, Vanden Hoek TL, Schumacker PT (2011) Mitochondrial oxidant stress triggers cell death in simulated ischemia-reperfusion. Biochim Biophys Acta 1813:1382–1394
Lyle AN, Deshpande NN, Taniyama Y, Seidel-Rogol B, Pounkova L, Du P, Papaharalambus C, Lassegue B, Griendling KK (2009) Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells. Circ Res 105:249–259
Macfarlane DP, Paterson KR, Fisher M (2007) Oral antidiabetic agents as cardiovascular drugs. Diabetes Obes Metab 9:23–30
Manea A, Raicu M, Simionescu M (2005) Expression of functionally phagocyte-type NAD(P)H oxidase in pericytes: effect of angiotensin II and high glucose. Biol Cell 97:723–734
Mariappan N, Elks CM, Sriramula S, Guggilam A, Liu Z, Borkhsenious O, Francis J (2010) NF-kappaB-induced oxidative stress contributes to mitochondrial and cardiac dysfunction in type II diabetes. Cardiovasc Res 85:473–483
Martyn KD, Frederick LM, von Loehneysen K, Dinauer MC, Knaus UG (2006) Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. Cell Signal 18:69–82
Matsuno K, Yamada H, Iwata K, Jin D, Katsuyama M, Matsuki M, Takai S, Yamanishi K, Miyazaki M, Matsubara H, Yabe-Nishimura C (2005) Nox1 is involved in angiotensin II-mediated hypertension: a study in Nox1-deficient mice. Circulation 112:2677–2685
Maxwell SR, Lip GY (1997) Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. Int J Cardiol 58:95–117
Mayhan WG, Arrick DM, Sharpe GM, Patel KP, Sun H (2006) Inhibition of NAD(P)H oxidase alleviates impaired NOS-dependent responses of pial arterioles in type 1 diabetes mellitus. Microcirculation 13:567–575
Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, Lan HY, Kivlighn S, Johnson RJ (2001) Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension 38:1101–1106
Meneshian A, Bulkley GB (2002) The physiology of endothelial xanthine oxidase: from urate catabolism to reperfusion injury to inflammatory signal transduction. Microcirculation 9:161–175
Miller FJ Jr, Filali M, Huss GJ, Stanic B, Chamseddine A, Barna TJ, Lamb FS (2007) Cytokine activation of nuclear factor kappa B in vascular smooth muscle cells requires signaling endosomes containing Nox1 and ClC-3. Circ Res 101:663–671
Ming XF, Barandier C, Viswambharan H, Kwak BR, Mach F, Mazzolai L, Hayoz D, Ruffieux J, Rusconi S, Montani JP, Yang Z (2004) Thrombin stimulates human endothelial arginase enzymatic activity via RhoA/ROCK pathway: implications for atherosclerotic endothelial dysfunction. Circulation 110:3708–3714
Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, Gutterman DD (2003) Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. Circ Res 92:e31–e40

Mollnau H, Wendt M, Szocs K, Lassegue B, Schulz E, Oelze M, Li H, Bodenschatz M, August M, Kleschyov AL, Tsilimingas N, Walter U, Forstermann U, Meinertz T, Griendling K, Munzel T (2002) Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. Circ Res 90:E58–E65

Monteiro P, Duarte AI, Goncalves LM, Providencia LA (2005) Valsartan improves mitochondrial function in hearts submitted to acute ischemia. Eur J Pharmacol 518:158–164

Montezano AC, Burger D, Paravicini TM, Chignalia AZ, Yusuf H, Almasri M, He Y, Callera GE, He G, Krause KH, Lambeth D, Quinn MT, Touyz RM (2010) Nicotinamide adenine dinucleotide phosphate reduced oxidase 5 (Nox5) regulation by angiotensin II and endothelin-1 is mediated via calcium/calmodulin-dependent, rac-1-independent pathways in human endothelial cells. Circ Res 106:1363–1373

Murdoch CE, Alom-Ruiz SP, Wang M, Zhang M, Walker S, Yu B, Brewer A, Shah AM (2011) Role of endothelial Nox2 NADPH oxidase in angiotensin II-induced hypertension and vasomotor dysfunction. Basic Res Cardiol 106:527–538

Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick AZ (2002) Characterization of the differentiated antioxidant profile of human saliva. Free Radic Biol Med 32:268–277

Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M (1991) Does superoxide underlie the pathogenesis of hypertension? Proc Natl Acad Sci USA 88:10045–10048

Nam D, Ni CW, Rezvan A, Suo J, Budzyn K, Llanos A, Harrison D, Giddens D, Jo H (2009) Partial carotid ligation is a model of acutely induced disturbed flow, leading to rapid endothelial dysfunction and atherosclerosis. Am J Physiol Heart Circ Physiol 297:H1535–H1543

Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 404:787–790

Nishino T (1994) The conversion of xanthine dehydrogenase to xanthine oxidase and the role of the enzyme in reperfusion injury. J Biochem 116:1–6

Oak JH, Cai H (2007) Attenuation of angiotensin II signaling recouples eNOS and inhibits nonendothelial NOX activity in diabetic mice. Diabetes 56:118–126

Ohashi M, Runge MS, Faraci FM, Heistad DD (2006) MnSOD deficiency increases endothelial dysfunction in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 26:2331–2336

Pagano PJ, Clark JK, Cifuentes-Pagano ME, Clark SM, Callis GM, Quinn MT (1997) Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. Proc Natl Acad Sci USA 94:14483–14488

Pain T, Yang XM, Critz SD, Yue Y, Nakano A, Liu GS, Heusch G, Cohen MV, Downey JM (2000) Opening of mitochondrial K(ATP) channels triggers the preconditioned state by generating free radicals. Circ Res 87:460–466

Park Y, Yang J, Zhang H, Chen X, Zhang C (2011) Effect of PAR2 in regulating TNF-alpha and NAD(P)H oxidase in coronary arterioles in type 2 diabetic mice. Basic Res Cardiol 106:111–123

Parmley LF, Mufti AG, Downey JM (1992) Allopurinol therapy of ischemic heart disease with infarct extension. Can J Cardiol 8:280–286

Pedruzzi E, Guichard C, Ollivier V, Driss F, Fay M, Prunet C, Marie JC, Pouzet C, Samadi M, Elbim C, O'Dowd Y, Bens M, Vandewalle A, Gougerot-Pocidalo MA, Lizard G, Ogier-Denis E (2004) NAD(P)H oxidase Nox-4 mediates 7-ketocholesterol-induced endoplasmic reticulum stress and apoptosis in human aortic smooth muscle cells. Mol Cell Biol 24:10703–10717

Perrelli MG, Pagliaro P, Penna C (2011) Ischemia/reperfusion injury and cardioprotective mechanisms: Role of mitochondria and reactive oxygen species. World J Cardiol 3:186–200

Peshavariya H, Dusting GJ, Jiang F, Halmos LR, Sobey CG, Drummond GR, Selemidis S (2009) NADPH oxidase isoform selective regulation of endothelial cell proliferation and survival. Naunyn Schmiedebergs Arch Pharmacol 380:193–204

Peterson JR, Burmeister MA, Tian X, Zhou Y, Guruju MR, Stupinski JA, Sharma RV, Davisson RL (2009) Genetic silencing of Nox2 and Nox4 reveals differential roles of these NADPH oxidase homologues in the vasopressor and dipsogenic effects of brain angiotensin II. Hypertension 54:1106–1114

Petry A, Djordjevic T, Weitnauer M, Kietzmann T, Hess J, Gorlach A (2006) NOX2 and NOX4 mediate proliferative response in endothelial cells. Antioxid Redox Signal 8:1473–1484

Powell CS, Jackson RM (2003) Mitochondrial complex I, aconitase, and succinate dehydrogenase during hypoxia-reoxygenation: modulation of enzyme activities by MnSOD. Am J Physiol Lung Cell Mol Physiol 285:L189–L198

Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG (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

Rathore R, Zheng YM, Niu CF, Liu QH, Korde A, Ho YS, Wang YX (2008) Hypoxia activates NADPH oxidase to increase [ROS]i and [Ca2+]i through the mitochondrial ROS-PKCepsilon signaling axis in pulmonary artery smooth muscle cells. Free Radic Biol Med 45:1223–1231

Ray R, Murdoch CE, Wang M, Santos CX, Zhang M, Alom-Ruiz S, Anilkumar N, Ouattara A, Cave AC, Walker SJ, Grieve DJ, Charles RL, Eaton P, Brewer AC, Shah AM (2011) Endothelial Nox4 NADPH oxidase enhances vasodilatation and reduces blood pressure in vivo. Arterioscler Thromb Vasc Biol 31:1368–1376

Romero MJ, Platt DH, Tawfik HE, Labazi M, El-Remessy AB, Bartoli M, Caldwell RB, Caldwell RW (2008) Diabetes-induced coronary vascular dysfunction involves increased arginase activity. Circ Res 102:95–102

Ryoo S, Lemmon CA, Soucy KG, Gupta G, White AR, Nyhan D, Shoukas A, Romer LH, Berkowitz DE (2006) Oxidized low-density lipoprotein-dependent endothelial arginase II activation contributes to impaired nitric oxide signaling. Circ Res 99:951–960

San Martin A, Foncea R, Laurindo FR, Ebensperger R, Griendling KK, Leighton F (2007) Nox1-based NADPH oxidase-derived superoxide is required for VSMC activation by advanced glycation end-products. Free Radic Biol Med 42:1671–1679

Santos CX, Tanaka LY, Wosniak J, Laurindo FR (2009) Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. Antioxid Redox Signal 11:2409–2427

Sawyer DB, Colucci WS (2000) Mitochondrial oxidative stress in heart failure: "oxygen wastage" revisited. Circ Res 86:119–120

Schroder K, Helmcke I, Palfi K, Krause KH, Busse R, Brandes RP (2007) Nox1 mediates basic fibroblast growth factor-induced migration of vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 27:1736–1743

Schroder K, Zhang M, Benkhoff S, Mieth A, Pliquett R, Kosowski J, Kruse C, Luedike P, Michaelis UR, Weissmann N, Dimmeler S, Shah AM, Brandes RP (2012) Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. Circ Res 110:1217–1225

Sheehan AL, Carrell S, Johnson B, Stanic B, Banfi B, Miller FJ Jr (2011) Role for Nox1 NADPH oxidase in atherosclerosis. Atherosclerosis 216:321–326

Sipkens JA, Hahn N, van den Brand CS, Meischl C, Cillessen SA, Smith DE, Juffermans LJ, Musters RJ, Roos D, Jakobs C, Blom HJ, Smulders YM, Krijnen PA, Stehouwer CD, Rauwerda JA, van Hinsbergh VW, Niessen HW (2011) Homocysteine-induced apoptosis in endothelial cells coincides with nuclear NOX2 and peri-nuclear NOX4 activity. Cell Biochem Biophys. doi:10.1007/s12013-011-9297-y

Sorescu D, Somers MJ, Lassegue B, Grant S, Harrison DG, Griendling KK (2001) Electron spin resonance characterization of the NAD(P)H oxidase in vascular smooth muscle cells. Free Radic Biol Med 30:603–612

Sorescu D, Weiss D, Lassegue B, Clempus RE, Szocs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, Griendling KK (2002) Superoxide production and expression of nox family proteins in human atherosclerosis. Circulation 105:1429–1435

Spallarossa P, Altieri P, Garibaldi S, Ghigliotti G, Barisione C, Manca V, Fabbi P, Ballestrero A, Brunelli C, Barsotti A (2006) Matrix metalloproteinase-2 and -9 are induced differently by

doxorubicin in H9c2 cells: the role of MAP kinases and NAD(P)H oxidase. Cardiovasc Res 69:736–745

Stirpe F, Della CE (1969) The regulation of rat liver xanthine oxidase. Conversion in vitro of the enzyme activity from dehydrogenase (type D) to oxidase (type O). J Biol Chem 244:3855–3863

Su J, Lucchesi PA, Gonzalez-Villalobos RA, Palen DI, Rezk BM, Suzuki Y, Boulares HA, Matrougui K (2008) Role of advanced glycation end products with oxidative stress in resistance artery dysfunction in type 2 diabetic mice. Arterioscler Thromb Vasc Biol 28:1432–1438

Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, Lambeth JD (1999) Cell transformation by the superoxide-generating oxidase Mox1. Nature 401:79–82

Suzuki H, DeLano FA, Parks DA, Jamshidi N, Granger DN, Ishii H, Suematsu M, Zweifach BW, Schmid-Schonbein GW (1998) Xanthine oxidase activity associated with arterial blood pressure in spontaneously hypertensive rats. Proc Natl Acad Sci USA 95:4754–4759

Swei A, Lacy F, Delano FA, Parks DA, Schmid-Schonbein GW (1999) A mechanism of oxygen free radical production in the Dahl hypertensive rat. Microcirculation 6:179–187

Szanto I, Rubbia-Brandt L, Kiss P, Steger K, Banfi B, Kovari E, Herrmann F, Hadengue A, Krause KH (2005) Expression of NOX1, a superoxide-generating NADPH oxidase, in colon cancer and inflammatory bowel disease. J Pathol 207:164–176

Taggart DP, Young V, Hooper J, Kemp M, Walesby R, Magee P, Wright JE (1994) Lack of cardioprotective efficacy of allopurinol in coronary artery surgery. Br Heart J 71:177–181

Takaya T, Hirata K, Yamashita T, Shinohara M, Sasaki N, Inoue N, Yada T, Goto M, Fukatsu A, Hayashi T, Alp NJ, Channon KM, Yokoyama M, Kawashima S (2007) A specific role for eNOS-derived reactive oxygen species in atherosclerosis progression. Arterioscler Thromb Vasc Biol 27:1632–1637

Takimoto E, Kass DA (2007) Role of oxidative stress in cardiac hypertrophy and remodeling. Hypertension 49:241–248

Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, Lazzarino G, Paolocci N, Gabrielson KL, Wang Y, Kass DA (2005) Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. J Clin Invest 115:1221–1231

Thomas SR, Chen K, Keaney JF Jr (2002) Hydrogen peroxide activates endothelial nitric-oxide synthase through coordinated phosphorylation and dephosphorylation via a phosphoinositide 3-kinase-dependent signaling pathway. J Biol Chem 277:6017–6024

Thomas M, Gavrila D, McCormick ML, Miller FJ Jr, Daugherty A, Cassis LA, Dellsperger KC, Weintraub NL (2006) Deletion of p47phox attenuates angiotensin II-induced abdominal aortic aneurysm formation in apolipoprotein E-deficient mice. Circulation 114:404–413

Thony B, Auerbach G, Blau N (2000) Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem J 347(Pt 1):1–16

Tojo T, Ushio-Fukai M, Yamaoka-Tojo M, Ikeda S, Patrushev N, Alexander RW (2005) Role of gp91phox (Nox2)-containing NAD(P)H oxidase in angiogenesis in response to hindlimb ischemia. Circulation 111:2347–2355

Usui M, Egashira K, Kitamoto S, Koyanagi M, Katoh M, Kataoka C, Shimokawa H, Takeshita A (1999) Pathogenic role of oxidative stress in vascular angiotensin-converting enzyme activation in long-term blockade of nitric oxide synthesis in rats. Hypertension 34:546–551

Van Buul JD, Fernandez-Borja M, Anthony EC, Hordijk PL (2005) Expression and localization of NOX2 and NOX4 in primary human endothelial cells. Antioxid Redox Signal 7:308–317

Vasquez-Vivar J, Kalyanaraman B, Martasek P, Hogg N, Masters BS, Karoui H, Tordo P, Pritchard KA Jr (1998) Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc Natl Acad Sci USA 95:9220–9225

Wang HD, Xu S, Johns DG, Du Y, Quinn MT, Cayatte AJ, Cohen RA (2001) Role of NADPH oxidase in the vascular hypertrophic and oxidative stress response to angiotensin II in mice. Circ Res 88:947–953

Wang P, Tang F, Li R, Zhang H, Chen S, Liu P, Huang H (2007) Contribution of different Nox homologues to cardiac remodeling in two-kidney two-clip renovascular hypertensive rats: effect of valsartan. Pharmacol Res 55:408–417

Watts GF, Playford DA, Croft KD, Ward NC, Mori TA, Burke V (2002) Coenzyme Q(10) improves endothelial dysfunction of the brachial artery in Type II diabetes mellitus. Diabetologia 45:420–426

Waud WR, Rajagopalan KV (1976) Purification and properties of the NAD + -dependent (type D) and O2-dependent (type O) forms of rat liver xanthine dehydrogenase. Arch Biochem Biophys 172:354–364

Waypa GB, Marks JD, Mack MM, Boriboun C, Mungai PT, Schumacker PT (2002) Mitochondrial reactive oxygen species trigger calcium increases during hypoxia in pulmonary arterial myocytes. Circ Res 91:719–726

Waypa GB, Guzy R, Mungai PT, Mack MM, Marks JD, Roe MW, Schumacker PT (2006) Increases in mitochondrial reactive oxygen species trigger hypoxia-induced calcium responses in pulmonary artery smooth muscle cells. Circ Res 99:970–978

Waypa GB, Marks JD, Guzy R, Mungai PT, Schriewer J, Dokic D, Schumacker PT (2010) Hypoxia triggers subcellular compartmental redox signaling in vascular smooth muscle cells. Circ Res 106:526–535

Weidemann A, Johnson RS (2008) Biology of HIF-1alpha. Cell Death Differ 15:621–627

Werner-Felmayer G, Golderer G, Werner ER (2002) Tetrahydrobiopterin biosynthesis, utilization and pharmacological effects. Curr Drug Metab 3:159–173

Wever RM, van Dam T, van Rijn HJ, de Groot F, Rabelink TJ (1997) Tetrahydrobiopterin regulates superoxide and nitric oxide generation by recombinant endothelial nitric oxide synthase. Biochem Biophys Res Commun 237:340–344

Widder JD, Chen W, Li L, Dikalov S, Thony B, Hatakeyama K, Harrison DG (2007) Regulation of tetrahydrobiopterin biosynthesis by shear stress. Circ Res 101:830–838. Epub 2007 Aug 2017

Widder JD, Fraccarollo D, Galuppo P, Hansen JM, Jones DP, Ertl G, Bauersachs J (2009) Attenuation of angiotensin II-induced vascular dysfunction and hypertension by overexpression of Thioredoxin 2. Hypertension 54:338–344

Wingler K, Wunsch S, Kreutz R, Rothermund L, Paul M, Schmidt HH (2001) Upregulation of the vascular NAD(P)H-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system in vitro and in vivo. Free Radic Biol Med 31:1456–1464

Wu R, Millette E, Wu L, de Champlain J (2001) Enhanced superoxide anion formation in vascular tissues from spontaneously hypertensive and desoxycorticosterone acetate-salt hypertensive rats. J Hypertens 19:741–748

Xia Y, Dawson VL, Dawson TM, Snyder SH, Zweier JL (1996) Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. Proc Natl Acad Sci USA 93:6770–6774

Xu S, He Y, Vokurkova M, Touyz RM (2009) Endothelial cells negatively modulate reactive oxygen species generation in vascular smooth muscle cells: role of thioredoxin. Hypertension 54:427–433

Youn JY, Gao L, Cai H (2012a) The p47(phox)- and NADPH oxidase organiser 1 (NOXO1)-dependent activation of NADPH oxidase 1 (NOX1) mediates endothelial nitric oxide synthase (eNOS) uncoupling and endothelial dysfunction in a streptozotocin-induced murine model of diabetes. Diabetologia 55(7):2069–2079

Youn JY, Wang T, Blair J, Laude KM, Oak JH, McCann LA, Harrison DG, Cai HL (2012b) Endothelium-specific sepiapterin reductase deficiency in DOCA-salt hypertension. Am J Physiol Heart Circ Physiol 302(11):H2243–H2249

Zalba G, Beaumont FJ, San Jose G, Fortuno A, Fortuno MA, Etayo JC, Diez J (2000) Vascular NADH/NADPH oxidase is involved in enhanced superoxide production in spontaneously hypertensive rats. Hypertension 35:1055–1061

Zhang J, Youn JY, Kim AY, Ramirez RJ, Gao L, Ngo D, Chen P, Scovotti J, Mahajan A, Cai H (2012) NOX4-dependent hydrogen peroxide overproduction in human atrial fibrillation and HL-1 atrial cells: relationship to hypertension. Front Physiol 3:140

Zheng JS, Yang XQ, Lookingland KJ, Fink GD, Hesslinger C, Kapatos G, Kovesdi I, Chen AF (2003) Gene transfer of human guanosine 5′-triphosphate cyclohydrolase I restores vascular tetrahydrobiopterin level and endothelial function in low renin hypertension. Circulation 108:1238–1245

Zimmerman MC, Dunlay RP, Lazartigues E, Zhang Y, Sharma RV, Engelhardt JF, Davisson RL (2004) Requirement for Rac1-dependent NADPH oxidase in the cardiovascular and dipsogenic actions of angiotensin II in the brain. Circ Res 95:532–539

Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ (2000) Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. J Exp Med 192:1001–1014

Zweier JL, Flaherty JT, Weisfeldt ML (1987) Direct measurement of free radical generation following reperfusion of ischemic myocardium. Proc Natl Acad Sci USA 84:1404–1407