The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases

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Activation of vascular NAD(P)H oxidases and the production of reactive oxygen species (ROS) by these enzyme systems are common in cardiovascular disease. In the past several years, a new family of NAD(P)H oxidase subunits, known as the non-phagocytic NAD(P)H oxidase (NOX) proteins, have been discovered and shown to play a role in vascular tissues. Recent studies make clearer the mechanisms of activation of the endothelial and vascular smooth muscle NAD(P)H oxidases. ROS produced following angiotensin II-mediated stimulation of NAD(P)H oxidases signal through pathways such as mitogen-activated protein kinases, tyrosine kinases and transcription factors, and lead to events such as inflammation, hypertrophy, remodeling and angiogenesis. Studies in mice that are deficient in p47^{phox} and gp91^{phox} (also known as NOX2) NAD(P)H oxidase subunits show that ROS produced by these oxidases contribute to cardiovascular diseases including atherosclerosis and hypertension. Recently, efforts have been devoted to developing inhibitors of NAD(P)H oxidases that will provide useful experimental tools and might have therapeutic potential in the treatment of human diseases.

In the past decade, it has become evident that reactive oxygen species (ROS) contribute to the pathogenesis of numerous cardiovascular diseases including hypertension, atherosclerosis, cardiac hypertrophy, heart failure and restenosis [1]. The NAD(P)H oxidases are a predominant source of ROS in these conditions, and activation of these enzymes leads to a variety of intracellular signaling events that ultimately cause dysfunction of the endothelium, proliferation of vascular smooth muscle cells, expression of pro-inflammatory genes and reconstruction of the extracellular matrix. A major stimulus for activation of NAD(P)H oxidases is the hormone angiotensin II (Ang II), and many of the untoward effects of Ang II seem to be mediated by an overproduction of ROS. Here, we explore the mechanisms of stimulation of these oxidases by Ang II, the downstream pathological events and the potential therapeutic consequences of inhibiting these enzyme systems.

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ROS and oxidant stress

Oxygen is fundamental to cellular respiration and cells have evolved several enzyme systems that use this ubiquitous substrate as an acceptor of electron transfer [2]. In addition to the mitochondrial electron-transport chain, other enzyme systems that participate in oxygen reduction include lipoxygenase and cyclooxygenase, which metabolize arachidonic acid, the cytochrome P450s, xanthine oxidase, NAD(P)H oxidases, nitric oxide synthases (NOSs), peroxidases and other hemoproteins [1]. As illustrated in Figure 1, reduction of oxygen by one electron leads to the formation of superoxide ($O_2^{\cdot -}$), which is a common progenitor of other ROS. In addition, some enzymes, such as xanthine oxidase and glucose oxidase, can directly donate two electrons to oxygen to produce hydrogen peroxide (H_2O_2). An important consequence of formation of ROS in vascular cells is the consumption of nitric oxide ($NO^{\cdot}$). This can occur via reactions with $O_2^{\cdot -}$ [3], lipid peroxy radicals and alkoxy radicals [4], and peroxidase-based reactions [5]. $NO^{\cdot}$ inhibits the development of vascular diseases, and loss of $NO^{\cdot}$ via these reactions is likely to be important in the pathogenesis of conditions such as atherosclerosis, hypertension and diabetic vascular disease [1]. Although ROS are often considered toxic products that lead to disease, there is evidence that they might play important roles in cell signaling. In particular, H_2O_2 is a signaling intermediate in many pathophysiological responses (reviewed in [6]).

The NAD(P)H oxidases as major sources of vascular ROS

Although each of the above-mentioned enzymes can produce ROS in vascular cells, it is generally recognized that the NAD(P)H oxidases are predominant sources of ROS in the vasculature. The structure and function of the NAD(P)H oxidases was well characterized initially in neutrophils where two membrane components, p22^{phox} and gp91^{phox}, comprise the cytochrome *b*558. Other important components include the cytoplasmic subunits p47^{phox}, p40^{phox}, p67^{phox} and the small GTP-binding protein Rac. When phagocytic cells are activated, the cytosolic subunits translocate to cytochrome *b*558 at the membrane, which results in activation of the oxidase and the well characterized oxidative burst [7]. An important initiating event is phosphorylation of p47^{phox}. The crystal structure of p47^{phox} reveals that intermolecular

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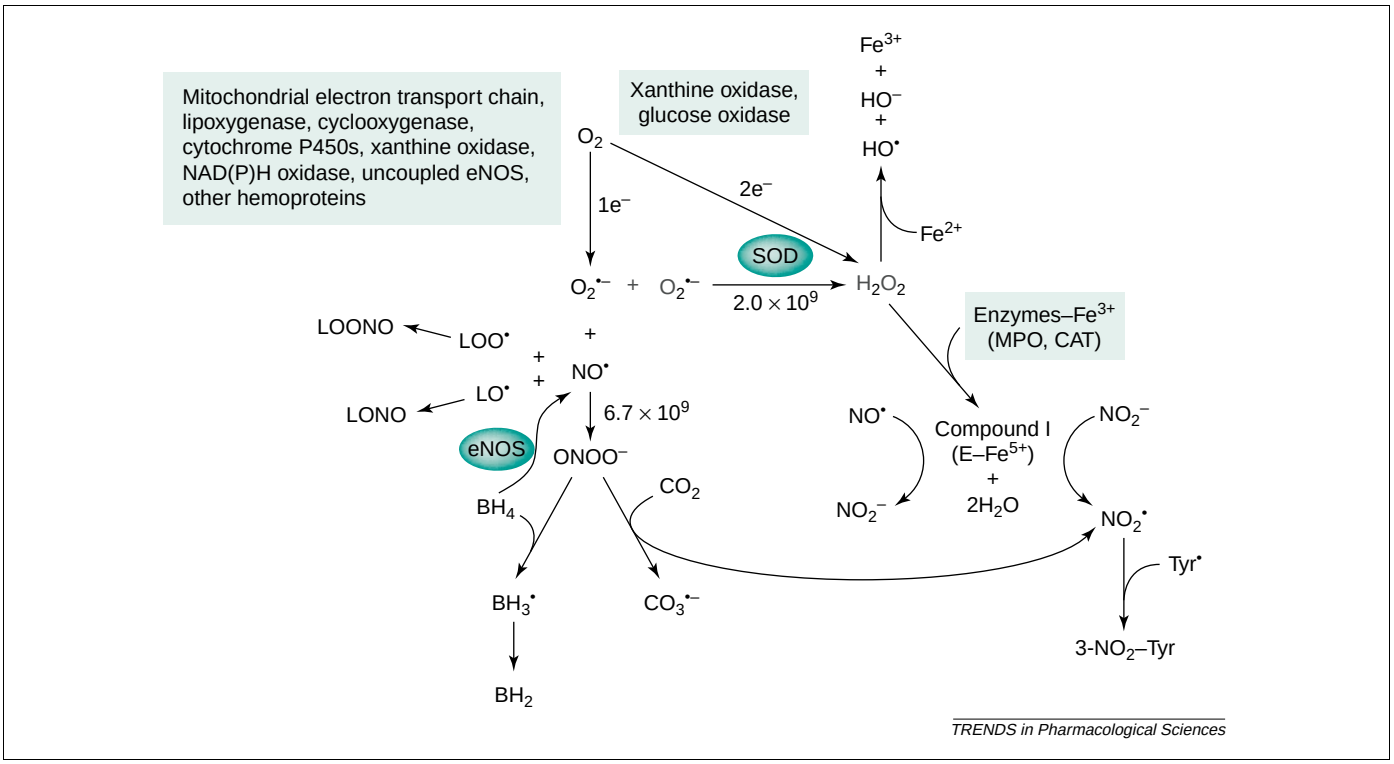

Fig. 1. Formation of reactive oxygen species (ROS). Several enzymes and enzyme systems can either generate or accept electrons, which leads to the production of ROS. The reduction of oxygen by one electron leads to the formation superoxide ($O_2^{\cdot-}$), which can be either dismutated to hydrogen peroxide (H_2O_2) spontaneously or in a reaction catalyzed by superoxide dismutase (SOD). Superoxide and nitric oxide ($NO^{\cdot}$) react with each other at a near diffusion-limited rate to form peroxynitrite ($ONOO^-$), which is a potent oxidant. Lipid radicals ($LO^{\cdot}$ and $LOO^{\cdot}$) can also react with $NO^{\cdot}$ to form LONO and LOONO, respectively. Mammalian peroxidases such as myeloperoxidase (MPO) and the eosinophil peroxidase are activated by H_2O_2 to form a highly reactive radical that can oxidize $NO^{\cdot}$ to $NO_2^{\cdot}$ and react with NO_2^- to form $NO_2^{\cdot}$. $NO_2^{\cdot}$ can, in turn, participate in nitrating events, such as the formation of nitrotyrosines. Abbreviation: CAT, catalase.

interaction between two tandem Src homology domains auto-inhibits binding to $p22^{phox}$ [8]. This auto-inhibitory interaction is lost on phosphorylation, which allows binding of $p47^{phox}$ and $p22^{phox}$ and initiates the cascade that leads to the respiratory burst [8].

The vascular NAD(P)H oxidases differ from the neutrophil NAD(P)H oxidase in several important respects. The neutrophil oxidase releases large amounts of $O_2^{\cdot-}$ in bursts, whereas the vascular NAD(P)H oxidases continuously produce low levels of $O_2^{\cdot-}$. Many of the neutrophil components, including $p22^{phox}$, $p47^{phox}$, $p40^{phox}$, $gp91^{phox}$ and Rac are present in vascular endothelial and smooth muscle cells [9–11]. Additionally, a $p67^{phox}$ subunit is found in aortic fibroblasts [12]. In the past few years, a family of $gp91^{phox}$-like proteins, termed the non-phagocytic NAD(P)H oxidase (NOX) proteins, has been discovered [13,14]. Based on homologies with each other and their apparent evolution from an ancestral NOX, these are named NOX1, NOX2 (also known as $gp91^{phox}$), NOX3, NOX4 and NOX5 [14,15]. Two related proteins, called DUOX1 and DUOX2, have NOX-homologous regions as well as regions with peroxidase activity [16]. Endothelial cells contain NOX1, NOX2, NOX4 and NOX5, whereas vascular smooth muscle cells express NOX1, NOX4 and NOX5 [15,17,18]. Adventitial fibroblasts and vascular smooth muscle cells from resistant arteries contain NOX2 [10,19]. Recently, two proteins with homology to $p47^{phox}$ and $p67^{phox}$, termed NOX organizer 1 and NOX activator 1, have been discovered and shown to regulate NOX1 activity [20–22]. These newly identified binding partners of the cytosolic proteins might modulate enzyme activity in either a tissue or stimulus-specific fashion.

The presence of NAD(P)H oxidases in different types of cells in the vessel wall is important because ROS have both autocrine and paracrine functions. Some ROS are produced intracellularly where they initiate cell-specific signaling events. Most ROS, such as $O_2^{\cdot-}$ and peroxynitrite ($ONOO^-$), are short lived and do not pass effectively from one cell to the next. Even when produced intracellularly, $O_2^{\cdot-}$ can have a paracrine effect by inactivating $NO^{\cdot}$ that is produced by adjacent cells. In contrast to other shorter-lived ROS, H_2O_2 is uncharged and sufficiently stable to diffuse from one cell to another. For example, it has been suggested recently that H_2O_2 acts as an endothelium-derived hyperpolarizing factor that is released by the endothelium and causes vasodilatation of vascular smooth muscle [23]. The roles of ROS vary depending on the cell type. In vascular smooth muscle cells, ROS produced by NAD(P)H oxidases contribute to hypertrophy [9], whereas in the endothelium, ROS seem to stimulate angiogenesis [24]. Of note, fibroblasts in the adventitia produce substantial amounts of ROS, which inactivate $NO^{\cdot}$ [19].

Ang II and modulation of vascular NAD(P)H oxidases

NAD(P)H oxidases are activated by mechanical forces, hormones and cytokines (reviewed in [1,6]). In particular, the octapeptide Ang II is an important activating stimulus for vascular NAD(P)H oxidases. Ang II is the major effector hormone of the renin–angiotensin system and has effects in the CNS, heart, vasculature and kidney [25]. Activation

of NAD(P)H oxidases by Ang II was demonstrated first by Griendling *et al.* [26], who showed that pathophysiologically relevant concentrations of Ang II increased NAD(P)H oxidase activity in rat vascular smooth muscle cells. Recently, several groups have provided insight into how Ang II activates NAD(P)H oxidases. In vascular smooth muscle cells, activation of the angiotensin AT_1 receptor leads to phosphorylation of $p47^{phox}$. Elegant studies from Touyz *et al.* indicate that c-Src is an important intermediate in this response because pharmacological inhibition of c-Src prevents phosphorylation of $p47^{phox}$ and the subsequent translocation of $p47^{phox}$ to the membrane in response to Ang II [27]. This important upstream role of c-Src in $p47^{phox}$ activation has been confirmed in vascular smooth muscle cells from c-$Src^{-/-}$ mice [28]. Activation of phospholipase D [28] and protein kinase C [29] are likely to precede phosphorylation of $p47^{phox}$.

In addition to its role in phosphorylation of $p47^{phox}$, c-Src activation also stimulates the transactivation of the epidermal growth factor receptor (EGFR), which evokes phosphatidylinositol 3-kinase-dependent production of phosphatidylinositol (3,4,5)-trisphosphate and, in turn activates Rac-1. Rac-1 then translocates to the membrane components, which leads to a second phase of oxidase activation [29]. Activation of c-Src is redox sensitive and stimulated by H_2O_2, which appears to represent a feed-forward mechanism whereby H_2O_2-mediated activation of c-Src amplifies NAD(P)H oxidase activity and H_2O_2 production. These signaling mechanisms involved in Ang II activation of vascular NAD(P)H oxidases are summarized in Figure 2.

A key role of $p47^{phox}$ in Ang II-mediated activation of vascular NAD(P)H oxidases is further illustrated in mice deficient in $p47^{phox}$ ($p47^{phox\ -/-}$). Vascular smooth muscle cells and endothelial cells from $p47^{phox\ -/-}$ mice do not produce $O_2^{\cdot-}$ in response to Ang II and other stimuli [30–32]. Li and Shah have shown that Ang II stimulates the association of $p47^{phox}$ with $p22^{phox}$ in endothelial cell membranes, and further confirmed its importance using cells from $p47^{phox\ -/-}$ mice and antisense techniques [32].

In addition to activating NAD(P)H oxidases, Ang II also increases the expression of NAD(P)H oxidase subunits when administered over hours to days. Chronic infusion of Ang II in rats increases the expression of $p22^{phox}$, NOX1 and NOX4 mRNA in aorta [33–35]. In addition, in cell-culture studies, Ang II upregulates the expression of the NAD(P)H oxidase subunits $gp91^{phox}$, $p22^{phox}$, $p47^{phox}$ $p67^{phox}$ and $p40^{phox}$ in smooth muscle cells isolated from human resistance arteries in a c-Src-dependent fashion [10]. Regulation of these subunits might be redox sensitive and their expression increased by the formation of ROS following initial activation of NAD(P)H oxidases. In the case of $p22^{phox}$, at least, treatment with a membrane-targeted form of superoxide dismutase (SOD) prevents the increase in its mRNA expression in response to Ang II [36].

Consequences of Ang II-mediated activation of NAD(P)H oxidase

The ROS produced by activated NAD(P)H oxidases can initiate numerous cellular events (Figure 3). H_2O_2 generated from NAD(P)H oxidases is essential for the hypertrophy of vascular smooth muscle cells caused by

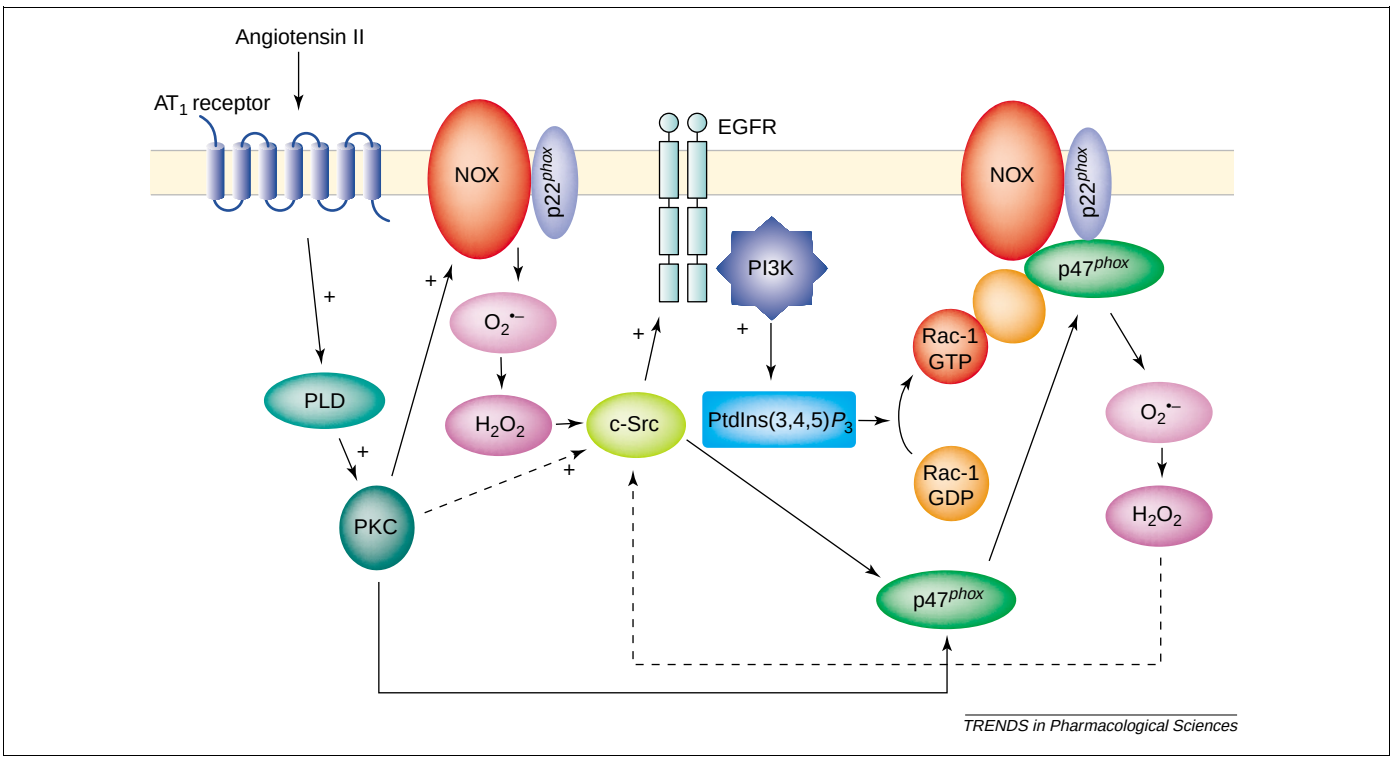

Fig. 2. Angiotensin II-stimulated activation of vascular NAD(P)H oxidases (NOXs). Activation of the angiotensin AT_1 receptor leads to phosphorylation of $p47^{phox}$. Crucial events between AT_1 receptor stimulation and phosphorylation of $p47^{phox}$ include the activation of phospholipase D (PLD), protein kinase C (PKC) and c-Src tyrosine kinase. Phosphorylation of $p47^{phox}$ promotes its binding to the membrane oxidase components. c-Src also transactivates the epidermal growth factor receptor (EGFR), which leads to activation of phosphatidylinositol 3-kinase (PI3K) and Rac-1. Translocation of Rac-1 to the membrane further activates the oxidase and sustains its function. Abbreviations: H_2O_2, hydrogen peroxide; $O_2^{\cdot-}$, superoxide; Ptd(3,4,5)P_3, phosphatidylinositol (3,4,5)-trisphosphate.

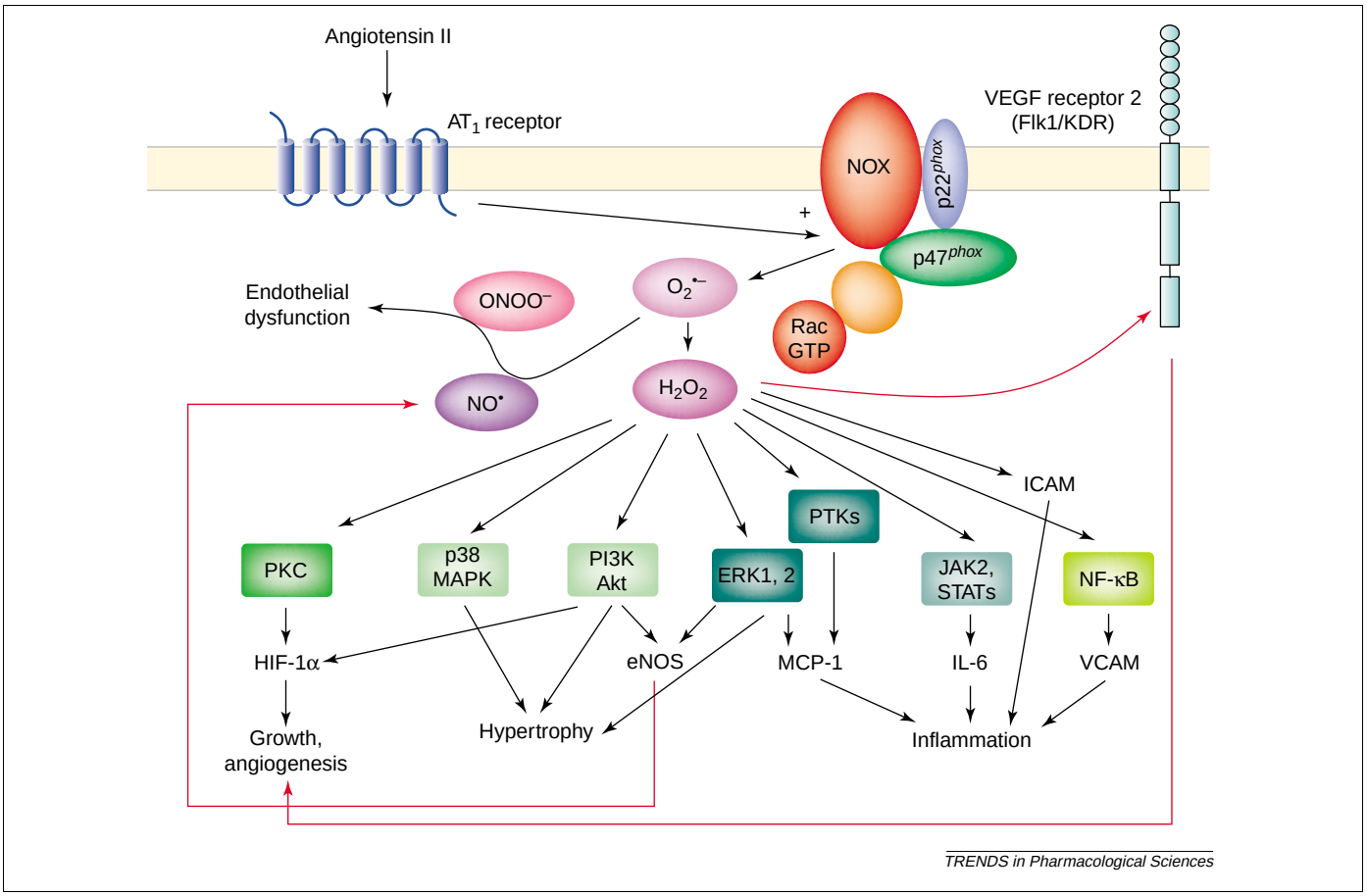

Fig. 3. Consequences of NAD(P)H oxidase (NOX) activation. Activation of vascular NAD(P)H oxidases by stimuli such as angiotensin II (Ang II) results in the formation of superoxide ($O_2^{\cdot-}$), which is dismutated to hydrogen peroxide (H_2O_2). $O_2^{\cdot-}$ can react with nitric oxide ($NO^{\cdot}$) to form peroxynitrite ($ONOO^-$), which leads to vasomotor alterations. Reactive oxygen species (ROS) produced by the oxidase regulate the expression of several pro-inflammatory mediators including monocyte chemoattractant 1 (MCP-1) [39], vascular cell adhesion molecule (VCAM) [40] and interleukin 6 (IL-6) [41]. Activation of nuclear factor κB (NF-κB) [39], extracellular signal-regulated kinase 1,2 (ERK1,2), protein tyrosine kinases (PTKs) [40] and the JAK2 (Janus-activated kinase 2)–STAT (signal transducers and activators of transcription) pathway [41] contributes to these signaling events. Activation of p38 mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), Akt and ERK1,2 by Ang II might contribute to hypertrophy [6,38]. In the endothelium, H_2O_2 can stimulate $NO^{\cdot}$ production by endothelial nitric oxide synthase (eNOS) via ERK1,2 and Akt [49,50]. H_2O_2 also stimulates angiogenesis by increasing the expression of hypoxia-induced factor 1α (HIF-1α), [44,45], which leads to an increase in the expression of vascular endothelial growth factor (VEGF) [46] and subsequent growth of vascular cells and angiogenesis. H_2O_2-dependent activation of the VEGF receptor 2 (Flk1/KDR) also contributes to angiogenesis [42]. These mechanisms at least partially account for the cardiovascular diseases induced by Ang II. Abbreviations: ICAM, intercellular cell adhesion molecule; PKC, protein kinase C.

Ang II [6,37]. Ang II also stimulates several cellular inflammatory responses via redox-sensitive mechanisms, including increased expression of monocyte chemoattractant 1 (MCP-1) [38], vascular cell-adhesion molecule 1 (VCAM-1) [39] and interleukin 6 [40]. Activation of nuclear factor κB (NF-κB) [38], extracellular signal-regulated kinase 1,2 (ERK1,2) and protein tyrosine kinases [39], and the JAK2 (Janus-activated kinase 2)–STAT (signal transducers and activators of transcription) pathway [40] contribute to these signaling events. ROS derived from NAD(P)H oxidases also seem to modulate angiogenesis via, for example, activation of vascular endothelial growth factor (VEGF) receptors [41], transactivation of EGFRs [42], and induction of hypoxia-induced factor 1α [43,44] and VEGF [45]. ROS both activate matrix metalloproteinases (MMPs) and increase their expression [46]. Very recently, Grote *et al.* have shown that mechanical stress rapidly activates ROS production in vascular smooth muscle cells and increases MMP-2 activation and mRNA expression [47], responses that are absent in cells from $p47^{phox\ -/-}$ mice [47]. A potentially important downstream effect of NAD(P)H oxidase activation that has been recognized recently is stimulation of NO^- production. H_2O_2 potently activates endothelial NOS (eNOS) via both Ca^{2+}-dependent and -independent mechanisms [48]. This occurs when H_2O_2 is administered extracellularly in addition to when production is stimulated by Ang II [49]. Ang II can, therefore, stimulate the simultaneous production of $O_2^{\cdot-}$ and $NO^{\cdot}$ by endothelial cells, which creates a situation that favors the formation of $ONOO^-$ [50–52].

Consequences of *in vivo* activation of vascular NAD(P)H oxidases

The effects of activation of the NAD(P)H oxidases are not simply a cell-culture phenomenon. They have also been demonstrated in several clinically relevant animal models of disease. Ang II-induced hypertension in rats is associated with a marked increase in vascular $O_2^{\cdot-}$ production and NAD(P)H oxidase activity [53,54]. Furthermore, intravascular administration of membrane-targeted forms of SOD lowers blood pressure in these animals [33,55]. Recently, using genetically modified mice that lack $p47^{phox}$, it has been demonstrated that NAD(P)H

oxidases are a major source of $O_2^{\cdot-}$ and contribute to hypertension caused by Ang II [31].

NAD(P)H oxidases seem to be important in other models of hypertension. For example, $O_2^{\cdot-}$ derived from NAD(P)H oxidases are increased in spontaneously hypertensive rats (SHRs) [56]. DOCA (deoxycorticosterone acetate)-salt hypertension is associated with oxidation of the NOS cofactor tetrahydrobiopterin (BH_4). This leads to a condition termed NOS uncoupling, whereby the NOSs produce $O_2^{\cdot-}$ rather than $NO^{\cdot}$. In $p47^{phox\ -/-}$ mice with DOCA-salt hypertension, BH_4 oxidation is reduced compared with wild-type mice and eNOS uncoupling does not occur. These data indicate that activation of NAD(P)H oxidases in hypertension can lead to BH_4 oxidation and the production of large amounts of $O_2^{\cdot-}$ from uncoupled eNOS [57].

In addition to hypertension, the vascular NAD(P)H oxidases contribute to excessive ROS production in other conditions in which the tissue concentrations of Ang II are increased. NAD(P)H oxidases are activated and $O_2^{\cdot-}$ production is increased in vessels of rabbits with experimental atherosclerosis. These abnormalities can be corrected by AT_1 receptor blockade [58]. In crosses between apolipoprotein E (ApoE)-deficient mice and $p47^{phox\ -/-}$ mice, atherosclerosis in the descending aorta is diminished compared with ApoE-deficient mice [59] whereas atherosclerosis near the origin of the aorta is unchanged [59,60]. This site-specific difference might be caused by either differences in the role of ROS at different sites of the circulation or differences in the time at which lesion formation was examined in these models. Interestingly, a cross between $gp91^{phox\ -/-}$ mice and ApoE-deficient mice yielded animals with similar amounts of atherosclerosis as mice that lack only ApoE [61]. This might be because other NOX proteins compensate for $gp91^{phox}$ disruption in the cells that are involved in the development of atherosclerotic lesions. Other cardiovascular diseases that are associated with activation of vascular NAD(P)H oxidases include diabetic vascular diseases [62–64], cardiac hypertrophy and heart failure [65,66], and nitrate tolerance [67].

NAD(P)H oxidase as a potential therapeutic target

In addition to the cardiovascular system, NOX proteins occur in many tissues and have been implicated in numerous disorders including cancer [14,68], bone resorption [69] and Alzheimer's disease [70]. Consequently, NOX inhibitors might have substantial clinical potential, particularly if they do not inhibit the oxidative burst of phagocytic cells (Figure 4).

There has been interest in the use of peptide-based inhibitors of NAD(P)H oxidase. The 26 residues at the N-terminus of the antibiotic peptide PR-39 bind the Src homology 3 (SH3) domain of $p47^{phox}$, which prevents association with $gp91^{phox}$ [71]. Initially, PR-39 was thought to inhibit NAD(P)H oxidases specifically, and it was used to examine the role of the NAD(P)H oxidases in myocardial ischemia reperfusion [72]. However, recent

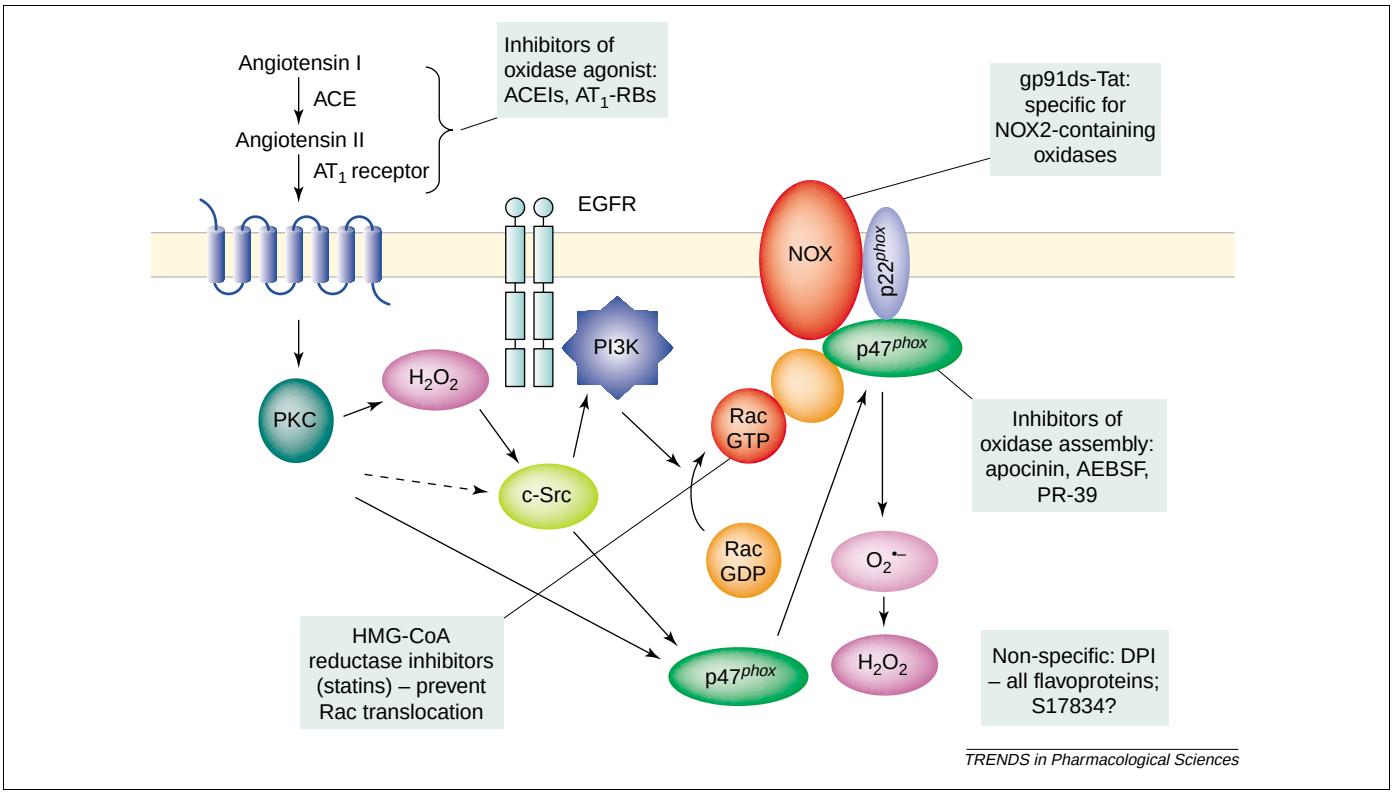

Fig. 4. Inhibitors of NAD(P)H oxidase (NOX). Angiotensin AT_1 receptor antagonists and angiotensin-converting enzyme inhibitors (ACEIs) prevent the activation of angiotensin AT_1 receptors and, thus, reduce this pathway for NAD(P)H oxidase activation. Statins prevent formation of geranylgeranyl diphosphate and, thus, inhibit association of the small GTP-binding protein Rac with the membrane. The peptide gp91ds-Tat inhibits the association between $gp91^{phox}$ (also known as NOX2) and $p47^{phox}$. Apocynin, PR-39 and AEBSF (also known as Pefabloc™) prevent the binding of $p47^{phox}$ to $p22^{phox}$. Diphenylene iodinium (DPI) is a flavoprotein inhibitor. Abbreviations: AEBSF, aminoethyl benzenesulfono fluoride; AT_1-RBs, AT_1 receptor blockers; EGFR, epidermal growth factor receptor; H_2O_2, hydrogen peroxide; $O_2^{\cdot-}$, superoxide; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C.

studies show that PR-39 has many effects, partly because it binds to the SH3 domains of other proteins and partly because it interacts with membrane lipids [73,74]. Therefore, PR-39 is not a specific inhibitor of NAD(P)H oxidases. Recently, Pagano and coworkers have developed a chimeric peptide called gp91ds-Tat that is composed of nine amino acids from the coat protein of HIV and nine amino acids of $gp91^{phox}$. This binds to $p47^{phox}$ and interferes with the assembly of $p47^{phox}$ and $gp91^{phox}$. The Tat portion allows gp91ds-Tat to enter cells readily, which makes it a useful, effective inhibitor of oxidases that contain $gp91^{phox}$. The gp91ds-Tat peptide has been used to show a role of $gp91^{phox}$-based oxidases in regulating the endothelial expression of MCP-1 [75], proliferation of the neointima following balloon injury [76] and Ang II-induced hypertension [77]. This inhibitor seems specific for oxidases that are dependent on $gp91^{phox}$, but is unlikely to inhibit NAD(P)H oxidases that use other NOX proteins.

Although peptide-based NAD(P)H oxidase inhibitors such as gp91ds-Tat are useful for experimental interventions, they cannot be administered orally and, therefore, have limited potential for clinical therapeutics. Several small molecules inhibit the NAD(P)H oxidases. Of these, apocynin, which is extracted from the leaves of *Picrorhiza kurroa*, seems particularly promising [78]. This agent blocks oxidase assembly [79], is effective when administered orally [80], and has been used extensively in several animal models of inflammation, including arthritis, septic shock and asthma. Apocynin might not be specific for the NAD(P)H oxidases because it inhibits thromboxane A_2 formation [81] and induces the AP-1 transcription factor [82]. However, it is not clear whether these effects are nonspecific or whether they occur as a result of ROS produced by NAD(P)H oxidases. Another agent, Pefabloc™ [aminoethyl benzenesulfono fluoride (AEBSF)], also inhibits the binding of cytochrome *b*558 to $p47^{phox}$ [83], but this is a serine protease inhibitor and has many effects in addition to NAD(P)H oxidase inhibition. Recently, a benzo(b)pyran-4-one derivative, S17834 has been shown to inhibit NAD(P)H oxidase activity in endothelial cells in culture. S17834 blocks tumor necrosis factor α-stimulated expression of VCAM-1 in these cells and reduces atherosclerosis in ApoE-deficient mice [84]. Diphenylene iodinium (DPI) is often used to inhibit NAD(P)H oxidases. However, this agent is an inhibitor of all flavoenzymes, including the NOSs, xanthine oxidase and the cytochrome P450 enzymes. Little is gained from using DPI, except to exclude a role of all flavin-containing enzymes.

Significantly, two commonly employed classes of drugs, HMG CoA reductase inhibitors (statins) and angiotensin receptor antagonists, lower NAD(P)H oxidase activity. AT_1 receptor antagonists reduce NADPH oxidase activity, even in control animals, and prevent their stimulation when Ang II is increased [53,58]. These observations support the importance of Ang II as a predominant modulator of the NAD(P)H oxidases *in vivo*. Statins inhibit the association of Rac-1 with the plasma membrane by reducing the production of geranylgeranyl diphosphate [85]. The clinical benefits associated with AT_1 receptor antagonists and statins might include their ability to inhibit NAD(P)H oxidases.

Concluding remarks

Our understanding of the vascular NAD(P)H oxidases has increased substantially during the past few years. However, much remains to be learned about the function of these enzymes and the stimuli, other than Ang II, that activate them. Different NOX proteins seem to have different cellular locations. How these affect the redox state at the subcellular level is likely to be important. The downstream targets of ROS produced by the NAD(P)H oxidases remain to be defined. The development of specific inhibitors of NOX-based oxidases could provide tools to elucidate the roles of these enzymes experimentally and would almost certainly be useful for treating a broad range of diseases.

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