NADPH oxidase-derived ROS and the regulation of pulmonary vessel tone

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Frazziano G, Champion HC, Pagano PJ. NADPH oxidase-derived ROS and the regulation of pulmonary vessel tone. Am J Physiol Heart Circ Physiol 302: H2166–H2177, 2012. First published March 16, 2012; doi:10.1152/ajpheart.00780.2011.—Pulmonary vessel constriction results from an imbalance between vasodilator and vasoconstrictor factors released by the endothelium including nitric oxide, endothelin, prostanoids, and reactive oxygen species (ROS). ROS, generated by a variety of enzymatic sources (such as mitochondria and NADPH oxidases, a.k.a. Nox), appear to play a pivotal role in vascular homeostasis, whereas elevated levels effect vascular disease. The pulmonary circulation is very sensitive to changes in the partial pressure of oxygen and differs from the systemic circulation in its response to this change. In fact, the pulmonary vessels contract in response to low oxygen tension, whereas systemic vessels dilate. Growing evidence suggests that ROS production and ROS-related pathways may be key factors that underlie this differential response to oxygen tension. A major emphasis of our laboratory is the role of Nox isozymes in cardiovascular disease. In this review, we will focus our attention on the role of Nox-derived ROS in the control of pulmonary vascular tone.

reactive oxygen species; vascular tone

Introduction

Since their discovery, reactive oxygen species (ROS) have aroused increasing interest because of evidence that they regulate important physiological and pathophysiological conditions. Under physiological conditions, the redox environment of vascular tissue is maintained in an overall reduced state via an interplay and balance between oxidants (reactive oxygen and nitrogen species) and antioxidant systems. Major ROS produced in the pulmonary vasculature are, among others, superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (HO·), and hydroperoxyl radical (HO$_2$), and the most important reactive nitrogen species emerge as nitric oxide (NO), nitrogen dioxide (NO$_2$), dinitrogen trioxide (N$_2$O$_3$), and peroxynitrite (Fig. 1). In this review, we decided to focus our attention on the ROS effects on pulmonary vascular tone. For a complete overview about reactive nitrogen species and their importance, the reader is referred to an excellent review on the topic (167). Endogenous antioxidant systems include enzymes such as superoxide dismutase [SOD, which dismutes superoxide anion (O$_2^-$) into H$_2$O$_2$, catalase (which converts H$_2$O$_2$ to water), peroxiredoxins (which catalobizes H$_2$O$_2$ to water), glutathione peroxidase, peroxiredoxin, heme oxygenase, glutaredoxin and thioredoxin (which reduce the disulfide bridges of various target proteins), and nonenzymatic agents such as glutathione, β-carotene, retinol, vitamin C, and vitamin E (109). When endogenous antioxidant systems are inadequate or overwhelmed by ROS generation, increased ROS steady-state levels initiate multiple pathologies including diabetes (53, 74, 146), hypertension (40, 96, 125, 169), atherosclerosis (44, 76, 159), and hypoxia/reperfusion injury (89, 95, 190). In the lungs and cardiovascular system, principal sources of ROS include mitochondrial electron transport, xanthine oxidase, cytochrome P-450, cyclooxygenases, uncoupled NO synthase, lipoxigenases, and the NADPH oxidase (Nox) family of proteins (5). It is known that these different sources of ROS modulate each other through mechanisms of negative feedback and feed-forward processes. Dysregulation in these processes contribute to the development of diseases. For example, ROS produced from Nox can stimulate ROS production by the other sources, and vice versa, increased levels of mitochondria-derived ROS lead to Nox activation. In this review, we will focus our attention in analyzing the effect of the Nox-derived ROS. In the pulmonary system, Nox-derived ROS production is involved in diseases such as pulmonary hypertension (57, 103, 120), fibrosis (4, 41, 110), chronic granulomatous disease (144, 154), acute lung injury and acute respiratory distress syndrome (81, 175, 178), emphysema and asthma (1, 88, 126, 141, 156, 165, 174), chronic obstructive pulmonary disease (45, 129, 130), and lung cancer (72, 107) (Table 1). On the other hand, in the pulmonary vasculature, Nox-derived ROS contribute to the maintenance of vascular tone and regulate important processes such as cytoskeletal organization, cell migration, cell growth, proliferation, differentiation, and apoptosis (71). These seemingly contradictory phenotypes can be explained by the current theory that gradation and spatial orientation of ROS levels elicit distinct out-
remains controversial whether these enzymes produce O$_2$·.

The involvement of Nox isoforms in lung disease is summarized in Table 1.

Table 1. Involvement of Nox isoforms in lung disease

<table>
<thead>
<tr>
<th>Nox Isoform</th>
<th>Localization</th>
<th>Pulmonary Disease Associated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nox2, Nox4</td>
<td>Pulmonary artery endothelial cells</td>
<td>PAH, ALI/ARDS</td>
<td>(51, 56, 70, 90, 105, 156)</td>
</tr>
<tr>
<td>Nox4</td>
<td>Pulmonary artery smooth muscle cells</td>
<td>PAH</td>
<td>(51, 105)</td>
</tr>
<tr>
<td>Nox2</td>
<td>Myofibroblasts</td>
<td>Pulmonary fibrosis</td>
<td>(3, 36, 95)</td>
</tr>
<tr>
<td>Nox3</td>
<td>Macrophages/neutrophils</td>
<td>Chronic granulomatous disease</td>
<td>(125, 135)</td>
</tr>
<tr>
<td>Nox3</td>
<td>Endothelial cells of airways</td>
<td>Emphysema</td>
<td>(1, 122, 143)</td>
</tr>
<tr>
<td>DUOX1 and -2</td>
<td>Airway epithelial cells</td>
<td>COPD and asthma</td>
<td>(66, 166)</td>
</tr>
<tr>
<td>DUOX1 and -2</td>
<td>Lung carcinoma cells</td>
<td>Lung cancer</td>
<td>(93)</td>
</tr>
</tbody>
</table>

Multiple studies demonstrate the involvement of various NADPH oxidase (Nox) isoforms in the development and progression of different pulmonary diseases, such as pulmonary arterial hypertension (PAH), acute lung injury (ALI), pulmonary fibrosis, chronic granulomatous disease, emphysema, obstructive lung disorders [chronic obstructive pulmonary disease (COPD)], asthma, and lung cancer. ARDS, acute respiratory distress syndrome; DUOX, dual oxidase.
expression level of p22

but the significance of this is unclear because changes in the

p22

lum (ER) and generates O2·

partially glycosylated, it is located to the endoplasmic reticu-

salivary glands (65), and prostate (177). When DUOX2 is

the airways (61, 66, 155), alveoli, intestinal tract (52, 65),

are widely expressed in epithelial tissues, including epithelia of

from the epithelium of the thyroid gland (31, 43, 49). DUOXs

-interacting protein-2 associates with p22

/H9254

ingly, Lyle and colleagues (108) showed that the polymerase

activation and thus appears to be constitutively active or

regulated by heretofore undefined cytosolic subunits. Intrigu-

ably, Lyle and colleagues (108) showed that the polymerase

δ-interacting protein-2 associates with p22

/H11002

of cytosolic subunits that are recognized as necessary for other

Nox isoforms (16). Nox5 has also been shown to bind p22

phox, but the significance of this is unclear because changes in the

expression level of p22

phox, or dominant negative constructs of p22

phox, do not affect Nox5 activity (21, 91). The elevation

of intracellular calcium promotes the occupation of the calcium-binding EF-hand domains of Nox5. This leads to

conformational changes among the domains that trigger O2·− production (16).

DUOX1 and DUOX2 were originally identified and cloned

from the epithelium of the thyroid gland (31, 43, 49). DUOXs

are widely expressed in epithelial tissues, including epithelia of the

airways (61, 66, 155), alveoli, intestinal tract (52, 65),

salivary glands (65), and prostate (177). When DUOX2 is

partially glycosylated, it is located to the endoplasmic reticu-

lum (ER) and generates O2·−, whereas when it is fully glyco-

sylated, it is transported to the plasma membrane and generates

H2O2 (7, 69, 196). DUOX proteins require the presence of two

maturation factors, DUOX activator 1 and 2 (DUOXA1 and

DUOXA2), which allow proper DUOX processing and traf-

ficking to the membrane (69). Only the combination of the

DUOXs with their corresponding DUOXAs renders the com-

plex active and allows exit from the ER (68). In experiments

with transfection of DUOX2 into PCCl3 cells and Cos7 cells,

an enhanced generation of H2O2 in response to Ca2+ has been

shown (42, 152). Moreover, site-directed mutation of the

predicted Ca2+-binding glutamate residues to glutamine in the

EF hands of DUOX1 (E839Q and E875Q) or DUOX2 (E843Q

and E879Q) resulted in an almost complete loss of function

(152). In addition, NOXA1 was recently shown to interact with

and inhibit DUOX1 in a Ca2+-dependent fashion, possibly

involving the COOH-terminal region of DUOX1 (133). The

levels of expression of DUOX1 and DUOX2 are selectively

upregulated by inflammatory cytokines (IL-4 and IL-13) and

interferon-γ, respectively (77).

Expression of Nox Isoforms in the Lung

In total lung tissue, mRNA for Nox1, Nox2, Nox4, DUOX1, DUOX2, p22

phox as well as the cytosolic subunits NOXA1, NOXO1, p47

phox, p40

phox, and p67

phox are expressed (51, 65, 120) (Table 2). In this section, we will discuss Nox isoform expression across the different sections of the lung tissue and in the different layers of the pulmonary vasculature.

Airway epithelium and alveolar cells. The primary sources of ROS production in airway epithelium are DUOX1 (66, 77, 98, 155) and DUOX2 (61, 77, 155). However, the expression of other Nox isoforms, Nox1, Nox2, and their regulatory proteins, has been reported (98). mRNA levels of DUOX1 and DUOX2 are regulated by cytokines. Reportedly, IL-4 and IL-13 (produced by Th2) induce a moderate increase of DUOX1 mRNA, whereas interferon-γ (from Th1) markedly

Table 2. Expression and function of Nox isoforms in lung tissue

<table>
<thead>
<tr>
<th>Nox Isoform</th>
<th>Distribution</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUOX1 and -2</td>
<td>Epithelial airway</td>
<td>Host defense, mucin production, cellular migration and differentiation</td>
<td>(60, 139, 176)</td>
</tr>
<tr>
<td>Nox2, Nox4</td>
<td>Pulmonary endothelial cells</td>
<td>Control of vascular tone, vascular cell growth and remodeling, angiogenesis, proinflammatory cytokine production</td>
<td>(24, 104, 118)</td>
</tr>
<tr>
<td>Nox4</td>
<td>Pulmonary smooth muscle cells</td>
<td>Differentiation and hypoxia-induced proliferation</td>
<td>(32, 144)</td>
</tr>
<tr>
<td>Nox4</td>
<td>Myofibroblasts</td>
<td>Differentiation</td>
<td>(42)</td>
</tr>
</tbody>
</table>

Distinct isoforms of Nox (especially Nox2, Nox4, DUOX1, and DUOX2) are expressed in lung and participate in multiple physiological and pathological processes such as control of vascular tone, cell growth, and differentiation.
increases mRNA for DUOX2, perhaps suggesting a constitutive role of DUOX1 in noninflamed airways and an inducible role of DUOX2 in infection and inflammation (77). Other Nox enzyme functions in airway epithelia include host defense (65), response to mechanical stress (34), regulation of matrix metalloproteinase-12 (MMP-12), MMP gene expression (98, 135), production of epithelial mucus (158), induction of cell death (199), regulation of lung development, epithelial cell differentiation (59, 77), and epithelial integrity (93, 188).

In type I pneumocytes, Nox2, p22phox, p67phox, p47phox and p40phox subunits, as well as Rac1, are present at substantially higher levels than in type II cells (168) in which ROS production is reportedly derived primarily from mitochondria (143). In type II cells DUOX1 is expressed (there is no significant expression of DUOX2), but the functions of this Nox isoform in alveolar cells remains unknown (58).

**Pulmonary vasculature.** In the pulmonary vasculature, Nox isoforms are activated by a wide range of stimuli including G protein-coupled receptor agonists, angiotensin II, thrombin, endothelin, serotonin, thromboxane A2 (38, 105), cytokines [tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β)] (166), mechanical forces, and shear stress. Proposed functions of Nox-derived ROS include NF-κB activation (25), MAPK activation (156), cell proliferation (119), and potassium channel regulation in response to changes in the oxygen concentration (9, 186).

**Pulmonary endothelium.** Generally speaking, endothelial cells express Nox1, Nox2, and Nox4 (and Nox5 in human cells) and their cofactors (70, 173). In human pulmonary artery endothelial cells and human lung microvascular endothelial cells, mRNA levels for Nox4 are much higher compared with mRNA for Nox2 (27, 139). Interestingly, in these cells, Nox4 silencing induces Nox2 mRNA upregulation, and in Nox2−/− mice mRNA Nox4 upregulation was described, suggesting that a compensatory regulation induced by their respective deficiency exists for Nox2 and Nox4 mRNA expression (138). Nox endothelial activity is increased in response to pulmonary ischemia (6) and hyperoxia (136), is associated with activation of ATP-sensitive K+ channels, and results in stimulated endothelial cell proliferation and NO production (119, 198). The roles of endothelial Noxs include control of vascular tone, vascular cell growth, angiogenesis, and inflammation. Studies suggest a role for both H2O2 (119) and O2− (79) in response to general pulmonary endothelial injury.

**PASMCs.** In PASMCs, both Nox1 and Nox4 are expressed, whereas Nox4 seems to be the most abundant isoform (24, 120, 139). Nox4 expression in PASMCs can be induced by several stimuli including hypoxia/ischemia, shear stress, and ER stress (19, 97, 120). Nox4 seems to be required for the expression of smooth muscle differentiation markers and maintenance of smooth muscle actin-based stress fibers as suggested by the correlation between the decrease of Nox4 expression and the loss of smooth muscle differentiation markers, such as smooth α-actin and myosin heavy chain, in multiple passages of freshly isolated PASMCs (37, 166). Both Nox1 and Nox4 induce proliferation of PASMCs (120). In particular, it has been shown that Nox4 is involved in this process through induction by TGF-β1 (166).

** Fibroblasts.** In lung fibroblasts, p47phox, p40phox, p22phox, Nox1, and Nox4 are expressed but not Nox2 (47), and Nox4 expression dominates Nox1. Oxidase-derived H2O2 generation is observed in response to TGF-β (170), irradiation with α-particles (127), rhinovirus infection (47), and hypoxia (99). Nox4 is upregulated by hypoxia, inflammatory mediators, and fibrotic stimuli, such as TNF-α and TGF-β, contributing to fibroblast activation and transdifferentiation. The consequences of Nox4-induced ROS by TGF-β in pulmonary fibroblasts include IL-8 upregulation (47) and induction of epithelial cell death through a paracrine mechanism (176). The TGF-β-induced Nox4-derived ROS production in human fibroblasts seems to be extracellular, in contrast to what is observed in human PASMCs in which the activation of Nox4 by TGF-β leads to intracellular ROS production (166, 176). Experiments using siRNA-mediated knockdown of Nox4 demonstrated that Nox4 contributed to the increase in ROS generation under hypoxic conditions, stimulated proliferation, and inhibited apoptosis in pulmonary fibroblasts (99).

**ROS-Induced Regulation of Pulmonary Vascular Tone**

Nox-derived ROS (both O2− and H2O2) play an important role as mediators and signaling molecules capable of activating multiple pathways involved in the control of pulmonary vascular tone, cell proliferation and apoptosis, inflammation, and fibrosis. One of the potential mechanisms by which ROS are able to regulate pulmonary vessel tone is via the control of the cellular redox potential (87). The pulmonary vascular responses to exogenously added ROS are heterogeneous because O2− or H2O2 can lead to contractile or relaxant responses. This heterogeneity might be explained by the nature (H2O2 or O2−) and dose of ROS applied on the existence of a previous contractile tone and on the nature of the preconstrictor used.

O2− induces constriction of pulmonary artery, triggering Rho-kinase-mediated Ca2+ sensitization (92), and is involved in hypoxia-induced vasoconstriction, as suggested by the fact that inhibitors of SOD, such as diethyldithiocarbamate or triethylentetramine, produce larger potentiation of the hypoxic contraction (2, 101) and that EC-SOD overexpression in the lung ameliorates monocrotaline-induced pulmonary hypertension (PH) in rats (90).

More controversial is the effect of H2O2 on the regulation of pulmonary vascular tone. Studies provide evidence that H2O2 constricts both isolated perfused rat lung (28) and isolated, perfused rabbit lung (157), and also isolated rat and rabbit pulmonary arteries (86, 132, 137, 145, 160). These findings are substantiated by our finding in systemic vessels (11, 12). The possible mechanisms involved in H2O2-induced vasoconstriction are stimulation of serine esterase with subsequent activation of phospholipase A2 and production of arachidonic acid products (33, 87, 160), activation of PKC, and activation of tyrosine kinases in smooth muscle and endothelium (32, 128, 195, 197, 200). H2O2 is also responsible for the elevation of intracellular calcium concentration ([Ca2+]i) in PASMC during hypoxia (150, 183), and multiple similarities have been drawn between H2O2-induced vasoconstriction and hypoxic pulmonary vasoconstriction. In contrast, it has been shown that H2O2 produces concentration-dependent relaxation of precontracted isolated bovine arteries by a mechanism independent of the endothelium or prostaglandin mediators but related to soluble guanylate cyclase and cGMP (30). A similar H2O2-induced vasodilatation is described in isolated perfused rabbit lungs (29). Finally, in arteries stimulated with PGF2α or phenyleph-
Hypoxic Pulmonary Vasoconstriction

The physiological response to short-term hypoxia is a reversible contraction of the PASMC, a compensatory physiological response that serves to redirect blood to better ventilated areas of the lung and preserve proper ventilation of the systemic circulation (54). The response of the pulmonary circulation to a decrease in partial pressure of oxygen is discordant with the response of the systemic circulation in which arterial smooth muscle cells (SMCs) relax in response to hypoxia maintaining a fairly constant blood flow to important organs and tissues. This suggests that oxygen-sensing mechanisms in vascular smooth muscle in each circulatory system are different. Despite many recent studies, the mechanism involved in hypoxic pulmonary vasoconstriction (HPV) is, to date, not fully understood. The redox theory of HPV involves a redox-based O2 sensor regulating the activity of effector proteins, and there is strong evidence indicating a role of ROS as signaling intermediates in this response (8, 118). The redox theory, initially proposed by Weir and colleagues (118), describes a hypoxia-induced decrease in ROS production leading to potassium channel inhibition, membrane depolarization, and cell contraction. Hypoxia has also been reported to decrease intracellular concentration of ROS ([ROS]) in isolated rat lungs and PASMCs (9, 116, 117), as well as in microsome-enriched fractions of calf pulmonary arteries (121, 124). Michelakis et al. (116) has shown that hypoxia decreases H2O2 in rat PASMCs but not in renal (systemic) artery SMCs. In addition, hypoxia has been found to decrease H2O2 levels in both cultured human pulmonary and coronary (systemic) artery SMCs (114). Conversely, Waypa et al. (182) showed that hypoxia produces an increase in ROS production in cultured rat pulmonary and renal artery SMCs, and other studies (149, 150) indicate that hypoxia causes a large increase in ROS and Nox activity in freshly isolated mouse PASMCs but not in mesenteric SMCs. Although still a matter of debate, the concept that a paradoxical increase in ROS arises during hypoxia is gaining traction (179). Many studies have revealed that hypoxia increases intracellular ROS concentrations ([ROS]) in isolated rabbit and goat lungs, rat and dog pulmonary arteries, and cultured calf, dog, and rat PASMCs (26, 62, 84, 102, 112, 134, 180–182). In accordance with these findings, studies using a novel, redox-sensitive, ratiometric fluorescent protein sensor (RoGFP) (181, 182) and a developed, genetically encoded, specific ROS biosensor HyPer (22) reveal that hypoxia augments ROS signals in PASMCs. The explanation for these potentially odd results could be the use of different experimental conditions, but also recent studies suggest that these contrary hypotheses may hold true. That is, hypoxia causes a decrease in ROS generation in the mitochondrial matrix compartment, whereas it increases ROS production in the mitochondrial intermembrane space, which diffuses to the cytosol (182). This can lead to the activation of Nox proteins and a further increase in ROS levels (150).

One of the mechanisms by which hypoxia-derived ROS may lead to pulmonary artery vasoconstriction involves inhibition of Kv channels (principally Kv 1.5 and Kv 2.1), depolarization, activation of Ca2+ voltage-dependent L-type channels, and an increase in [Ca2+], in PASMCs (8). In support of this notion, many research groups have reported that exogenous H2O2, similar to hypoxia, inhibits Kv (38), induces an increase in [Ca2+], in PASMCs (183) (100), and vasoconstriction in pulmonary arteries (28, 86, 151, 157, 160, 189, 194).

Role of Nox-derived ROS in HPV. Many studies show that HPV is biphasic with an acute phase occurring in seconds/minutes and a sustained phase developing over several minutes to hours. Nox and mitochondria are the major sources of intracellular ROS generation mediating hypoxic responses in PASMCs (185, 191), and they are interdependent at least in acute HPV. The first evidence of a role for Nox in HPV came from experiments in which iodonium compounds or apocynin (both nonspecific Nox inhibitors) attenuates the hypoxic increase in [ROS] (112, 123), inhibits Kv currents (39, 184), increases [Ca2+], and induces contraction of PASMCs (112) and HPV in isolated lungs and pulmonary arteries (63, 73, 122, 123, 185, 186). Experiments using other Nox inhibitors, such as 4-(2-aminoethyl)benzenesulfonyl fluoride in isolated rabbit lung (186) and cadmium sulfate in isolated rat pulmonary artery further support the role of Nox in HPV. Although, Nox2–/– mice show normal or reduced hypoxic responses (10, 103); in p47phox–/– mice, acute but not sustained HPV is inhibited (185), suggesting that a Nox isoform other than Nox2 using p47phox contributes to the acute phase of HPV. Recently, a role for Nox4 in HPV has been shown by Ahmad et al. (3). They showed that Nox4-derived H2O2 maintains a basal relaxation under normoxic condition, which is removed by hypoxia leading to acute HPV. Increasing Nox4 expression with TGF-β was also observed to enhance HPV (192). Wolin et al. (75, 192) suggest that the mechanism of HPV in isolated bovine pulmonary arteries originates from the ability of pulmonary arteries to maintain increased smooth muscle levels of cytosolic NADPH, which sustain Nox4-derived H2O2 production. Moreover, Diebold et al. (48) showed that hypoxia induces the upregulation of Nox4 mRNA though hypoxia-inducible factor-1α (HIF-1α), which stimulates the synthesis of matrix metalloproteinases (MMPs), activation of MAPKs, transactivation of growth factor receptors, and signaling molecules and transcription factors involved in the proliferation of PASMCs.
and angiogenesis (109). In human lung adenocarcinoma A549 cells, an upregulation of Nox1 mRNA and protein occurred during hypoxia, accompanied by the activation of HIF-1-dependent target gene expression (heme oxygenase-1 mRNA, hypoxia-responsive element reporter gene activity), followed by enhanced ROS generation. HIF-1 induction is inhibited by diphenylene iodonium (DPI) and catalase, suggesting that hypoxic upregulation of Nox1 and subsequently augmented ROS generation may activate HIF-1-dependent pathways (67). Rathore et al. (150) recently revealed that hypoxia markedly increases Nox activity in PASMCs through activation of protein kinase C ε (PKCε) (150). Previous findings from the same group showed that the hypoxic activation of PKCε is secondary to increasing mitochondrial ROS (149), suggesting that hypoxia may enhance mitochondrial ROS generation, which activates PKCε and then augments Nox activity to cause further generation of intracellular ROS. Furthermore, several inhibitors of PKC isoforms inhibit HPV (17), and studies show the role for PKCζ in the activation of Nox induced by hypoxia (62). ROS also can activate a number of PKCs, and PKCα is involved in H₂O₂-induced contraction in pulmonary arteries (145). Whether various PKCs are simultaneously required remains unknown.

Role of Nox-derived ROS in pulmonary vascular remodeling. Chronic hypoxia induces irreversible change in vascular remodeling characterized by medial and adventitial thickening of the muscular and elastic vessels and muscularization of previously nonmuscular more distal small vessels. This leads to a thickening of the vessel wall and reduction of the lumen area, increased vascular resistance, and thus development of pulmonary hypertension and right ventricular hypertrophy. Distinct segments of the pulmonary artery appear to contribute to this process. Endothelial cells from the intima exhibit atypical proliferation and participate in the formation ofplexiform lesions. Nox2, Nox4, and p22phox are pivotal for proliferation of pulmonary endothelial cells (18, 142); SMCs from the medial layer exhibit hyperplasia and enhanced contractility (50). Both Nox1 and Nox4 are involved in the proliferation of SMCs (115, 120, 166). In particular, Nox4 is known to be induced by TGF-β and has been related to the TGF-β-dependent proliferation of SMCs in the pulmonary artery (82). In contrast to the mechanisms of TGF-β1 signaling observed in normoxic human PASMCs (166), the hypoxic release of TGF-β1 increases insulin-like growth factor binding protein (IGFBP-3) expression through phosphatidylinositol 3-kinase (PI3K) signaling with subsequent serine/threonine kinase (Akt) phosphorylation and further Nox4 activation. Moreover, IGFBP-3 increases Nox4 gene expression, resulting in PASMC proliferation (82).

The adventitial layer is thickened via hypoxia-induced proliferation of adventitial fibroblasts (153, 164). Furthermore, transdifferentiation of adventitial fibroblasts into myofibroblasts or SMCs appears to be critically involved in this process (162). Several studies demonstrate a role of Nox4 in the pathogenesis of hypoxia-induced pulmonary arterial remodeling and pulmonary hypertension, as well as idiopathic pulmonary arterial hypertension (IPAH) (120). In adventitial fibroblasts, a higher level of Nox4 mRNA expression than Nox1 is observed under normal conditions and this expression is significantly upregulated under hypoxic conditions (99). Consistent with this, a significant increase of Nox4 mRNA expression was observed under hypoxic conditions in adventitial fibroblasts from the lungs of patients with IPAH compared with healthy donors (99). Silencing of Nox4 causes reduction of H₂O₂ levels under normoxic and hypoxic conditions, suppression of the hypoxia-induced ROS increase, a decrease in adventitial fibroblast proliferation, and enhanced apoptosis (99).

Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is characterized by vascular remodeling and enhanced constrictor vasoactivity. In PAH, there is a progressive decrease in arterial lumen and an increase of pulmonary arterial pressure that ultimately leads to right ventricular failure and death (148). Three mechanisms principally contribute to the decrease of the arterial lumen: the increase in contractility of the pulmonary resistance arteries, the proliferation and remodeling of pulmonary endothelial SMCs and fibroblasts, and the thrombosis. Accumulating evidence indicates that ROS contributes to all three mechanisms mentioned above, which are involved in the pathogenesis of PAH. Liu et al. (103) show that a disruption of the murine Nox2 gene completely prevents chronic hypoxia-induced PAH and vascular remodeling. In addition to studies supporting a role for Nox2 in PAH in response to hypoxia (103), it has been demonstrated that PAH is also characterized by the induction of Nox4, perhaps in response to an initial activation of Nox2 within the pulmonary endothelium, which is responsible for increased ROS generation and SMC proliferation (120). Hypoxia-dependent development of PAH in mice is linked to increased Nox4 expression in PASMCs (120), suggesting a role for Nox4 in the vascular remodeling associated with hypoxia-induced PAH. As mentioned above, hypoxia increases the expression of TGF-β (85) and Nox4 expression (166); Nox4 has also been shown to be critical for hypoxia-inducible factor 2α (HIF-2α) expression and transcriptional activation in renal carcinoma cells (111). This suggests that in hypoxic vascular remodeling, a mechanism involving HIF-2α, TGF-β, and Nox4 is operant.

One of the early events contributing to the pathophysiology of all forms of PAH is endothelial dysfunction (148). A role for Nox2 in hypoxia-induced endothelial dysfunction involving intrapulmonary arteries has been established. In addition, ROS generation from Nox-independent sources, xanthine oxidase (83), or uncoupled endothelial NO synthase (eNOS) (94) may also contribute to hypoxia-induced vascular dysfunction and PAH.

Several strategies designed to diminish ROS levels, through scavenging ROS or through the inhibition of the sources of ROS, have been proposed as potential therapeutic treatments for PAH. For example, treatment with recombinant human SOD is able to enhance ROS clearance, which leads to enhanced eNOS expression and function in animal models (neonatal lambs) of persistent pulmonary hypertension of the newborn (PPHN) (55). Interestingly, Kamezaki et al. (90) recently reported that gene transfer of extracellular SOD (EC-SOD) protects rats against monocrotaline-induced PH, suggesting that extracellular O₂⁻ is important in the disease process. Manipulation of EC-SOD in models of vascular and lung diseases confirm a role for extracellular O₂⁻ in the pathogenesis of a number of disease models associated with fibrosis and tissue remodeling (13, 23, 56, 60, 147). Using wild-type (WT)
and transgenic mice overexpressing human EC-SOD in the lung, Nozik-Grayck et al. (131) show a protective effect of lung EC-SOD overexpression on chronic hypoxic-induced PAH and vascular remodeling. This protective effect is suggested to involve the suppression of redox-sensitive transcription factor early growth response-1 (Egr-1) pathway (131). In addition, catalase and SOD mimetics have proven beneficial in PH (46). Moreover, inhibition of xanthine oxidase (83) or eNOS (193) has shown beneficial effects in rat neonates with PH and in caveolin-1 knockout mice with PH, respectively. Also, soluble guanylate cyclase activators have yielded beneficial effects in preclinical studies in different animal models of PAH (163). Finally, in the clinic, use of phosphodiesterase-5 inhibitors has also been reported to reduce ROS levels (80).

Conclusion

For nearly two decades, Nox-derived ROS have been described as critical mediators of systemic vascular hypertension. While ROS vasoconstrictor and vasoactivator effects in pulmonary vessels have been studied for at least as long, interest in the role of Nox in the complex orchestration of events leading to pulmonary hypertension has burgeoned in recent years. Nox isoforms appear to subserve a pivotal role in lung signaling, leading to marked changes in pulmonary airway, pneumocyte, and vascular cell phenotypes, including proliferation, hypertrophy, and apoptosis, resulting in acute lung injury, emphysema, and pulmonary hypertension among other pathologies. Evidence has emerged for a highly influential role of Nox in pulmonary hypertension; however, much of these data are either associative or are gleaned from in vitro studies. Studies designed to modulate Nox isoforms in a pulmonary vascular cell-specific manner will be needed to draw more definitive conclusions. Even less well studied is the direct or indirect contribution Nox isoforms play in right ventricular failure, the major cause of death in patients with PH. As past drug therapy has been focused solely on various pulmonary vascular targets and has largely been disappointing, emphasis should be placed on targeting individual or combinations of Nox in both the pulmonary vasculature and the right ventricle. For this reason, major priority is now being given to the discovery of specific Nox small molecule and peptidic inhibitors and therapies targeting these drugs to the pulmonary vasculature and right ventricle.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.F. drafted manuscript; H.C.C. and P.J.P. edited and revised manuscript; P.J.P. approved final version of manuscript.

REFERENCES

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