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A BRIEF OVERVIEW OF NITRIC OXIDE AND REACTIVE OXYGEN SPECIES SIGNALING IN HYPOXIA-INDUCED PULMONARY HYPERTENSION

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Abstract

Pulmonary hypertension (PH) is characterized by increased vasoconstriction and smooth muscle cell hyperplasia driving pathological vascular remodeling of arterial vessels. In this short review, we discuss the primary source of reactive oxygen species (ROS) and nitric oxide (NO) relevant to PH and the mechanism by which dysregulation of their production contributes to PH. Specifically, hypoxia-induced PH is associated with diminished endothelial nitric oxide synthase (eNOS)-derived NO production and increased production of superoxide ($O_2^{\cdot -}$) through eNOS uncoupling and defective mitochondrial respiration. This drives the inhibition of the NO/soluble guanylate cyclase (sGC) pathway and activation of the transcription factor hypoxia-inducible factor-1α (HIF-1α) with consequential dysregulation of the pulmonary vasculature. Therapeutics aimed at increasing NO or cGMP bioavailabilities are amenable to hypoxia disease-induced PH. Similarly, strategies targeting HIF-1α are now considered. Overall, pulmonary hypertension including hypoxia-induced PH offers unique opportunities for the rational development of therapeutics centered on modulating redox signaling.

Keywords

pulmonary hypertension; vascular remodeling; smooth muscle; hypoxia; nitric oxide; nitrite; mitochondria; reactive oxygen species; superoxide; hydrogen peroxide; HIF-1α

1. Introduction

In general, chronic hypoxia leads to pulmonary artery remodeling driven by smooth muscle proliferation and increase in wall thickness, which causes increase in flow resistance. This phenomenon leads to pressure overload of the heart right ventricle (RV) potentially causing its failure, the main driver of mortality in patients with chronic obstructive disease (Naeije, 2005). The remodeling process is initiated by oxygen sensors present in vascular cells that detect a decrease in partial pressure of oxygen in the blood (pO_2), and then activate a signaling system that leads to acute constriction of pulmonary arteries (Prabhakar and Semenza, 2012). Eventually, this acute phase is "consolidated" by architectural remodeling

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of the vascular wall that perpetuates lumen narrowing (Prabhakar and Semenza, 2012). Interestingly, although RV dysfunction seems to be caused by pressure overload secondary to increase in pulmonary vascular resistance, new evidence suggest that these two phenomena could develop independently in such a way that RV dysfunction occurs even if PH is prevented (Ball et al., 2014).

Among many signaling molecules, the contribution of reactive oxygen species (ROS) and NO to the pathophysiology of PH is complex and partially elucidated in the context of hypoxia-mediated pulmonary hypertension (World Health Organization Class 3). Some of the cellular signals found to participate in the hypoxia-induced PH seem to be also relevant in other models of pulmonary vascular remodeling that occur under normoxia (Bonnet et al., 2006). In this short review, we will outline the salient results that provide a foundation to the delineation of the mechanisms by which aberrant production of NO and ROS may contribute to the pathogenesis of PH.

2. Nitric oxide and pulmonary hypertension

2.1 Relevant Nitric oxide biochemistry

Nitric oxide (NO) is a paracrine and autocrine messenger molecule that is derived from the five electron oxidation of L-arginine (Moncada et al., 1991). This reaction is catalyzed by nitric oxide synthase (NOS; Figure 1), of which 3 isoforms have been described in mammals, neuronal NOS (nNOS, NOS1), inducible NOS (iNOS, NOS2), and endothelial NOS (eNOS, NOS3) (Nathan and Xie, 1994). Tissues and cells conserve NO through nitrosylation and nitrosation of biomolecules and NO itself can be released upon reductive decomposition of functional groups such as S-nitrosothiols (Figure 1; (Feelisch et al., 2002). The reaction of NO with metals to form nitrosyl or nitroso species is also an important step determining many of the functional effects of NO such as the activation of soluble guanylate cyclase (sGC) or inhibition of cytochrome c oxidase (Grisham et al., 1999). In addition, the nitrosation of thiols by reactive species derived from NO serves as a posttranslational modification that modulates protein function such as certain caspases (Foster et al., 2003).

An alternative source of NO is derived from the reduction of nitrite (NO_2^-; Figure 1) that proceeds at low pH and under hypoxia (Zweier et al., 1995). The significance of this reaction is as an alternative source of NO at sites where NOS might be inhibited due to the lack of molecular oxygen but where hypoxic and acidic reduction of diet or pharmacologically-derived NO_2^- is possible. Deoxyhemoglobin is an important site upon which NO_2^- is reduced to NO, although the chemical pathway and mechanism by which NO may escape erythrocytes still need clarification (Huang et al., 2005). Whether non-erythrocytic cells mediate hypoxic NO_2^- reduction in the lung is also under investigation with multiple intracellular activities identified consisting of additional globins (Myoglobin (Rassaf et al., 2014), Neuroglobin (Tiso et al., 2011), and Cytoglobin (Li et al., 2012)) and molybdenum-containing proteins (xanthine dehydrogenase (Li et al., 2001), sulfite oxidase (Wang et al., 2015), aldehyde oxidase and mitochondria amidoxine reducing component (Sparacino-Watkins et al., 2014)) potentially contributing to this activity in the pulmonary vasculature.

One of the most significant reactions of nitric oxide (NO) is its combination with superoxide ($O_2^{\cdot -}$) at a diffusion-limited rate (Figure 1; (Beckman et al., 1990)). The product of this reaction, peroxynitrite ($ONOO^-$/ONOOH), is a one and two electron oxidant, which modifies DNA, proteins, lipids, and sugars by way of oxidation, nitration, and nitrosation (Beckman et al., 1990, Beckman et al., 1992, Gow et al., 1997). The biochemical reactivity of peroxynitrite in physiologically relevant settings may be dominated by its reaction with thiols and transition metals but also with excess carbon dioxide to yield a nitrosoperoxocarboxylate anion ($ONOOCO_2^-$) that partially decomposes to nitrogen dioxide and the carbonate radical (Denicola et al., 1996). Under most conditions, peroxynitrite might not coexist with NO or $O_2^{\cdot -}$ because superoxide dismutase and oxyhemoglobin insure limited availability of these molecules in excess of peroxynitrite. However, during conditions characterized by high NO synthase activity and multiple cellular sources of $O_2^{\cdot -}$, peroxynitrite-mediated reactions combined with those of excess NO or $O_2^{\cdot -}$ may become important. The formation of peroxynitrite in vivo, inferred from the formation of stable footprints such as 3-nitrotyrosine, represents an important mediator of tissue injury and dysfunction that limits NO bioavailability (Beckman and Koppenol, 1996).

2.2 Nitric oxide signaling in pulmonary hypertension

The bioavailability and signaling of NO is decreased in experimental models and in patients with PH (Xue and Johns, 1995, Kharitonov et al., 1997, Fagan et al., 1999, Quinlan et al., 2000). For example, Giaid and Saleh provided some evidence that the expression of eNOS was decreased in the vascular endothelium of pulmonary arteries in a cohort of patients with pulmonary hypertension with different grades of arteriopathy (Giaid and Saleh, 1995). The dysfunction is usually considered to be decreased vaso-protection including depleted vasodilatory, anti-migratory, and anti-proliferative functions. However, loss of eNOS is also associated with a decrease in muscularization of small pulmonary vessels during chronic hypoxia in the mouse due to a decrease in proliferative capacity (Quinlan et al., 2000). The underlying mechanism has been relatively well-studied in animal models and is usually considered to be multifactorial through changes in eNOS expression and uncoupling (Zhao et al., 2009), alteration in L-arginine metabolism (Block et al., 1995, Xu et al., 2004), and increased NO consumption through $O_2^{\cdot -}$. Although conflicting results exist regarding the levels of eNOS expression during PH, it is possible that – if increased - eNOS in the context of PH is uncoupled, meaning that a fraction of its activity is diverted towards the production of other reactive species such as $O_2^{\cdot -}$. Increased ROS production is associated with endothelial dysfunction and NADPH oxidase (NOX)-derived $O_2^{\cdot -}$ limits NO production and downstream signaling through eNOS uncoupling (Landmesser et al., 2003). Overall, this provides conditions conducive to decrease NO bioavailability and increase oxidative and nitrative stress through the formation of peroxynitrite or metal-catalyzed nitration. Insufficient stimulation of sGC by NO reduces cGMP production and downstream effector activation such as cGMP-dependent protein kinase (PKG) (Zhao et al., 2009). In addition, downstream nitration or oxidation of target molecules such as PKG may lead to amplification of the inhibitory effect associated with NO inactivation (Zhao et al., 2009).

2.3 Therapeutics based on direct targeting of cGMP and NO surrogates' delivery

With a key role for the dysregulation of NO signaling in PH (including WHO Group 3 PH), therapeutic strategies aimed at restoring the NO/cGMP pathway have received increasing attention. A novel class of drugs that directly stimulates sGC independently of NO is now aggressively pursued for the treatment of PH. One such molecule, riociguat (Figure 2), has been approved for the treatment of pulmonary artery hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH)(Wardle et al., 2016, Tsugu et al., 2016). Riociguat has also shown significant therapeutic effects in patients with other types of PH including interstitial lung disease PH and PH associated with chronic obstructive pulmonary disease (COPD) (Benza et al., 2016). The NO/cGMP pathway may also be targeted by inhibitors of phosphodiesterase type 5 (PDE-5) causing inhibition of the breakdown of cGMP by PDE-5. One such molecule, Tadalafil (Figure 2), has been shown to reduce clinical worsening and improve hemodynamic outcomes in patients with PAH and is use in the clinic in this specific setting (Galie et al., 2009).

A number of preclinical studies have also indicated the beneficial effect of providing an alternate source of NO in the form of pharmacological delivery of NO_2^- or nitrate (NO_3^-) to alleviate PH (Zuckerbraun et al., 2010, Baliga et al., 2012, Pankey et al., 2012). In the context of hypoxia-induced PH in the mouse, inhaled nebulized NO_2^- inhibits and reverse pre-establish PH and high right ventricular pressure. In this case, the effect of NO_2^- has been shown to be inhibited by a xanthine oxidase inhibitor or through diet-mediated inhibition of molybdenum-containing enzymes (Zuckerbraun et al., 2010). Dietary NO_3^- (which can be reduced to NO_2^-through the entero-salivary cycle) also reduced pulmonary vascular remodeling in mouse exposed to hypoxia for three weeks (Baliga et al., 2012). Interestingly, this effect required eNOS in addition to xanthine oxidase, suggesting a role for eNOS as a nitrite reductase. In a recent early phase II pilot study, Simon and coworkers have shown that inhaled NO_2^- provides some hemodynamics improvement in a small group (n=6) of patients with PH due to lung disease or hypoxia, although these effects were less than those observed in patients with WHO Group 2 PH (Simon et al., 2016).

3. Reactive oxygen species and pulmonary hypertension

3.1 Significant sources of ROS

While signaling pathways centered on NO bioavailability are key therapeutic targets for the treatment of PH, the production of ROS is also an essential contributor to hypoxia-induced PH. In this case, cellular respiration is an important source of ROS (Waypa et al., 2016). Accordingly, ROS role in the development of PH is strongly suggested by studies showing profound alteration in mitochondrial structure and function in that context. Using human and rodent models, Ryan and colleagues found evidence of mitochondrial fragmentation (Ryan et al., 2013), which is associated with a decrease in the expression of mitofusin-2 (MFN2), a molecular regulator that promotes the fusion of mitochondria into long tubular structures. Also, PGC1α, a transcriptional activator of mitochondrial biogenesis was found to be downregulated in that context. Adenoviral overexpression of MFN2 increased mitochondrial fusion, decreased proliferation, lessened the severity of PH, and improved exercise capacity in the rodent model (Ryan et al., 2013). These results suggest that decreases in MFN2 and

PGC1α contribute to pulmonary vascular remodeling and provide indirect evidence of a potential role of mitochondrial-derived ROS in the pathophysiology of PH.

Although not completely elucidated in one single model, compelling evidence from different laboratories indicates that low oxygen stimulation leads to mitochondrial production of ROS, which serves as an activator of prolyl-4-hydroxylases which in turn induce activation of Hypoxia-inducible factor 1 (HIF-1), a necessary event that triggers vascular remodeling and narrowing of the pulmonary arteries (Figure 3). We present, in the following paragraphs, a concise review of the current evidence supporting this mechanism.

During hypoxia, mitochondria from vascular cells release superoxide ($O_2^{\cdot -}$) from complex III to the intermembrane space, where it is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (Thompson, 2016, Waypa et al., 2016). The H_2O_2 then enters the cytosol, where it activates multiple responses contributing to smooth muscle contraction and remodeling. The mechanism of hypoxia-driven ROS generation was first suspected using pulmonary artery cell homogenates, which suggested that superoxide generation increased during hypoxia in an effect that was inhibited by diphenylene iodonium, a flavoptrotein inhibitor of NADPH oxidase but not by the mitochondrial inhibitor myxothiazol, which blocks electron entry into complex III (Marshall et al., 1996). Chandel et al first demonstrated that mitochondrial ROS signals control gene transcription in hypoxia (Chandel et al., 1998). Specifically, using mitochondrial inhibitors, ρ0 cells lacking a functional electron transport chain (ETC), and ROS-sensitive fluorescent chemical probes, they showed that under hypoxia ETC is required for ROS-dependent stabilization of the HIF-1α transcription factor subunit (Chandel et al., 1998). Also, the same group found that even though anoxia is the most extreme form of hypoxia, these two stimulations operate differently on downstream signaling: the activation of HIF-1 under hypoxia requires mitochondrial ETC whereas, under anoxia, HIF-1 is activated without involvement of ETC (Schroedl et al., 2002).

The specific mechanisms linking hypoxia and ROS generation are not completely understood, but evidence indicates that they involve oxidation of complex III, which requires cytochrome c and leads to the formation of $O_2^{\cdot -}$ that is later ejected to the intermembrane space due to electrical gradient. Indeed, when cytochrome c is absent, complex III remains fully reduced, which prevents ROS generation under hypoxia and are unable to stabilize HIF-1α (Mansfield et al., 2005). These findings implicate electron flux through complex III as a critical event in the detection of hypoxia in cells (Mansfield et al., 2005). Waypa et al. demonstrated that hypoxic pulmonary vasoconstriction required electron flux through complex III, and that increases in ROS generation were responsible for eliciting the hypoxic response (Waypa et al., 2010, Waypa et al., 2013). Specifically, these authors demonstrated that acute production of ROS during hypoxia pulmonary artery smooth muscle cells depends on the Rieske iron-sulfur protein subunit of complex III, as reflected by PH attenuation in animals with deletion of this gene using a Cre/loxP system (Waypa et al., 2013).

3.2 Signaling pathways associated with increased ROS in pulmonary hypertension

3.2.1 ROS cause HIF-1 activation—HIF-1 is a highly conserved transcription factor present in almost all cell types (Prabhakar and Semenza, 2012). It is tightly regulated by O_2

availability, and modulates the expression of hundreds of genes. HIF-1 exists as a heterodimer, consisting of HIF-1α and HIF-1β subunits. HIF-1β is constitutively expressed, whereas HIF-1α is found at very low levels under normoxic conditions (Shimoda and Semenza, 2011). In this context, HIF-1α protein is ubiquitinated and degraded by the proteasomal pathway; however, acute exposure of pulmonary arterial smooth muscle cells (PASMCs) or endothelial cells (ECs) to hypoxia (1% O_2) causes increased HIF-1α protein levels and HIF-1 DNA-binding activity. Thus, HIF-1α confers sensitivity and specificity for hypoxic induction of HIF-1 transcriptional activity (Prabhakar and Semenza, 2012, Shimoda and Semenza, 2011).

Under normoxia, HIF-1 is associated to von Hippel-Lindau protein (VHL), which recruits an E3-ubiquitin protein ligase Elongin 2 and 3, Cullin 2, and RBX1(Kamura et al., 2000, Maxwell et al., 1999). Binding of VHL depends on hydroxylation of HIF-1 proline-402 and 564 in well-oxygenated cells (Ivan et al., 2001). Three prolyl-4-hydroxylase domain proteins (PHDs) that hydroxylate proline-402 and 564 in an O_2-dependent manner are identified in mammalian cells (Ivan et al., 2002, Epstein et al., 2001). These proteins, known as PHD1, PHD2, and PHD3 are members of a superfamily of dioxygenases that contain Fe(II) in their catalytic center and utilize O_2 and α-ketoglutarate as substrates. Reduction of Fe(III) to Fe(II) in the catalytic center by ascorbate is required for a subsequent catalytic cycle. The observed reduction in hydroxylase activity under hypoxic has been proposed to be due to substrate (O_2) limitation (Epstein et al., 2001, Chua et al., 2010) and/or by an increase in mitochondrial production of ROS that may oxidize Fe(II) and inactivate the PHDs (Brunelle et al., 2005, Guzy et al., 2005, Mansfield et al., 2005). Thus, hypoxia can lead to PDHs deactivation and HIF-1 stabilization via a direct effect of either low oxygen, or also ROS on PDHs (Figure 3). Importantly, at least in the acute phase, the generation of ROS appears to be a necessary step in the process of PH under hypoxia (Waypa et al., 2013).

3.2.2 HIF-1 mediates hypoxia-driven pulmonary hypertension—Seminal work by Shimoda and coworkers (Shimoda et al., 2001) established the effects of chronic hypoxia (CH) on heterozygous mice lacking one copy of the HIF-1 gene (homozygous animals could not be used due to intrauterine lethality). Compared with wild-type control animals, heterozygous HIF-1a mice demonstrated impaired lung vascular remodeling in chronic hypoxia and attenuated RV hypertrophic responses (Shimoda et al., 2001, Kline et al., 2002, Yu et al., 1999, Shimoda et al., 2006). This was associated with lower level of vascular smooth muscle hypertrophy, attenuated up-regulation of transient potential receptor proteins and Na^+/H^+ exchanger-isoform 1, and failure to suppress the expression of plasma membrane K1 channels during CH (Shimoda et al., 2001). Recently, using a Cre/loxP system smooth muscle-specific conditional deletion of HIF-1, Ball et al demonstrated that HIF-1 is critical as a mediator of pulmonary arterial remodeling under hypoxia. Interestingly, they also found that loss of HIF-1 function in smooth muscle did not affect hypoxic cardiac remodeling (Ball et al., 2014); suggesting that the cardiac hypertrophy response is not directly coupled to the increase in pulmonary artery pressure (Figure 3). This last finding challenges the "hemodynamic dogma" that states that the right ventricular hypertrophy and eventual failure depend purely on pressure overload due to increase of pulmonary vascular resistance and suggests that ventricular and vascular remodeling are

distinct and somewhat independent processes. Similar challenges are emerging regarding the left ventricular remodeling in connection to systemic hypertension (Popov et al., 2014).

3.2.3 Mechanisms of HIF1-driven vascular wall remodeling—Chronic hypoxia induces functional and structural changes in the endothelial and smooth muscle cells, and fibroblasts that make up the intima, media, and adventitia of pulmonary arterial wall thus contributing to pulmonary hypertension (Morrell et al., 2009). The effect of hypoxia on vascular remodeling has been mostly studied in PASMC. Acute hypoxia leads to an increase in intracellular calcium $[Ca^{2+}]_i$ that is reversible upon reoxygenation. In contrast, chronic hypoxia causes a sustained increase on $[Ca^{2+}]_i$ which remains elevated even after return to normoxia (Shimoda et al., 2000). This effect is mediated by store-operated Ca^{2+} channels, which are activated by depletion of intracellular Ca^{2+} stores during chronic hypoxia (Wang et al., 2006). These channels are composed of transient receptor potential (TRP) proteins, which are under the control of HIF-1 (Wang et al., 2006). Indeed, infection with an adenovirus encoding a constitutively active form of HIF-1α increases TRPC1 and TRPC6 expression under non-hypoxic conditions.

Hypoxia inhibits opening of voltage-gated K^+ channels Kv1.5 and Kv2.1, which contributes to PASMC depolarization (Archer et al., 1998). The expression of these channels is also decreased in PASMCs subjected to chronic hypoxia in vivo or ex vivo (Reeve et al., 2001, Yuan et al., 1998), and these changes in gene expression are also HIF-1α dependent (Bonnet et al., 2006). In addition to increased $[Ca^{2+}]_i$, chronic hypoxia also results in increased intracellular pH (pHi) in PASMCs, an effect that is due to HIF-1-dependent expression of the sodium-hydrogen exchanger NHE1 (Shimoda et al., 2006). Increased $[Ca^{2+}]_i$ and pH_i contribute to the activation of signal transduction pathways that promote PASMC hypertrophy and hyperplasia, which leads to the medial thickening of pulmonary arterioles, which is the pathological hallmark of hypoxia-induced PAH. Indeed, exposure of WT but not *Hif-1*$^{-/-}$ mice to chronic hypoxia induces PASMC alkalinization and hypertrophy (Shimoda et al., 2006, Shimoda et al., 2001).

The demonstration that mice partially deficient in HIF-1α expression are protected against the development of PAH suggests that pharmacological inhibition of HIF-1 may be of therapeutic benefit in this clinical context. This hypothesis was tested by the daily administration of digoxin, a cardiac glycoside that has been used to treat congestive heart failure and cardiac arrhythmias for decades, which inhibits the synthesis of HIF-1α protein (Zhang et al., 2008). Treatment with digoxin attenuated the development of right ventricular hypertrophy and prevented the changes in pulmonary vascular $[Ca^{2+}]_i$ and pH_i, remodeling, and pressure that occur in mice exposed to chronic hypoxia (Abud et al., 2012).

The role of HIF-1 in the pathogenesis of PH is not restricted to hypoxia-induced PH. The spontaneous development of PAH in fawn-hooded rats is associated with increased HIF-1α expression, HIF-dependent reductions in K^+ currents and Kv1.5 expression, increased PDK1 expression, and a switch from oxidative to glycolytic metabolism in pulmonary artery smooth muscle cells (Bonnet et al., 2006). These metabolic changes appear to play a critical role in the pathogenesis of PAH because treatment of animals with dichloroacetate, an inhibitor of PDK1, leads to regression of PAH (Michelakis et al., 2002).

4. Conclusion

Dysregulation in NO and ROS production have been established in the context of PH including hypoxia/disease-induced PH and there is now sufficient evidence to indicate that decreased NO bioavailability and increased mitochondrial-derived $O_2^{\cdot -}/H_2O_2$ production are central to the pathogenesis of PH. Whether these two arms of redox biology converge on common molecular pathways and pathologies is still debatable and will require additional investigation. What is clear is that the elucidation of the signaling pathways downstream of NO and ROS and their pathophysiological alterations will continue to provide a foundation for the rational design and clinical implementation of new therapies targeting PH.

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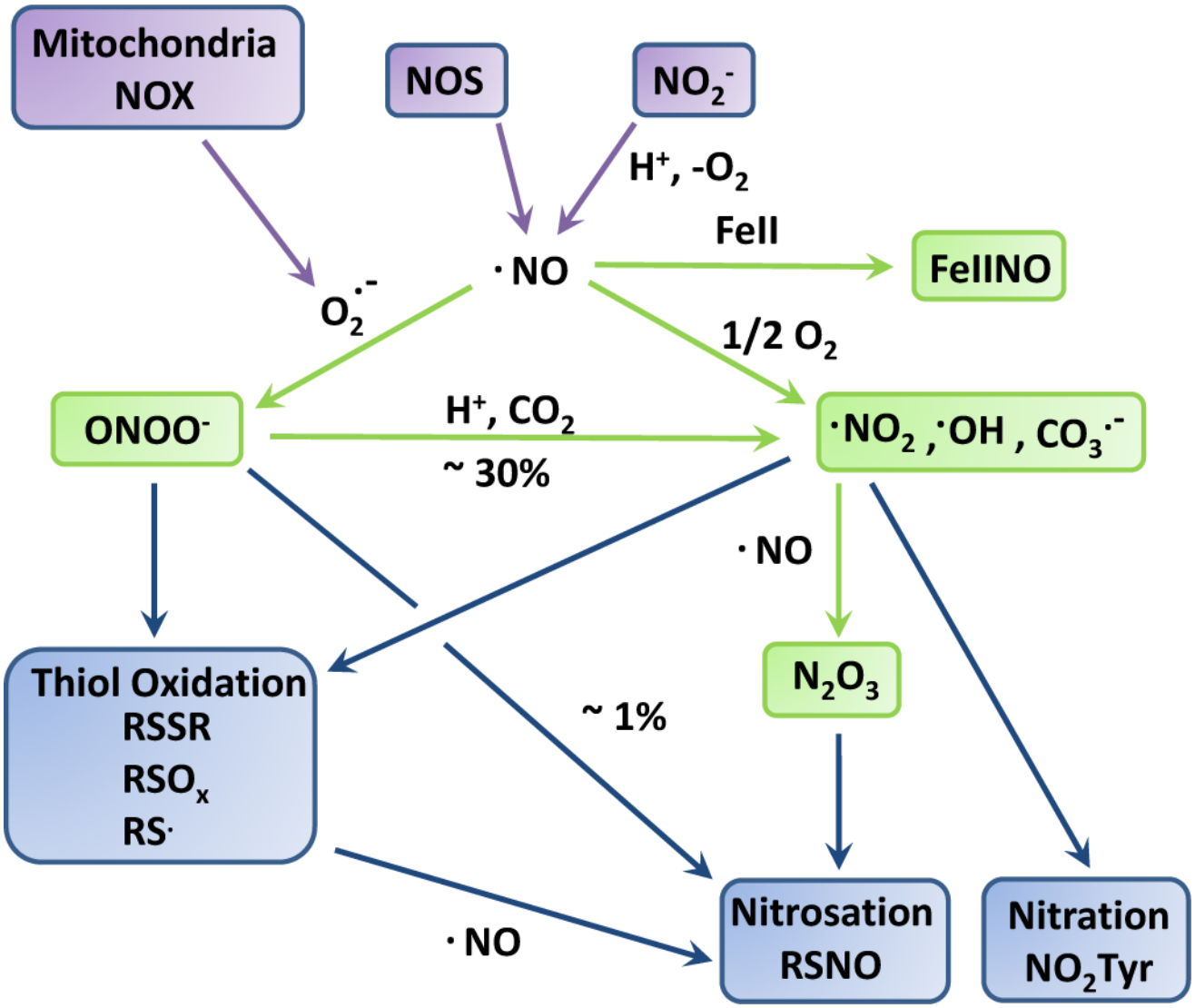

Fig. 1. Biological chemistry of nitric oxide relevant to pulmonary hypertension

Important sources of nitric oxide (NO) include nitric oxide synthase (NOS) activity and nitrite (NO_2^-)-reduction under hypoxia and in acidic environments. NO reacts with superoxide ($O_2^{\cdot -}$) to form peroxynitrite ($ONOO^-$). Superoxide may be derived from multiple sources including mitochondrial and NADPH oxidase (NOX) activities. Peroxynitrite - upon protonation or combination with carbon dioxide (CO_2) – yields a number of free radicals, including nitrogen dioxide ($\cdot NO_2$), and the hydroxyl ($\cdot OH$) and carbonate $CO_3^{\cdot -}$) radicals. NO reacts with metals such as iron (FeII) to form a metal-nitrosyls (FeIINO) such as the one found in soluble guanylate cyclase. Nitric oxide also reacts with molecular oxygen to form nitrogen dioxide and dinitrogen trioxide (N_2O_3). All together these species may be involved in oxidative (such as thiol oxidation), nitrosation (thiol nitrosation, RSNO), and nitration (tyrosine nitration, NO_2Tyr) reactions with biological targets. See Text for details.

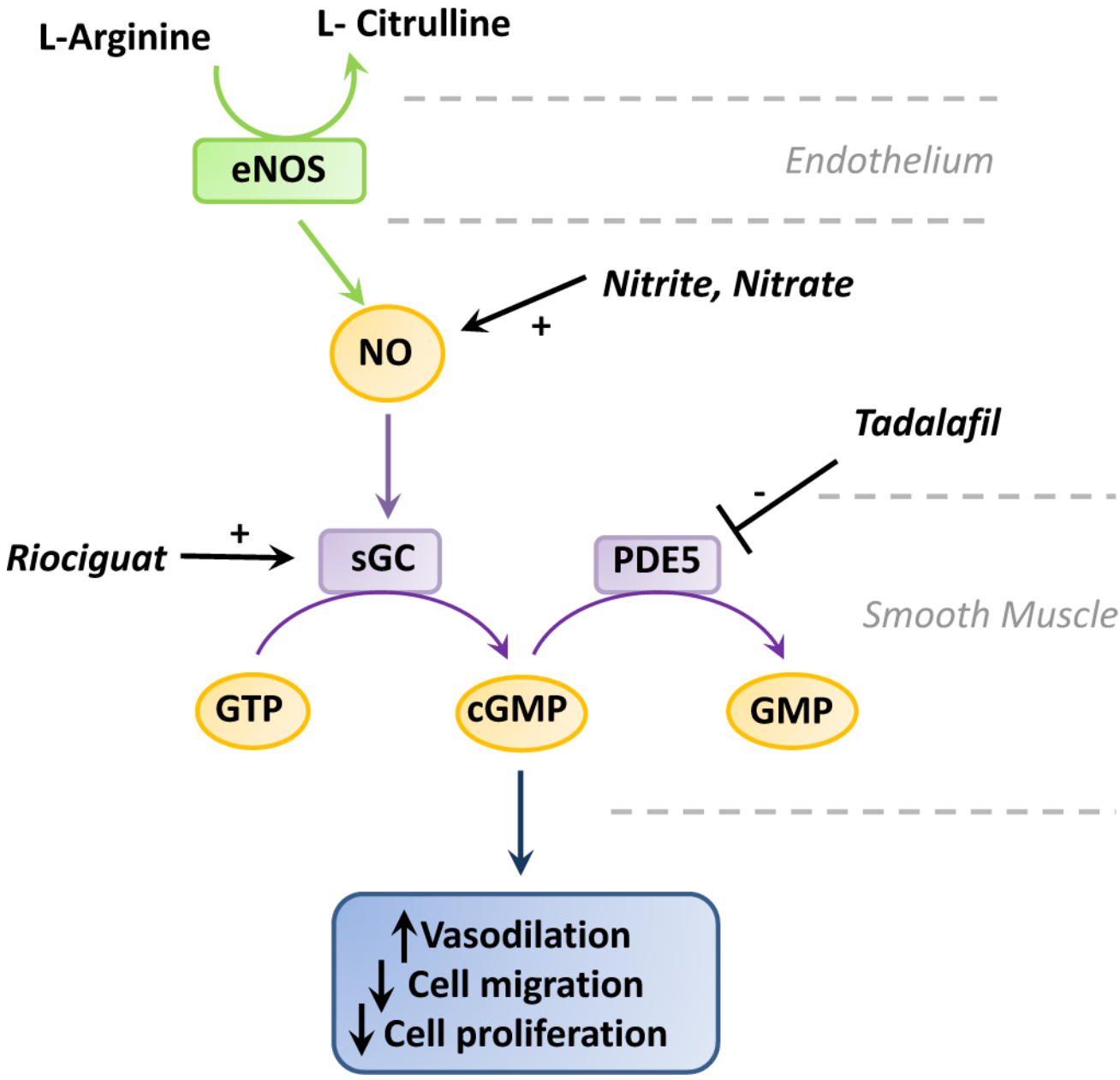

Fig. 2. Mechanism of action of NO in the vasculature and therapeutic targets

Nitric oxide (NO) is generated from the oxidation of L-arginine to L-citrulline by endothelium Nitric Oxide Synthase (eNOS). NO diffuses into target cells such as smooth muscle cells to bind and activate soluble guanylate cyclase (sGC), which in turn generates cyclic GMP (cGMP) from GTP to promote vasodilation, and inhibit cell migration and proliferation. The signal is turned off upon cGMP hydrolysis to GMP by phosphodiesterase 5 (PDE5). Inhibition of this pathway is thought to contribute to the pathogenesis of pulmonary hypertension (PH). Increase NO biovailability through nitrite or nitrate delivery, stimulating sGC with Riociguat, or inhibiting PDE5 with Tadalafil all provides therapeutic means for the treatment of certain type of PH. See Text for details.

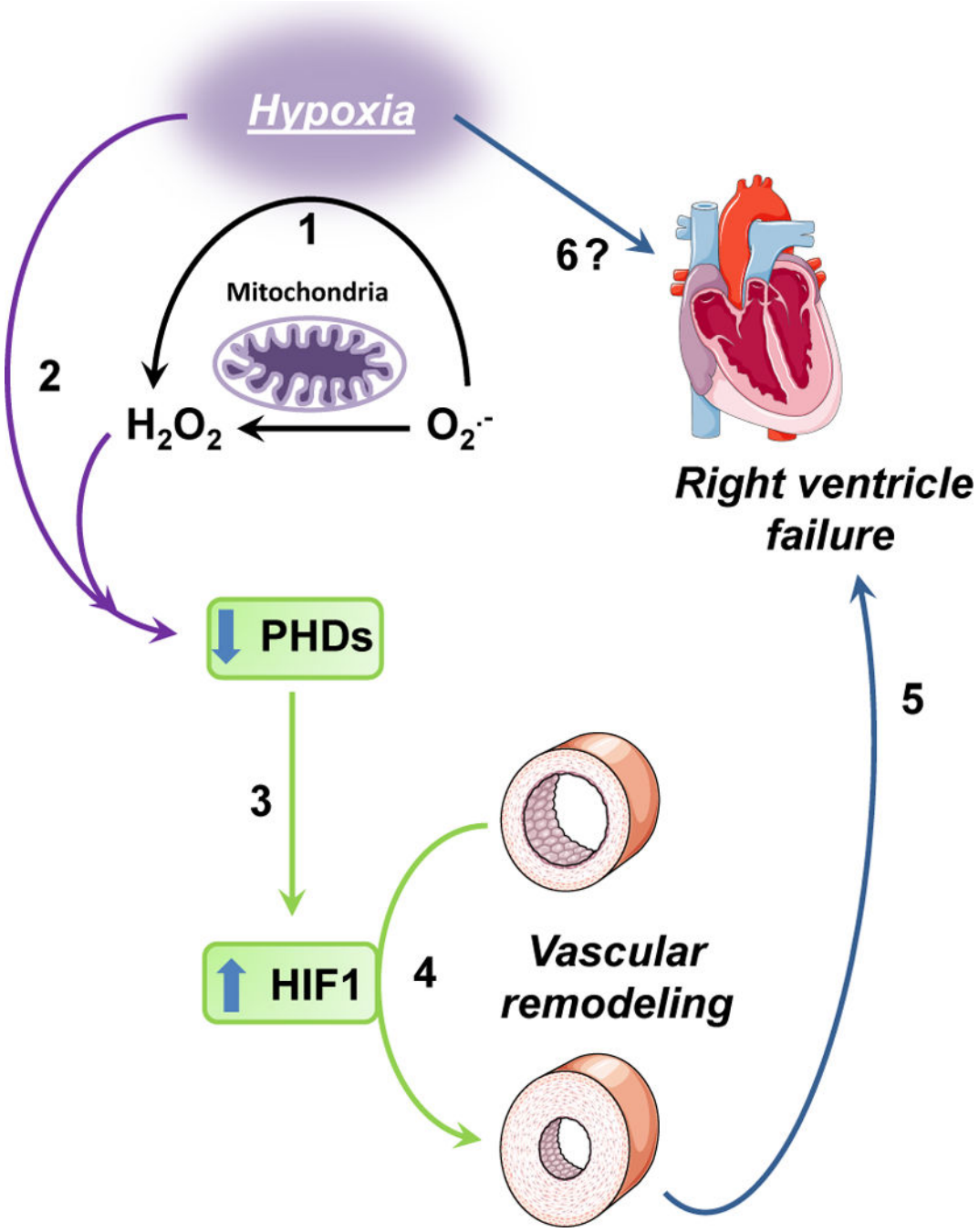

Fig. 3. Potential mechanisms of hypoxia-induced pulmonary hypertension

1: hypoxia causes cells to release superoxide ($O_2^{\cdot-}$) which is converted to hydrogen peroxide (H_2O_2). 2: Both low oxygen and ROS production cause a reduction in hydroxylase activity of PHDs. 3: Lower PHDs activity causes a reduction of HIF-1α proline-402 and proline-564 hydroxylation, which leads to dissociation from VHL and stabilization of the transcriptional activity of HIF-1α. 4: Increase in the transcriptional activity of HIF-1α causes pulmonary vascular remodeling. 5: Increased pulmonary vascular resistance leads to right ventricle

failure. 6: Hypoxia could also contribute to right ventricle remodeling and failure independent of HIF-1α (see Ball et.al.).