

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Nitric Oxide in Pathophysiology and Treatment of Pulmonary Hypertension

---

Junko Maruyama, Ayumu Yokochi, Erquan Zhang,  
Hirofumi Sawada and Kazuo Maruyama

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55680>

---

## 1. Introduction

All conditions causing pulmonary hypertension (PH) are characterized by three major changes in the pulmonary vasculature: vasoconstriction, vascular remodeling, and thrombosis [1,2,3]. Vascular remodeling includes muscularization of normally non-muscular peripheral pulmonary arteries, increase in medial wall thickness of muscular arteries, and increase in vascular connective tissue such as collagen and elastin [1,2,3]. Imbalance of vasoconstrictive and vasodilatory mediators might explain the increased vascular tone [1,2,3]. Endothelial cells synthesize and release prostacyclin and nitric oxide for vasodilation as well as endothelin and thromboxane for vasoconstriction. Approved treatments for pulmonary arterial hypertension (PAH) include prostacyclins, endothelin receptor blockers, and phosphodiesterase-5 inhibitors as well as inhaled NO for persistent pulmonary hypertension of the neonate (PPHN) [2].

Studies have demonstrated that short- and long-term NO inhalation improves arterial oxygenation and reduces pulmonary artery (PA) pressure in animal models of PH [4,5,6,7,8,9,10] and clinical disease such as post-operative congenital heart disease [11,12], chronic obstructive pulmonary disease (COPD) [13], pulmonary fibrosis [14], and acute respiratory distress syndrome (ARDS) [15]. In chronic hypoxia-induced PH in rats, we showed that low-dose NO (less than 5ppm) induces a submaximal reduction in pulmonary artery pressure, which does not correlate with the severity of pulmonary vascular changes [4]. Clinically, the effect of inhaled NO is based on pulmonary vasorelaxation. In experimental settings, NO inhibits vascular smooth muscle cell proliferation directly through regulating protein kinases modulating gene expression for cell growth and/or indirectly through reducing pressure on the vascular cells by cyclic guanosine-3',5'-monophosphate (cGMP) dependent

vascular relaxation. In this chapter we will discuss NO and its regulation and function with special references to the development of PH as well as pulmonary vascular reactivity in PH.

## 2. Biological effects of NO

### 2.1. NO acts through the sGC pathway and S-nitrosylation of target proteins

NO activates soluble guanylyl cyclase (sGC) stimulating cGMP production and subsequent activation of cGMP-dependent protein kinase (PKG). This sGC-cGMP-PKG pathway plays a major role in NO-mediated regulation. In addition to this pathway, NO directly binds to proteins and induces conformational changes with subsequent functional alterations, like phosphorylation. Thus, S-nitration is also called S-nitrosylation, the term which emphasizes a biological effect of the chemical reaction of S-nitration [16]. S-nitrosylation modifies the activity of some kinases and phosphatases, thus raising the possibility that NO modifies phosphorylation and dephosphorylation through S-nitrosylation.

NO reacts with oxygen, transitional metal ions, thiols, and superoxides, exerting its effects via cGMP-dependent and/or -independent pathways. cGMP effector molecules include cGMP-dependent protein kinases type-I and -II, cGMP-activated phosphodiesterases, and cGMP-gated ion channels. Similar to phosphorylation, S-nitrosylation regulates protein function allosterically or by direct modification of cysteine.

In the vascular system, NO reacts with sGC forming cGMP, which activates cGMP-dependent protein kinase decreasing vascular smooth muscle cell cytoplasmic  $\text{Ca}^{2+}$  concentration by 1) activation of proteins such as  $\text{Ca}^{2+}$ -sensitive potassium channels which decrease membrane potential thereby causing hyperpolarization and closing voltage dependent  $\text{Ca}^{2+}$  channels; 2) phosphorylation of voltage- and receptor-operated sarcolemmal  $\text{Ca}^{2+}$  channels, causing them to close; 3) inhibition of the inositol 1,3,5-trisphosphate-sensitive  $\text{Ca}^{2+}$  release channel of the sarcoplasmic reticulum [17].

### 2.2. NO prevents the development of PH

NO mediates vasorelaxation, anticoagulation, and anti-proliferation, as well as neurotransmission. Several earlier studies demonstrated that NO inhibits smooth muscle cell growth by a cGMP-dependent mechanism [18] in addition to inhibiting growth regulating enzymes such as ribonucleotide reductase and thymidine kinase [19,20]. NO also suppresses the hypoxia-induced increase in ET-1 and platelet-derived growth factor-B, both of which have vasoconstriction and growth effects [21]. These effects of NO led investigators to determine whether administration of NO prevents the development of PH. Chronic NO inhalation ameliorates the development of hypertensive pulmonary vascular changes of chronic hypoxia-induced PH in rats [22], but not in monocrotaline (MCT)-induced PH [23]. In contrast, supplementation with the NO precursor, L-arginine, but not D-arginine prevented the development of PH in both models [24]. The reason for the different effects of NO inhalation is unclear, but may be a result of differing pathogenic mechanisms in the two models of PH: the increase in pulmo-

nary pressures precedes the vascular structural changes in chronic hypoxia-induced PH, whereas the reverse sequence of events occurs in MCT-induced PH. Endogenous NO from L-arginine could prevent the development of new muscularization of peripheral pulmonary arteries in both models, whereas exogenous inhaled NO would be effective only in hypoxia-induced PH because of the reduction in pulmonary vascular pressures caused by NO mediated vasodilation.

Inhaled NO likely attenuates the hypertensive vascular structural changes through pulmonary vasodilation by a cGMP-mediated mechanism. Endogenous NO from L-arginine might also prevent the development of structural changes through a cGMP-mediated mechanism. This hypothesis is supported by another study that showed that pulmonary gene transfection of atrial natriuretic peptide (ANP), another inducer of cGMP, attenuates the development of chronic hypoxia-induced pulmonary vascular changes [25]. Treatment to increase NO production in the pulmonary vascular bed by eNOS gene transfection ameliorates the development of PH. Studies have demonstrated that eNOS transfected smooth muscle cell administration prevented the development of MCT-induced PH [26] and that eNOS transfected bone marrow-derived endothelial-like progenitor cell venous administration reversed established MCT-induced PH [27].

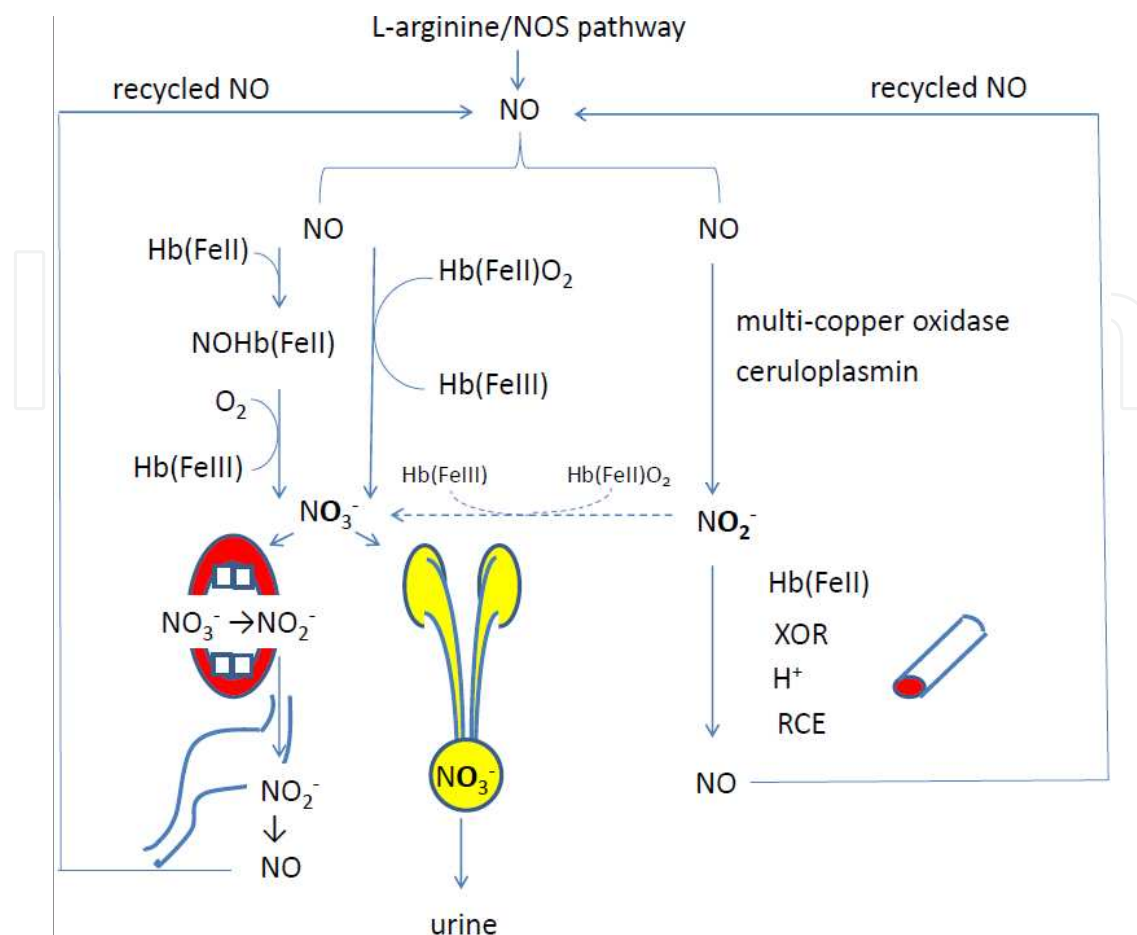
### 3. Endogenous NO production

#### 3.1. Nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) as sources of NO (Figure 1)

NO is produced from L-arginine by nitric oxide synthase (NOS) in the presence of oxygen, tetrahydrobiopterin ( $\text{BH}_4$ ), and reduced NADPH[3]. Recent studies have indicated that inorganic anions, nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ), can be recycled to NO in vivo as alternative sources of NO in addition to the classical NOS-NO pathway. The source of nitrate includes the endogenous NOS-NO synthase pathway and the diet. Green vegetables such as lettuce and spinach provide nitrate and preservatives in cured meat and bacon include nitrite. Basically reduction of nitrate and nitrite produce NO, thus nitrate and nitrite are considered an 'endocrine reservoir' of NO [28].

Nitrate in the plasma is excreted into the saliva, whereas nitrate is reduced by the oral anerobic bacteria producing nitrite. These bacteria use nitrate as an electron acceptor instead of oxygen during respiration. During its subsequent movement into the stomach, nitrite undergoes further reduction to NO, thus leading to gastric NO formation, which may play a role in gastric mucosa maintenance. This is a entero-salivary circulation of nitrate. In the systemic circulation intravascular nitrite is reduced to NO by deoxyHb, respiratory chain enzymes, xanthine oxidoreductase, deoxygenated myoglobin, and protons ( 29 ). They facilitate the transfer of protons to  $\text{NO}_2^-$ , causing NO production which is intensified under acidic and hypoxic states. Artery-to-vein gradients in nitrite are observed.

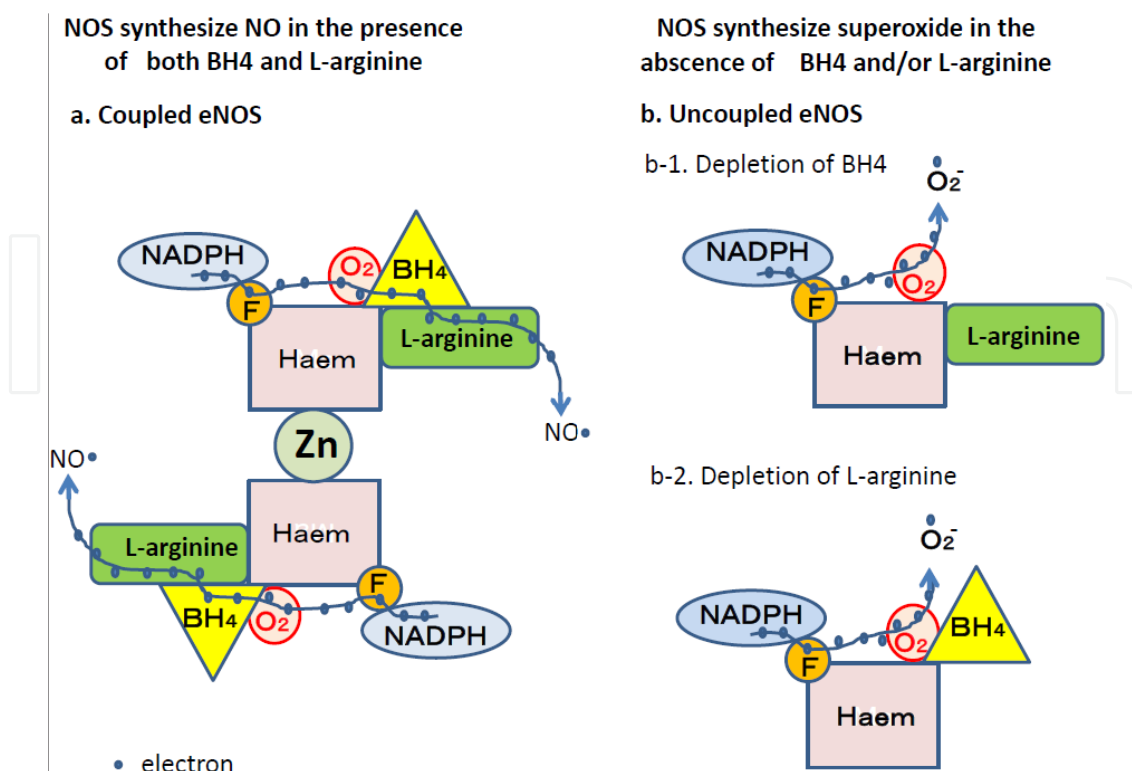
Nitrite has a vasodilatory effect. Inhaled nebulized sodium nitrite reduces pulmonary artery pressure (PAP) without changes in systemic artery pressure in hypoxia- or thromboxane-



**Figure 1.** Recycling of NO from NO<sub>2</sub><sup>-</sup>. Endogenous NO includes NO produced from L-arginine by NOS and recycled NO from NO<sub>2</sub><sup>-</sup>. NO is converted to NO<sub>3</sub><sup>-</sup> by the reaction with the Hb and /or to NO<sub>2</sub><sup>-</sup> by the oxidation in the plasma with the aid of multicopper oxidase and NO oxidase ceruloplasmin. NO<sub>3</sub><sup>-</sup> is excreted into urine by kidney and/or into oral cavity by salivary gland. In the oral cavity anaerobic bacteria reduces NO<sub>3</sub><sup>-</sup> converting to NO<sub>2</sub><sup>-</sup>, which goes down into stomach and is protonated under the gastric acidic state forming nitrous acid (HNO<sub>2</sub>) with further decomposition to NO and/or other nitrogen oxides. NO<sub>2</sub><sup>-</sup> in the plasma is reduced and converted to NO by the reductase activity of deoxygenated hemoglobin, xanthine oxidoreductase, respiratory chain enzymes, and hydrogen ion. Hb(FeII), deoxygenated hemoglobin; Hb(FeII)O<sub>2</sub>, oxygenated hemoglobin; NO, nitric oxide; NOHb(FeII), nitrosylhemoglobin; NOS, nitric oxide synthase; NO<sub>2</sub><sup>-</sup>, nitrite, NO<sub>3</sub><sup>-</sup>, nitrate; XOR, xanthine oxidoreductase; Hb(FeIII), methemoglobin; REC, respiratory chain enzymes

induced PH [30]. Intravenous administration of sodium nitrite reverses PH induced by hypoxia or thromboxane analogs [31]. Furthermore, intermittent nebulization of sodium nitrite ameliorated the muscularization and hyperplasia of small pulmonary arteries, the development of right ventricular hypertrophy, and the rise in right ventricular pressure in chronic hypoxia- or MCT-induced PH in rats [32], which is similar to L-arginine administration[33].

The effects of inhaled NO are not restricted to the lung. Recent studies have shown that inhaled NO improves neurological and left ventricular dysfunction after successful cardiopulmonary resuscitation [34] as well as liver function after liver transplantation [35]. Inhaled NO is converted to nitrate and nitrite when it enters the blood [36, 37]. NO can be recycled from nitrite and be used to protect organs from ischemia reperfusion injury.



**Figure 2. Coupled eNOS (eNOS homodimer) produces NO.** (a) eNOS homodimer produces NO, whereas eNOS monomer produces superoxide. eNOS uncoupling occurs during the conversion of eNOS homodimer to eNOS monomer. Two eNOS monomers are connected with the aid of Zn<sup>2+</sup>, making eNOS homodimer. BH<sub>4</sub> strengthens the Zn<sup>2+</sup> connection, maintaining the dimer form. In coupled NOS, an electron is transferred to L-arginine, producing NO and L-citrulline. (b) electron(+) from NADPH is transferred to O<sub>2</sub> in the uncoupled eNOS in absence of BH<sub>4</sub>(b-1) and/or L-arginine(b-2), thereby producing superoxide. BH<sub>4</sub>, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; F, flavin; NADPH, nicotinamide adenine dinucleotide phosphate

### 3.2. NOS uncoupling: NOS produces NO and superoxide depending on whether it is a homodimer or monomer (Figure2)

In the process of NO formation from oxygen and L-arginine, oxygen molecules are incorporated in both NO and L-citrulline, showing that NOS is a dioxygenase [38]. NOS contains both a reductase domain and an oxygenase domain, where electron transfer occurs from the reductase domain to the oxygenase domain. NADPH and flavin bind to the reductase domain, while oxygen, BH<sub>4</sub> and L-arginine bind to the oxygenase domain. Electrons are transferred from NADPH through the flavin containing reductase domain to the oxygenase domain [39]. Then two cascades of further electron transfer occur depending on the presence or absence of BH<sub>4</sub> and L-arginine. When both BH<sub>4</sub> and L-arginine are present, NO is synthesized by oxidative deamination of arginine by NOS, where the electron is transferred to L-arginine. The initial step of L-arginine oxidation is donation of electrons to the ferrous-dioxygen complex from BH<sub>4</sub>, where trihydrobiopterin is produced and the electron is supplied through flavin regaining BH<sub>4</sub> [40]. In contrast, in the absence of L-arginine or BH<sub>4</sub>, NOS synthesizes the superoxide, where the electron is transferred to ferrous oxygen. Intracellular deficiency of BH<sub>4</sub> induces superoxide generation from eNOS [40]. The term “eNOS uncoupling” means func-



tionally that electron transfer to L-arginine is uncoupled, when the electron is transferred to ferrous-dioxygen instead of L-arginine, producing superoxide. NOS homodimer produces NO from L-arginine and oxygen, whereas NOS monomer produces superoxide [41]. Thus, the molecular basis of eNOS uncoupling is conversion of the NOS dimer to the NOS monomer. To maintain the NOS dimer, BH4 is essential and dihydrobiopterin (BH2) is the oxidized form of BH4. Peroxynitrite oxidizes BH4 to BH2, reducing the BH4 amount and/or the BH2/BH4 ratio, both of which induce eNOS uncoupling [42]. The effects of BH4 are mediated through the regulation of NO compared with superoxide synthesis by endothelial NOS. Since BH4 might both augment NO synthesis and decrease superoxide production, BH4 deficiency may play a role in the pathogenesis of PH.

eNOS uncoupling is evaluated by the eNOS dimer/monomer ratio in cold SDS-PAGE Western blot analysis. While oxidative stress reduces the eNOS dimer/monomer ratio in a cardiac hypertrophic model suggesting eNOS uncoupling, exogenous BH4 restored the eNOS dimer/monomer ratio [43]. Administration of exogenous BH4 might be used for eNOS uncoupling diseases. BH4 deficiency might cause PH in mice and BH4 augmentation might ameliorate the development of PH. Mice with low BH4 tissue levels develop PH which is reversed by increasing BH4 with targeted transgenic overexpression of the rate-limiting enzyme in BH4 synthesis, guanosine triphosphate (GTP) cyclohydrolase [44]. Lung BH4 availability is controlled by pulmonary vascular tone, right ventricular hypertrophy, and vascular structural remodeling. BH4 is a cofactor of NOS in the production of NO. BH4 deficiency causes decreased NO production with concomitant production of superoxide by NOS. Chronic administration of BH4 analogues improves NO-mediated pulmonary artery dilatation in rats with chronic hypoxic pulmonary hypertension [45]. Copresence of increased levels of NOS and reduced NO bioactivity might be explained by the deficiency of BH4 and/or L-arginine.

Long-term increases in NO might increase eNOS expression and eNOS uncoupling, thereby producing superoxide. Long-term administration of nitroglycerin (TNG) increased eNOS mRNA and protein expression and vascular superoxide ( $O_2^{\bullet-}$ ) in intact vessels monitored using ESR spectroscopy [46]. An earlier study showed that endothelial denudation improves vascular relaxation induced by TNG in isolated vessels from nitrate-tolerant animals [47].

### 3.3. Caveolin and NOS (Figure 3)

Caveolae are flask-shaped invaginations on the cell surface, which contain structural proteins called caveolin and other signaling proteins. In endothelial cells, eNOS is inactivated when it is conjugated to caveolin-1, a structural protein of endothelial caveolae; eNOS is activated when it dissociates from caveolae. Stimulation of  $\beta_2$  adrenergic receptors cause this dissociation through phosphorylation of Tyr in caveolin-1. The mouse pulmonary endothelial  $\beta_2$  adrenergic receptor coupled to Gi/o proteins causes phosphorylation of caveolin-1 by Src kinase and eNOS phosphorylation at ser<sup>1177</sup> by the Src kinase - phosphatidylinositol 3 kinase (PI3kinase) - Akt kinase pathway [48]. Thus, stimulation of the  $\beta_2$  adrenergic receptor causes endothelial NO synthase-dependent relaxation.

Loss of caveolin-1 induces chronic activation of eNOS and subsequent tyrosine nitration of PKG in lungs from patients with idiopathic pulmonary hypertension, where activated eNOS

is uncoupled eNOS, producing superoxide [49]. Genetic deletion of caveolin in mice causes PH and treatment with a superoxide scavenger and/or a NOS inhibitor prevents PH associated vascular remodeling [49]. Although caveolin expression in total lung determined by Western blotting is not altered in severe PH, its immunohistological expression in plexiform lesions is absent or decreased [50].

A 90-kDa heat shock protein (HSP90) is a molecular chaperone of proteins that modulates protein functions. Along with many other proteins, eNOS and sGC are targets for HSP90. HSP90 interacts with eNOS and HSP90 facilitates the displacement of eNOS from caveolin 1, activating eNOS. HSP90 activity is dependent on adenosine triphosphate (ATP). Asymmetric dimethylarginine (ADMA) inhibits HSP90 activity in pulmonary endothelial cells through mitochondrial dysfunction, caused by ADMA induced eNOS uncoupling with subsequent superoxide production and nitration of mitochondrial protein, which reduce ATP production [51].

### **3.4. eNOS expression and activity in PH**

To examine whether the change in eNOS expression and its activity is associated with vascular endothelial dysfunction in PH, many studies have been performed in several species of animals and humans, using isolated lung, isolated pulmonary artery, and in vivo. eNOS is expressed in not only vascular endothelial cells, but lung epithelial cells. In addition, eNOS expression and/or activity might be different between conduit PAs and resistance PAs.

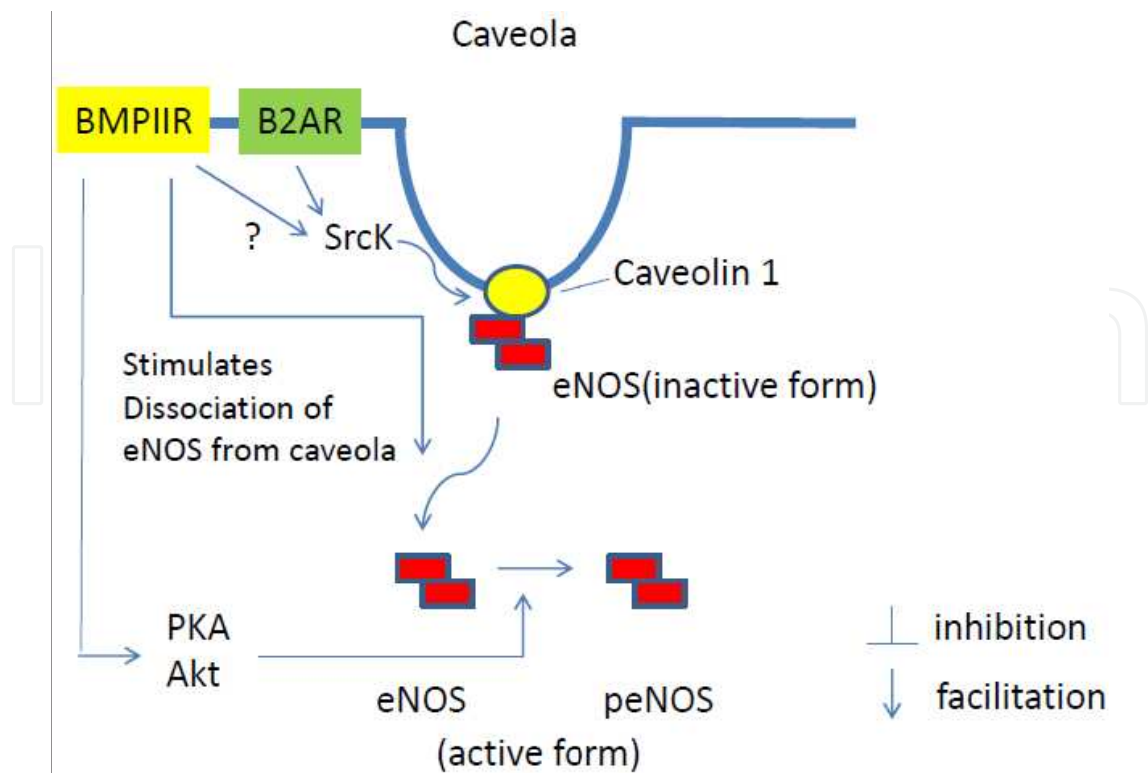
#### **Animal models**

mRNA and protein expression of eNOS in rat lung and eNOS expression localized in pulmonary vascular endothelial cells and epithelial cells is upregulated in acute hypoxia [52]. In that study, nitrate/nitrite in rat lung homogenate also increased, suggesting augmented eNOS activity. The enhancement of eNOS activity in hypoxic pulmonary vasoconstriction (HPV) in normal rat lung also has been shown in other studies using NOS inhibitors [53, 54] (see sect. 3.1). eNOS protein expression was time-dependently increased in rats in chronic hypoxia-induced PH [55,56], while phosphorylated eNOS (peNOS), active form, was impaired [55]. MCT-induced PH rats showed decreased expression of both eNOS [57,58,59] and peNOS [59].

#### **Human**

Many studies of eNOS expression and its activity have been performed in adult human PAH. However, the results are not consistent: eNOS expression is reduced in pulmonary vessels from adults with primary and secondary PH, but is increased in plexiform lesions [60]. Western blot analysis showed that eNOS expression is not changed in the lung tissue of idiopathic PAH (IPAH) patients [61]. However, several studies reported lower exhaled nitrate/nitrite (NOx) in PAH patients [62,63]. Overall, these results suggest that eNOS activity might be depressed in adult human PAH.





**Figure 3. Inactive form of eNOS associated with caveola.** eNOS is associated with caveola, which is the inactive form of eNOS. The active form of eNOS is dissociated from caveola. Stimulation of BMPIIR induces dissociation of eNOS from caveola as well as phosphorylation of eNOS through PKA and/or Akt activation. eNOS, endothelial nitric oxide synthase; B2-AR, beta 2-adrenergic receptor; SrcK, src kinase; peNOS, phosphorylated eNOS; BMPIIR, bone morphogenetic protein II receptor; PKA, cyclic AMP-dependent protein kinase

## 4. Endothelium-dependent and -independent NO-mediated relaxation in pulmonary circulation

### 4.1. Role of endothelium-derived NO in basal tone

L-NMMA (N omega-monomethyl-L-arginine), L-NNA (N omega-nitro-L-arginine), L-NAME (N omega-nitro-L-arginine methyl ester), L-NA (N omega-nitro-L-arginine) and other NOS inhibitors have been used to examine the physiological role of NO in pulmonary vascular tone. The increase in vascular tone in the presence of NOS inhibitors may indirectly represent NO production and/or release in the pulmonary circulation.

#### Animals

L-NMMA [53] and L-NNA [64,65] did not change pulmonary basal tone in normal rat PA rings. Normal isolated perfused lungs were not affected by NOS inhibitors such as L-NMMA [53], L-NNA [64], and L-NA [66] except for a few studies showing a moderate increase with L-NAME [67]. In chronic hypoxia, many studies showed markedly enhanced vascular tone by L-NNA [64] or L-NAME [67]. Although these NOS inhibitors caused different results, the findings suggested that 1) NO might not be involved in vascular basal tone in normal pulmonary circulation, and 2) basal NO production might be increased in hypoxia-induced chronic

PH. On exposure to acute hypoxia, NOS inhibitors augmented vascular contraction in normal [53,67,68] and hypoxia-induced PH rat models [67]. This finding suggests that NO production in HPV is increased in both normal and hypoxic PH rats.

## **Humans**

Inhibition of NO production by L-NMMA caused the reduction of pulmonary flow in conscious healthy adults [69,70], suggesting the possible role of continuous production of NO in maintaining basal vascular tone. In PAH patients, several studies reported decreased expression of NOS. Although several studies reported decreased exhaled nitrogen oxide (NO<sub>x</sub>) levels in PAH patients, others have reported higher levels. The results therefore remain inconclusive.

### **4.2. Vasoreactivity to endothelium-dependent and independent NO-related relaxing substances in rat lung**

Many studies have been performed using acetylcholine (Ach) and sodium nitroprusside (SNP), endothelium-dependent and -independent NO-related vasorelaxants, to examine functional changes in vascular endothelial and smooth muscle cells in PH. As Ach-induced relaxation was abolished by NOS inhibitors [64] and restored with L-arginine [71,72], reactivity may partly reflect changes in NOS expression and/or activity.

#### **Rats with hypoxic PH**

The relaxation response to Ach is impaired in rat isolated conduit pulmonary arteries (PAs) [65,73,74,75,76]. Many of these studies also described an impaired relaxation response to SNP in conduit PAs [65,74,76]. These results suggested 1) decreased production and release of NO in endothelial cells or 2) decreased responsiveness to NO in smooth muscle cells, or both. Impaired relaxation in Ach and SNP was partially restored after exposure to chronic hypoxia. As the recovery process was different between the responses of Ach and SNP [65], it was speculated that NO-related functional abnormalities in endothelial and smooth muscle cells occurred independently.

In contrast, in hypoxic vasoconstriction resistant rat PA rings, the relaxation response to Ach was not changed [74,75] or augmented [77] in chronic hypoxia. It is likely that Ach-reactive NO production and/or release varies in a vascular site-specific manner. Conduit arteries produce and release more eNOS than peripheral arteries. The vascular functional change in response to stimuli such as abnormal shear stress, circumferential wall stretch and hypoxia itself may occur in conduit PAs more than in peripheral resistant arteries. Although conduit arteries do not directly relate to pulmonary vascular resistance, the pathophysiological change in conduit arteries may play a key role in pulmonary vascular remodeling [78].

Impaired response to Ach was partly restored in the presence of a non-selective inhibitor of cyclooxygenase (COX) [65] or prostaglandin (PG) H<sub>2</sub> / thromboxane (TX) A<sub>2</sub> receptor antagonist [79], suggesting the possibility of 1) imbalance between the production of vasoconstricting and vasorelaxing prostanoid in vascular endothelial cells, and 2) simultaneously release of vasoconstricting prostanoids such as PGH<sub>2</sub> and/or TXA<sub>2</sub>. Pidgeon et al. showed that the basal

expression of COX2, otherwise known as PGH synthase, was increased in rat lungs in chronic hypoxia, and a PGH<sub>2</sub>/TXA<sub>2</sub> receptor antagonist attenuated the rise in PAP induced by chronic hypoxia [80].

### **MCT-induced PH in rats**

PA vascular functional changes in rats with MCT-induced PH have been compared with PAs from animals with chronic hypoxia-induced PH. Many vasodilation studies have reported a depressed relaxation response to Ach in MCT-induced rat conduit PA rings [76,81,82,83,84]. Many of these studies described impaired SNP relaxation, [76,82] with the exception of one study [84]. While Ach-induced relaxation was impaired in the pulmonary circulation in MCT-induced PH, the SNP relaxation response has been reported to be impaired [85] or not impaired [86]. Taken together, in MCT-induced PH, vascular endothelial dysfunction is observed from proximal to distal PAs; however, smooth muscle functional alteration is not apparent in peripheral PAs.

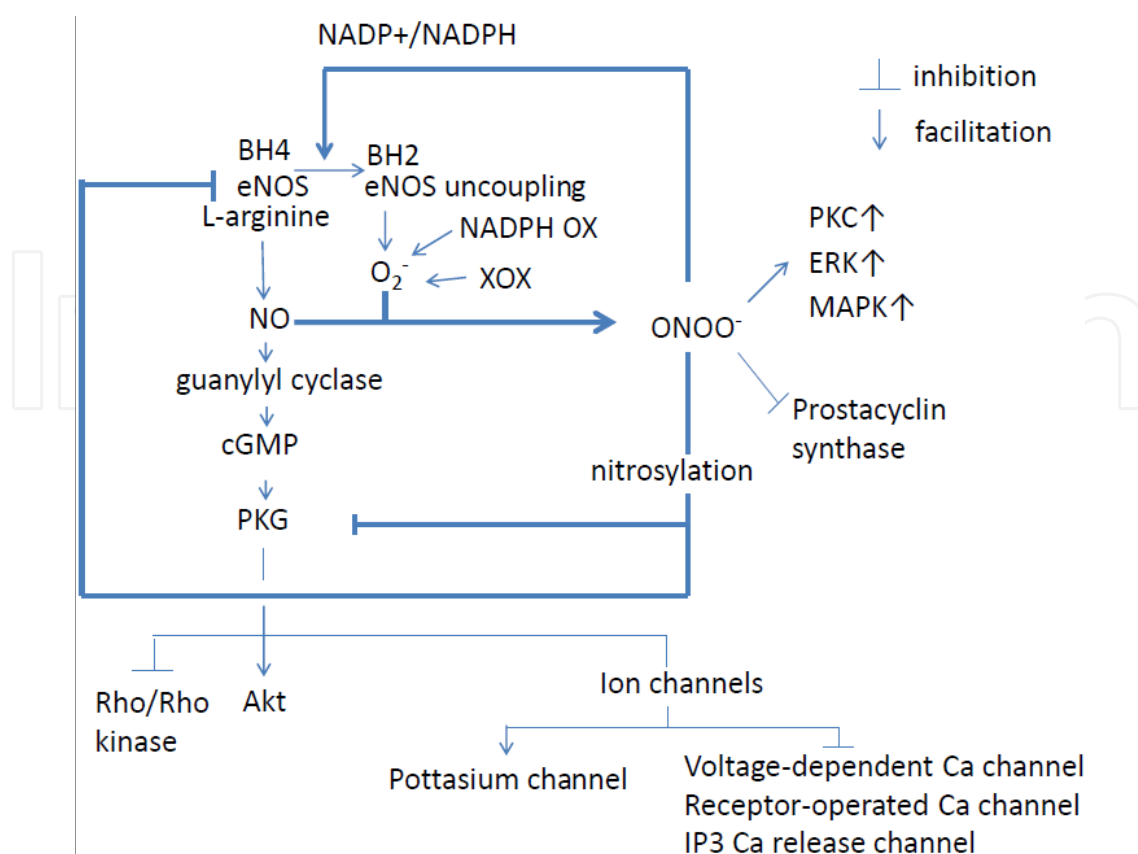
## **5. Superoxide scavenges NO producing peroxynitrite (Figure 4)**

### **5.1. Oxidative stress**

In pulmonary hypertension, endothelial NOS expression is increased, which may not necessarily indicate an increase in NO production [87]. NOS might produce superoxide, which is due to uncoupling of NOS [88]. Increased levels of NOS and reduced NO bioactivity might be explained by the deficiency of BH4 and/or L-arginine. Oxidative stress induces the changes of BH4 to BH2. Oxidative stress also induces S-glutathionylation and subsequent eNOS uncoupling [39], in which S-glutathionylation of eNOS reversibly decreases NOS activity with an increase in O<sub>2</sub><sup>•-</sup> generation primarily from the reductase and endothelium-dependent relaxation is impaired. Oxidative stress upregulates nuclear factor (NF)-kappaB, a key transcription factor that is involved in vascular tissue remodeling. NF-kappaB nuclear localization and vascular cell adhesion molecule 1(VCAM-1) expression is temporally and spatially associated with the development of MCT-induced PH in rats, which is ameliorated by administering a NF-kappaB inhibitor, pyrrolidine dithiocarbamate(PDTC)[89].

### **5.2. Production of superoxide in PH: role of NADPH oxidase and SOD**

NAD(P)H oxidase enzyme complex catalyzes one electron reduction of oxygen using NADPH or NADH as an electron donor, which produces superoxide :  $\text{NAD(P)H} + 2\text{O}_2 \rightarrow \text{NAD(P)}^+ + \text{H}^+ + 2\text{O}_2^{\cdot-}$  NADPH oxidase expression is increased in pulmonary arteries from a lamb model of persistent pulmonary hypertension of the newborn (PPHN) [90]. The expression was determined by the Western blotting of the levels of p67<sup>phox</sup> a subunit of the NADPH oxidase complex and immunostaining of the pulmonary vessels in lung sections. Another study demonstrated that expression and activity of the NADPH oxidase complex are upregulated in PH with increased pulmonary blood flow [91].



**Figure 4. Peroxynitrite production from NO and superoxide.** Superoxide (O<sub>2</sub><sup>-</sup>) is produced by uncoupled eNOS, NADPH oxidase, and xanthine oxidase. NO reacts with O<sub>2</sub><sup>-</sup> producing peroxynitrite(ONOO<sup>-</sup>) with subsequent nitrosylation of protein kinases, thereby activating or suppressing their activities. PKG phosphorylates Rho kinase, Akt, and ion channels. Phosphorylation of ion channels makes Ca<sup>2+</sup> ion channels closed and potassium channel open. Peroxynitrite further oxidize BH<sub>4</sub> to BH<sub>2</sub>, inducing eNOS uncoupling with subsequent superoxide production. BH<sub>4</sub>, tetrahydrobiopterin; BH<sub>2</sub>, dihydrobiopterin; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; IP<sub>3</sub>, inositol triphosphate receptor; MAPK, mitogen-activated protein kinase; OX, oxidase; ONOO<sup>-</sup>, peroxynitrite; PKC, protein kinase C; PKG, cyclic-GMP dependent protein kinase( protein kinase G); XOX, xanthine oxidase;

Deficiency of superoxide dismutase (SOD) may play a role in the development of PH. Expression and activity of mitochondrial SOD2 in patients and animal models of PH is decreased [92,93] in pulmonary arteries and plexiform lesions. SOD produces H<sub>2</sub>O<sub>2</sub> from mitochondrial superoxide. H<sub>2</sub>O<sub>2</sub> is less potent than superoxide and acts as a signaling molecule to inhibit transcriptional factors such as hypoxia-inducible factor-1 $\alpha$ . Epigenetic suppression of SOD with selective hypermethylation of CpG islands in SOD2 gene induces excessive proliferation and decreases apoptosis in pulmonary artery smooth muscle cells [92], suggesting a causative role of SOD deficiency in PH.

NO reacts with superoxide more rapidly than SOD producing peroxynitrite. Peroxynitrite is a more potent and versatile oxidant than NO or superoxide, in which HO<sup>+</sup> and NO<sub>2</sub> produced from peroxynitrous acid (HOONO) and/or its reactive activated isomer (HOONO\*) attacks biological targets [94] including cyclic GMP-dependent protein kinase (PKG). In the setting of eNOS uncoupling, eNOS synthesizes superoxide which reacts with NO to create peroxynitrite. Nitrosylation of PKG by ONOO<sup>-</sup> depresses the function of PKG ( 42 ).

## 6. Prevention of hypertensive pulmonary vascular remodeling through NOS/NO pathway

### 6.1. NO precursor L-arginine ameliorates PH

Arginase, an enzyme in the urea cycle, converts arginine to ornithine and urea. NO<sub>x</sub> concentrations in exhaled gas and serum are decreased in PH patients compared with normal persons [95], suggesting decreased NO availability in PH. The deficiency of the NO precursor L-arginine, the substrate depletion of NOS, might partly explain the decrease in NO availability. Lower levels of arginine in the cell might be due to the increased activity of arginase. In PH patients, lower levels of arginine correlate with higher pulmonary artery pressures. Serum arginase activity is higher and the serum arginine-ornithine ratio is lower in PH patients than in healthy controls, indirectly suggesting increased intracellular arginase activity [33]. Animal studies showed that prolonged administration of L-arginine ameliorated the development of monocrotaline-induced PH [24,96] and chronic hypoxia-induced PH [96]. In patients with PH L-arginine treatment reduces PAP [97]. In addition to functioning as the substrate for NO formation, L-arginine prevents eNOS uncoupling, serves as a direct radical scavenger, and competes with the endogenous eNOS inhibitor ADMA, which decreases superoxide and increases NO formation [41].

### 6.2. ATRA increases NO production (Figure 5)

The level of asymmetrical dimethylarginine (ADMA) is increased in patients with PAH and MCT-induced PH in rats [98]. Since ADMA is an endogenous competitive inhibitor of NOS and suppresses NOS activity, increases in ADMA inhibit NO production. In atherosclerotic arteries from patients with high serum ADMA, endothelium-dependent relaxation by acetylcholine was impaired and O<sub>2</sub><sup>•-</sup> production was increased [99]. Dysregulation of ADMA might cause PH through the decrease in NO in the lung as well. Dimethylarginine dimethylaminohydrolase (DDAH) is a metabolizing enzyme of ADMA. Thus the increase in DDAH activity reduces ADMA and induces subsequent increases in NOS activity. DDAH has two isoforms: DDAH1 and DDAH2. DDHA1 and DDAH2 are expressed predominantly in tissues containing neuronal NOS (nNOS) and eNOS, respectively [100]. Phosphodiesterase (PDE) 3/4 inhibitors reduce ADMA and raise NO/cGMP levels [2]; PDE3/4 inhibitors activate the cAMP/protein kinase A (PKA) pathway and induce subsequent activation of the promoter region of DDAH2. Western blot analysis of lung from PH rats 28 days after the injection of MCT showed decreases in eNOS, pNOS, AKT, and DDAH2 and increases in lung and serum ADMA levels [101]. In this PH model, 1) decreased Akt reduces eNOS phosphorylation and thereby decreases eNOS activity 2) decreased DDAH2 reduces ADMA breakdown and thereby the increase in ADMA inhibits eNOS activity. This study showed that rosuvastatin ameliorates MCT-induced PH through the normalization of Akt, eNOS and DDAH2 expression and ADMA levels [101].

Endothelial cells express retinoid receptors and all-trans-retinoic acid (ATRA) increased DDHA2 mRNA levels in endothelial cells. Although eNOS mRNA expression is not increased



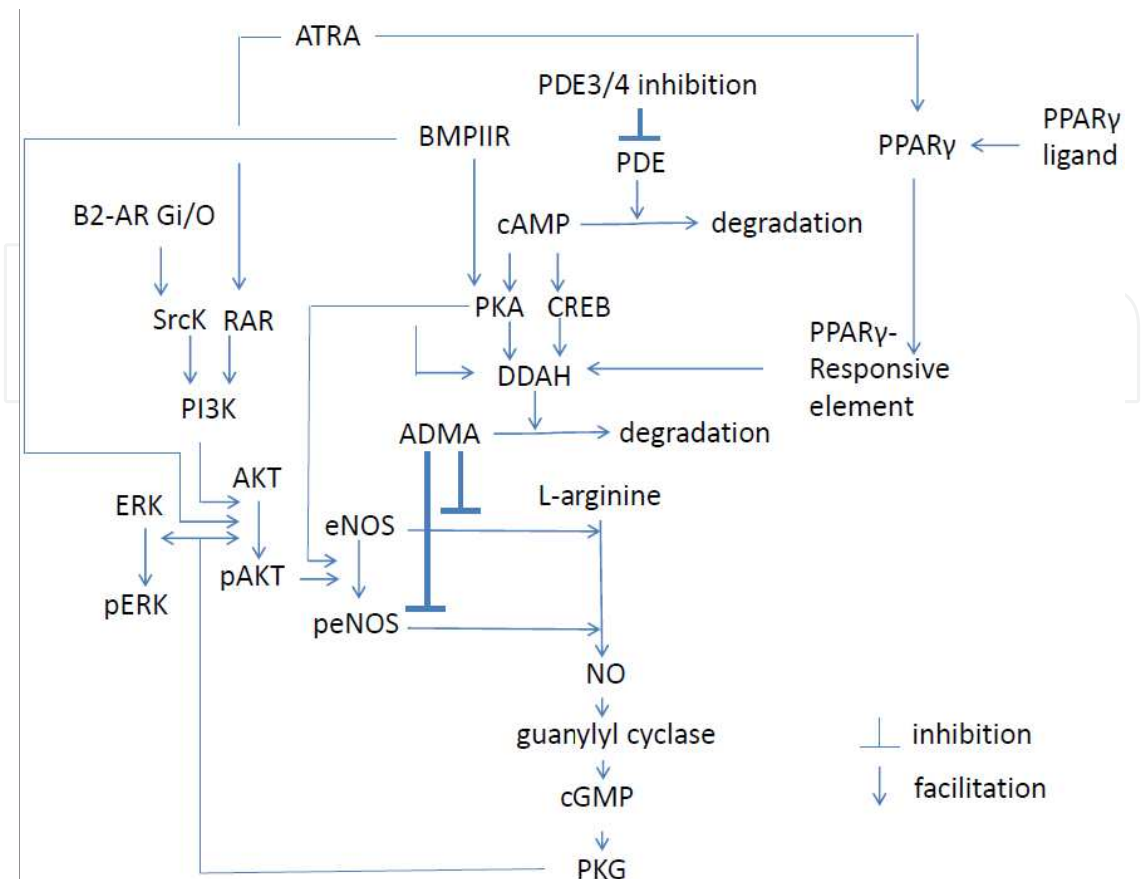
with ATRA treatment, ATRA increases NO production, suggesting that ATRA increases activity of expressed eNOS indirectly through the decrease in ADMA due to increased DDHA2 [102]. ATRA also upregulates NO production in vascular endothelial cells through the PI3 kinase/Akt pathway [103]. ATRA induces eNOS phosphorylation at ser<sup>1177</sup> and Akt phosphorylation at ser<sup>473</sup> without changes in protein expression such as occur during DDAH2 upregulation. In terms of inducible NOS(iNOS), interleukin(IL)-1 $\beta$  increases iNOS mRNA levels and ATRA reduces this increase in vascular smooth muscle cell culture [104]. Because iNOS inhibition by the iNOS inhibitor N6-(1-iminoethyl-L-lysine, dihydrochloride(L-NIL) prevented the development of PH [105], the inhibitory effect of ATRA on iNOS expression might reduce the development of PH. Peroxisome proliferator-activated receptors (PPAR $\gamma$ s) are a nuclear hormone receptor superfamily of ligand-activated transcription factors of retinoid hormone receptors other than steroid and thyroid hormones. PPAR $\gamma$  or retinoid X receptor (RXR) agonists inhibit smooth muscle proliferation. The PPAR $\gamma$  agonist rosiglitazone attenuates the development of chronic hypoxia-induced vascular structural remodeling [106], although it has little effect on the vasoconstriction component of PH. Since PPAR $\gamma$  mediates effects through the RXR, retinoids might also ameliorate PH vascular changes. PPAR $\gamma$  ligands increase the release of NO from endothelial cells through a transcriptional mechanism probably through the increase in DDAH mRNA expression without changes in eNOS expression [107]. These results suggest that ATRA might prevent the development of experimental PH in rats. ATRA ameliorated the development of MCT-induced PH [108], but not chronic hypoxia-induced PH [109]. These differences in the effect of ATRA on the development of PH may be due to a more pronounced inflammatory response in MCT-induced PH and a more subtle inflammatory reaction in chronic hypoxia-induced PH; endothelial damage precedes the rise in PAP in MCT model whereas the rise in PAP precedes endothelial changes in the chronic hypoxic model [110,111].

### 6.3. BMPIIR activates eNOS

Mutation of the bone morphogenetic protein receptor type II (BMPIIR) gene is one of the causes of familial PAH. The link between BMPIIR and eNOS partly explains the mechanism for the development of PH caused by BMPIIR mutations. Stimulation of BMPIIR induces eNOS phosphorylation, primarily through the cyclic-AMP dependent protein kinase and partially through serine-threonine kinase Akt [112]. Stimulation of BMPIIR also causes dissociation of eNOS from caveolin-1 and increases the eNOS-HSP90 interaction, which facilitates electron transfer through eNOS[112]. Thus, impaired BMPIIR or loss of BMPIIR stimulation might disturb the pulmonary vascular homeostasis, thereby causing PH.

### 6.4. VEGF increases eNOS expression

Vascular endothelial growth factor (VEGF) stimulates NO production initially by increasing intracellular Ca<sup>++</sup> levels and subsequent Ca<sup>++</sup>-calmodulin dependent activation of eNOS, and later by increasing intracellular eNOS message and protein levels [113]. VEGF stimulates vasodilation, microvascular hyperpermeability, and angiogenesis. Plexiform lesions show striking expression of VEGF associated with endothelial proliferation. NOS inhibition prevents

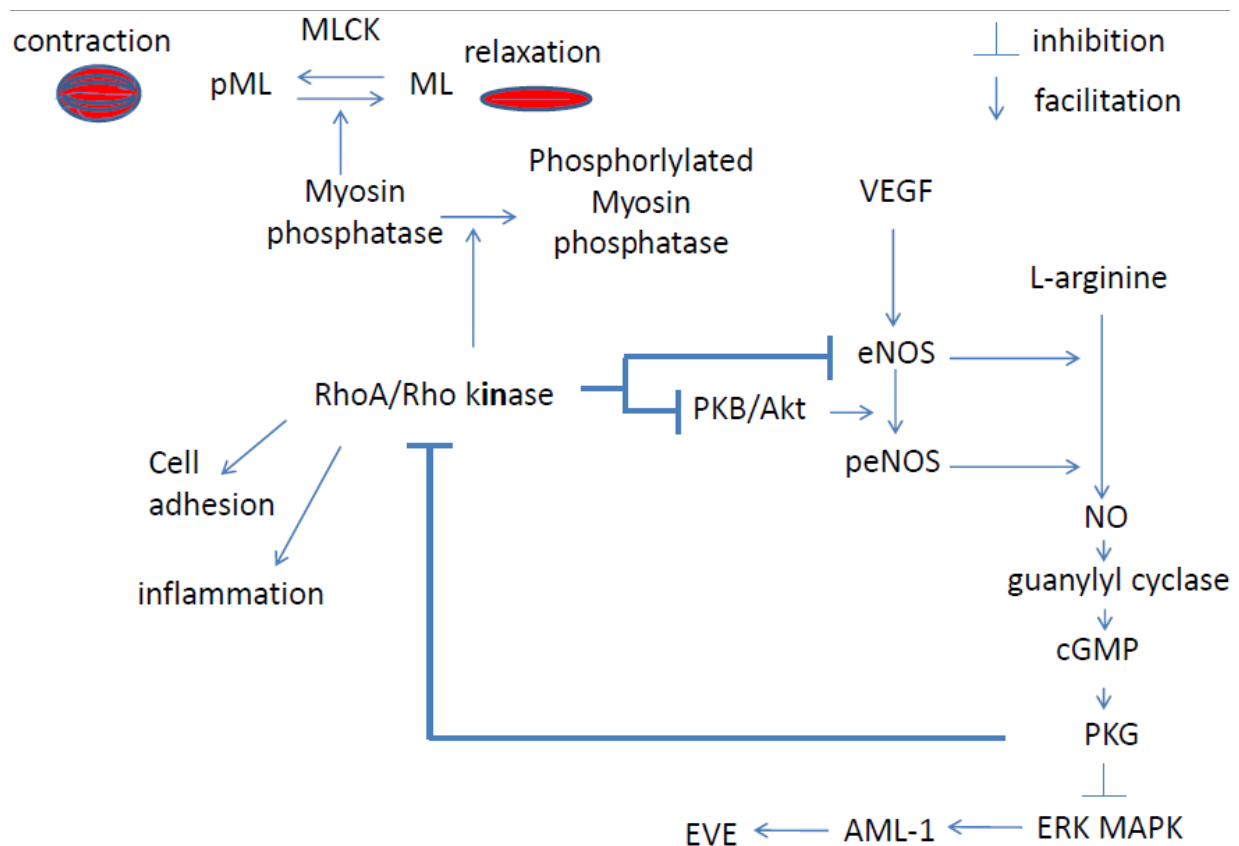


**Figure 5. Possible pathway to enhance NO production by ATRA, DDAH, PDE3/4 inhibition, and BMPIIR.** ADMA suppresses NOS activity. DDAH is the enzyme that metabolizes ADMA. The cAMP/PKA pathway activates the promoter region of DDAH2, thereby increasing DDAH2 expression. ATRA increases DDAH2 mRNA, stimulates RAR with a subsequent increase in PI3K activity as well as PI3K protein and mRNA expression, and thereby enhances Akt and eNOS phosphorylation without increasing eNOS expression. Phosphorylated Akt(pAkt) phosphorylates eNOS making peNOS, the activated form of NOS. B2-AR stimulation activates SrcK via Gi/o protein. Activated SrcK phosphorylates PI3K and induces subsequent its downstream eNOS phosphorylation as well as phosphorylation of caveolin -1 to dissociate eNOS from caveola (Figure 3]. ADMA, asymmetric dimethylarginine; ATRA, all trans retinoic acid; B2-AR, beta 2-adrenergic receptor; BMPIIR, bone morphogenetic ptotein II receptor; CREB, cAMP responsive element binding protein; cAMP, cyclic adenosine monophosphate; DDAH, dimethylarginine dimethylaminohydrolase; eNOS, endothelial nitric oxide synthase; peNOS, phosphorylated eNOS; ERK, extracellular signal-regulated kinase; pERK, phosphorylated ERK; Gi/o, GTP binding protein subunit Gi/o; PDE, phosphodiesteras; PI3K, phosphoinositide 3-kinase; PKA, cAMP dependent protein kinase; PKG, cyclic GMP-dependent protein kinase ; PPARγ, peroxisome proliferator-activated receptor; SrcK, src kinase; RAR, retinoic acid receptor;

VEGF-induced proliferation in cultured microvascular endothelial cells, associated with the decrease in cGMP levels [114], suggesting that VEGF-induced proliferation is in part mediated by the NOS-NO-cGMP pathway. VEGF induces translocation of eNOS and caveolin-1 from caveola to the nucleus, where NO production activates transcriptional factors thereby inducing the early growth response gene, c-fos [115] and possibly inducing angiogenesis, and endothelial cell growth. VEGF receptor 2 (VEGF2R) blockade combined with chronic hypoxic exposure causes PH with plexiform like lesions, where decreased expression of VEGF2R, Src, Akt, phosphorylated Akt protein in lung have been demonstrated [116]. Studies have demonstrated that reduced Src and Akt attenuate eNOS phosphorylation [101].

### 6.5. Elastase inhibition by NO

Earlier studies have shown that vascular elastase activity is increased in MCT-induced PH and chronic hypoxia-induced PH in rats [3,117], and that elastase inhibition prevents the development of pulmonary hypertension, right ventricular hypertrophy, muscularization of peripheral pulmonary arteries and medial hypertrophy of muscular arteries [3,117,118]. NO might reduce the elastase activity through its scavenging effect of superoxide. Reactive oxygen species inactivates endogenous elastase inhibitor,  $\alpha$ 1-protease inhibitor, and might increase elastase activity [119]. Furthermore NO might reduce elastase expression by inhibiting its transcriptional factor, acute myeloid leukemia factor 1 (AML-1), through extracellular signal-regulated kinase mitogen-activated protein kinase (ERK MAPK) inhibition which is mediated by cGMP dependent protein kinase activation [120].



**Figure 6. Rho/Rho kinase pathway inhibits eNOS/NO/cGMP pathway** Rho kinase is activated by the guanosine triphosphate (GTP)-bound, active form of RhoA (GTP RhoA). Activated Rho kinase phosphorylates and subsequently inactivates myosin phosphatase, causing smooth muscle contraction, which is the RhoA/Rho kinase pathway. PKG phosphorylates Rho A at Ser<sup>188</sup> and inhibits Rho A function, thereby inactivating the RhoA/Rho kinase pathway. Activated RhoA/Rho kinase decreases eNOS mRNA and protein expression, inactivates Akt, and inhibits PKG activity, thereby suppressing the eNOS/ NO/cGMP pathway. VEGF upregulates eNOS mRNA and protein expression. AML-1, acute myeloid leukemia factor 1(transcriptional factor); EVE, endogenous vascular elastase; PKG, cyclic GMP-dependent protein kinase(G kinase); PKB, protein kinase B(=Akt), AML-1, acute myeloid leukemia factor 1; ML, myosin light chain; pML, phosphorylated myosin light chain; MLCK, myosin light chain kinase; ERK MAPK, extracellular signal-regulated kinase mitogen activated protein kinase; VEGF, vascular endothelial growth factor.

### 6.6. Rho-kinase inhibitor upregulates NOS in PH (Figure 6)

Myosin light chain (MLC) phosphorylation by myosin light chain kinase (MLCK) causes vascular smooth muscle contraction. In contrast, myosin light chain dephosphorylation by myosin light chain phosphatase causes relaxation. The phosphorylation status of MLC phosphatase determines the contractility of smooth muscle at the same  $\text{Ca}^{++}$  concentration, thereby regulating the  $\text{Ca}^{++}$  sensitivity for contraction; the stronger the phosphatase activity, the weaker the vascular tone at the same  $\text{Ca}^{++}$  concentration. RhoA/Rho-kinase activation augments the phosphorylation of MLC phosphatase, which results in inhibition of MLC phosphatase. Studies have shown that Rho-kinase in circulating neutrophils is increased in patients with PH and that Rho-kinase expression is upregulated in isolated lung tissue on transplantation [121]. Rho-kinase activity in pulmonary arteries is enhanced in experimental PH [122,123]. NO-cGMP-cGMP dependent protein kinase pathway suppresses Rho/Rho kinase activity [124]. On the other hand Rho/Rho-kinase activation downregulates eNOS expression and eNOS phosphorylation through the inhibition of the protein kinase B/Akt pathway [125].

### Author details

Junko Maruyama, Ayumu Yokochi, Erquan Zhang, Hirofumi Sawada and Kazuo Maruyama

Department of Anesthesiology and Critical Care Medicine, Mie University School of Medicine and Department of Clinical Engineering, Suzuka University of Medical Science, Mie, Japan

### References

- [1] Farber HW, Loscalzo J. Pulmonary arterial hypertension. *N Engl J Med*. 2004 Oct 14;351(16):1655-65.
- [2] Archer SL, Weir EK, Wilkins MR. Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies. *Circulation*. 2010 May 11;121(18):2045-66.
- [3] Rabinovitch M. Molecular pathogenesis of pulmonary arterial hypertension. *J Clin Invest*. 2008 Jul;118(7):2372-9.
- [4] Jiang BH, Maruyama J, Yokochi A, Amano H, Mitani Y, Maruyama K. Correlation of inhaled nitric-oxide induced reduction of pulmonary artery pressure and vascular changes. *Eur Respir J*. 2002 Jun;20(1),52-8.

- [5] Jiang BH, Maruyama J, Yokochi A, Iwasaki M, Amano H, Mitani Y, Maruyama K. Prolonged nitric oxide inhalation fails to regress hypoxic vascular remodeling in rat lung. *Chest*. 2004 Jun;125(6):2247-52.
- [6] Kobayashi T, Gabazza EC, Shimizu S, Yasui H, Yuda H, Hataji O, Maruyama K, Yamauchi T, Suzuki K, Adachi Y, Taguchi O. Long-term inhalation of high-dose nitric oxide increases intraalveolar activation of coagulation system in mice. *Am J Respir Crit Care Med*. 2001 Jun;163(7):1676-82.
- [7] Maruyama J, Jiang BH, Maruyama K, Takata M, Miyasaka K. Prolonged nitric oxide inhalation during recovery from chronic hypoxia does not decrease nitric oxide-dependent relaxation in pulmonary arteries. *Chest*. 2004 Dec;126(6):1919-25.
- [8] Maruyama J, Maruyama K, Mitani Y, Kitabatake M, Yamauchi T, Miyasaka K. Continuous low-dose NO inhalation does not prevent monocrotaline-induced pulmonary hypertension in rats. *Am J Physiol*. 1997 Jan;272(1 Pt 2):H517-24.
- [9] Katayama Y, Hatanaka K, Hayashi T, Onoda K, Namikawa S, Yuasa H, Yada I, Maruyama K, Kitabatake M, Kusagawa M. Effects of inhaled nitric oxide in single lung transplantation in rats with monocrotaline-induced pulmonary hypertension. *J Heart Lung Transplant*. 1995 May-Jun;14(3):486-92.
- [10] Katayama Y, Hatanaka K, Hayashi T, Onoda K, Yada I, Namikawa S, Yuasa H, Kusagawa M, Maruyama K, Kitabatake M. Effects of inhaled nitric oxide in rats with chemically induced pulmonary hypertension. *Respir Physiol*. 1994 Aug;97(3):301-7.
- [11] Shimpo H, Mitani Y, Tanaka J, Mizumoto T, Onoda K, Tani K, Yuasa H, Yada I, Maruyama K. Inhaled low-dose nitric oxide for postoperative care in patients with congenital heart defects. *Artif Organs*. 1997 Jan;21(1):10-3.
- [12] Ashida Y, Miyahara H, Sawada H, Mitani Y, Maruyama K. Anesthetic management of a neonate with vein of Galen aneurysmal malformations and severe pulmonary hypertension. *Paediatr Anaesth*. 2005 Jun;15(6):525-8.
- [13] Yoshida M, Taguchi O, Gabazza EC, Kobayashi T, Yamakami T, Kobayashi H, Maruyama K, Shima T. Combined inhalation of nitric oxide and oxygen in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1997 Feb;155(2):526-9.
- [14] Yoshida M, Taguchi O, Gabazza EC, Yasui H, Kobayashi T, Kobayashi H, Maruyama K, Adachi Y. The effect of low-dose inhalation of nitric oxide in patients with pulmonary fibrosis. *Eur Respir J*. 1997 Sep;10(9):2051-4.
- [15] Maruyama K, Zhang E, Maruyama J. Clinical application of inhaled nitric oxide. In Yoshikawa/Naito (eds) *Gas Biology Research in Clinical Practice*. Basel Karger 2011, pp43-55
- [16] Nakamura T, Lipton SA. Redox modulation by S-nitrosylation contributes to protein misfolding, mitochondrial dynamics, and neuronal synaptic damage in neurodegenerative diseases. *Cell Death and Differentiation* 18; 1478-1486, 2011



- [17] Hampl V, Herget J. Role of nitric oxide in the pathogenesis of chronic pulmonary hypertension. *Physiol Rev.* 2000 Oct;80(4):1337-72.
- [18] Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest.* 1989 May;83(5):1774-7.
- [19] Garg UC, Hassid A. Mechanisms of nitrosothiol-induced antimitogenesis in aortic smooth muscle cells. *Eur J Pharmacol.* 1993 Jun 24;237(2-3):243-9.
- [20] Kwon NS, Stuehr DJ, Nathan CF. Inhibition of tumor cell ribonucleotide reductase by macrophage-derived nitric oxide. *J Exp Med.* 1991 Oct 1;174(4):761-7.
- [21] Kourembanas S, McQuillan LP, Leung GK, Faller DV. Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. *J Clin Invest.* 1993 Jul;92(1):99-104.
- [22] Kouyoumdjian C, Adnot S, Levame M, Eddahibi S, Bousbaa H, Raffestin B. Continuous inhalation of nitric oxide protects against development of pulmonary hypertension in chronically hypoxic rats. *J Clin Invest.* 1994 Aug;94(2):578-84.
- [23] Maruyama J, Maruyama K, Mitani Y, Kitabatake M, Yamauchi T, Miyasaka K. Continuous low-dose NO inhalation does not prevent monocrotaline-induced pulmonary hypertension in rats. *Am J Physiol.* 1997 Jan;272(1 Pt 2):H517-24.
- [24] Mitani Y, Maruyama K, Sakurai M. Prolonged administration of L-arginine ameliorates chronic pulmonary hypertension and pulmonary vascular remodeling in rats. *Circulation.* 1997 Jul 15;96(2):689-97.
- [25] Mitani Y, Maruyama J, Jiang BH, Sawada H, Shimpo H, Imanaka-Yoshida K, Kaneda Y, Komada Y, Maruyama K. Atrial natriuretic peptide gene transfection with a novel envelope vector system ameliorates pulmonary hypertension in rats. *J Thorac Cardiovasc Surg.* 2008 Jul;136(1):142-9. Epub 2008 May 12.
- [26] Campbell AI, Kuliszewski MA, Stewart DJ. Cell-based gene transfer to the pulmonary vasculature: Endothelial nitric oxide synthase overexpression inhibits monocrotaline-induced pulmonary hypertension. *Am J Respir Cell Mol Biol.* 1999 Nov;21(5):567-75.
- [27] Zhao YD, Courtman DW, Deng Y, Kugathasan L, Zhang Q, Stewart DJ. Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. *Circ Res.* 2005 Mar 4;96(4):442-50. Epub 2005 Feb 3.
- [28] Zuckerbraun BS, George P, Gladwin MT. Nitrite in pulmonary arterial hypertension: therapeutic avenues in the setting of dysregulated arginine/nitric oxide synthase signalling. *Cardiovasc Res.* 2011 Feb 15;89(3):542-52. Epub 2010 Dec 22. Review.
- [29] Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov.* 2008 Feb;7(2):156-67. Review.

- [30] Hunter CJ, Dejam A, Blood AB, Shields H, Kim-Shapiro DB, Machado RF, Tarekegn S, Mulla N, Hopper AO, Schechter AN, Power GG, Gladwin MT. Inhaled nebulized nitrite is a hypoxia-sensitive NO-dependent selective pulmonary vasodilator. *Nat Med*. 2004 Oct;10(10):1122-7. Epub 2004 Sep 12.
- [31] Casey DB, Badejo AM Jr, Dhaliwal JS, Murthy SN, Hyman AL, Nossaman BD, Kadowitz PJ. Pulmonary vasodilator responses to sodium nitrite are mediated by an allopurinol-sensitive mechanism in the rat. *Am J Physiol Heart Circ Physiol*. 2009 Feb; 296(2):H524-33. Epub 2008 Dec 12
- [32] Zuckerbraun BS, Shiva S, Ifedigbo E, Mathier MA, Mollen KP, Rao J, Bauer PM, Choi JJ, Curtis E, Choi AM, Gladwin MT. Nitrite potently inhibits hypoxic and inflammatory pulmonary arterial hypertension and smooth muscle proliferation via xanthine oxidoreductase-dependent nitric oxide generation. *Circulation*. 2010 Jan 5;121(1): 98-109. Epub 2009 Dec 21.
- [33] Xu W, Kaneko FT, Zheng S, Comhair SA, Janocha AJ, Goggans T, Thunnissen FB, Farver C, Hazen SL, Jennings C, Dweik RA, Arroliga AC, Erzurum SC. Increased arginase II and decreased NO synthesis in endothelial cells of patients with pulmonary arterial hypertension. *FASEB J*. 2004 Nov;18(14):1746-8. Epub 2004 Sep 13.
- [34] Minamishima S, Kida K, Tokuda K, Wang H, Sips PY, Kosugi S, Mandeville JB, Buys ES, Brouckaert P, Liu PK, Liu CH, Bloch KD, Ichinose F. Inhaled nitric oxide improves outcomes after successful cardiopulmonary resuscitation in mice. *Circulation*. 2011 Oct 11;124(15):1645-53. Epub 2011 Sep 19.
- [35] Lang JD Jr, Teng X, Chumley P, Crawford JH, Isbell TS, Chacko BK, Liu Y, Jhala N, Crowe DR, Smith AB, Cross RC, Frenette L, Kelley EE, Wilhite DW, Hall CR, Page GP, Fallon MB, Bynon JS, Eckhoff DE, Patel RP. Inhaled NO accelerates restoration of liver function in adults following orthotopic liver transplantation. *J Clin Invest*. 2007 Sep;117(9):2583-91
- [36] Yoshida K, Kasama K, Kitabatake M, Okuda M, Imai M. Metabolic fate of nitric oxide. *Int Arch Occup Environ Health*. 1980;46(1):71-7.
- [37] Yoshida K, Kasama K, Kitabatake M, Imai M. Biotransformation of nitric oxide, nitrite and nitrate. *Int Arch Occup Environ Health*. 1983;52(2):103-15.
- [38] Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*. 1991 Jun;43(2):109-42.
- [39] Chen CA, Wang TY, Varadharaj S, Reyes LA, Hemann C, Talukder MA, Chen YR, Druhan LJ, Zweier JL. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. *Nature*. 2010 Dec 23;468(7327):1115-8.
- [40] Crabtree MJ, Tatham AL, Al-Wakeel Y, Warrick N, Hale AB, Cai S, Channon KM, Alp NJ. Quantitative regulation of intracellular endothelial nitric-oxide synthase (eNOS) coupling by both tetrahydrobiopterin-eNOS stoichiometry and biopterin re-

- dox status: insights from cells with tet-regulated GTP cyclohydrolase I expression. *J Biol Chem*. 2009 Jan 9;284(2):1136-44. Epub 2008 Nov 14
- [41] Gielis JF, Lin JY, Wingler K, Van Schil PE, Schmidt HH, Moens AL. Pathogenetic role of eNOS uncoupling in cardiopulmonary disorders. *Free Radic Biol Med*. 2011 Apr 1;50(7):765-76. Epub 2010 Dec 21.
- [42] Tabima DM, Frizzell S, Gladwin MT. Reactive oxygen and nitrogen species in pulmonary hypertension. *Free Radic Biol Med*. 2012 May 1;52(9):1970-86. Epub 2012 Mar 6.
- [43] Moens AL, Takimoto E, Tocchetti CG, Chakir K, Bedja D, Cormaci G, Ketner EA, Majmudar M, Gabrielson K, Halushka MK, Mitchell JB, Biswal S, Channon KM, Wolin MS, Alp NJ, Paolocci N, Champion HC, Kass DA. Reversal of cardiac hypertrophy and fibrosis from pressure overload by tetrahydrobiopterin: efficacy of recoupling nitric oxide synthase as a therapeutic strategy. *Circulation*. 2008 May 20;117(20):2626-36. Epub 2008 May 12
- [44] Khoo JP, Zhao L, Alp NJ, Bendall JK, Nicoli T, Rockett K, Wilkins MR, Channon KM. Pivotal role for endothelial tetrahydrobiopterin in pulmonary hypertension. *Circulation*. 2005 Apr 26;111(16):2126-33. Epub 2005 Apr 11.
- [45] Kunuthur SP, Milliken PH, Gibson CL, Suckling CJ, Wadsworth RM. Tetrahydrobiopterin analogues with NO-dependent pulmonary vasodilator properties. *Eur J Pharmacol*. 2011 Jan 10;650(1):371-7. Epub 2010 Oct 13.
- [46] Münzel T, Li H, Mollnau H, Hink U, Matheis E, Hartmann M, Oelze M, Skatchkov M, Warnholtz A, Duncker L, Meinertz T, Förstermann U. Effects of long-term nitroglycerin treatment on endothelial nitric oxide synthase (NOS III) gene expression, NOS III-mediated superoxide production, and vascular NO bioavailability. *Circ Res*. 2000 Jan 7;86(1):E7-E12.
- [47] Münzel T, Sayegh H, Freeman BA, Tarpey MM, Harrison DG. Evidence for enhanced vascular superoxide anion production in nitrate tolerance. A novel mechanism underlying tolerance and cross-tolerance. *J Clin Invest*. 1995 Jan;95(1):187-94.
- [48] Banquet S, Delannoy E, Agouni A, Dessy C, Lacomme S, Hubert F, Richard V, Muller B, Leblais V. Role of G(i/o)-Src kinase-PI3K/Akt pathway and caveolin-1 in  $\beta$ 2-adrenoceptor coupling to endothelial NO synthase in mouse pulmonary artery. *Cell Signal*. 2011 Jul;23(7):1136-43. Epub 2011 Mar 6
- [49] Zhao YY, Zhao YD, Mirza MK, Huang JH, Potula HH, Vogel SM, Brovkovich V, Yuan JX, Wharton J, Malik AB. Persistent eNOS activation secondary to caveolin-1 deficiency induces pulmonary hypertension in mice and humans through PKG nitration. *J Clin Invest*. 2009 Jul;119(7):2009-18.
- [50] Achcar RO, Demura Y, Rai PR, Taraseviciene-Stewart L, Kasper M, Voelkel NF, Cool CD. Loss of caveolin and heme oxygenase expression in severe pulmonary hypertension. *Chest*. 2006 Mar;129(3):696-705.

- [51] Sud N, Wells SM, Sharma S, Wiseman DA, Wilham J, Black SM. Asymmetric dimethylarginine inhibits HSP90 activity in pulmonary arterial endothelial cells: role of mitochondrial dysfunction. *Am J Physiol Cell Physiol*. 2008 Jun;294(6):C1407-18. Epub 2008 Apr 2
- [52] Rus A, Peinado MA, Castro L, Del Moral ML. Lung eNOS and iNOS are reoxygenation time-dependent upregulated after acute hypoxia. *Anat Rec (Hoboken)*. 2010 ; 293(6):1089-98.
- [53] Archer SL, Tolins JP, Raij L, Weir EK. Hypoxic pulmonary vasoconstriction is enhanced by inhibition of the synthesis of an endothelium derived relaxing factor. *Biochem Biophys Res Commun*. 1989 15;164(3):1198-205.
- [54] Fox GA, Paterson NA, McCormack DG. Effect of inhibition of NO synthase on vascular reactivity in a rat model of hyperdynamic sepsis. *Am J Physiol*. 1994 ;267(4 Pt 2):H1377-82.
- [55] Murata T, Kinoshita K, Hori M, Kuwahara M, Tsubone H, Karaki H, Ozaki H. Statin protects endothelial nitric oxide synthase activity in hypoxia-induced pulmonary hypertension. *Arterioscler Thromb Vasc Biol*. 2005 ;25(11):2335-42.
- [56] Blumberg FC, Wolf K, Arzt M, Lorenz C, Riegger GA, Pfeifer M. Effects of ET-A receptor blockade on eNOS gene expression in chronic hypoxic rat lungs. *J Appl Physiol*. 2003 ;94(2):446-52.
- [57] Kanno S, Wu YJ, Lee PC, Billiar TR, Ho C. Angiotensin-converting enzyme inhibitor preserves p21 and endothelial nitric oxide synthase expression in monocrotaline-induced pulmonary arterial hypertension in rats. *Circulation*. 2001;104(8):945-50.
- [58] Mawatari E, Hongo M, Sakai A, Terasawa F, Takahashi M, Yazaki Y, Kinoshita O, Ikeda U. Amlodipine prevents monocrotaline-induced pulmonary arterial hypertension and prolongs survival in rats independent of blood pressure lowering. *Clin Exp Pharmacol Physiol*. 2007 ;34(7):594-600.
- [59] Pei Y, Ma P, Wang X, Zhang W, Zhang X, Zheng P, Yan L, Xu Q, Dai G. Rosuvastatin attenuates monocrotaline-induced pulmonary hypertension via regulation of Akt/eNOS signaling and asymmetric dimethylarginine metabolism. *Eur J Pharmacol*. 2011 ;666(1-3):165-72.
- [60] Mason NA, Springall DR, Burke M, Pollock J, Mikhail G, Yacoub MH, Polak JM. High expression of endothelial nitric oxide synthase in plexiform lesions of pulmonary hypertension. *J Pathol*. 1998 ;185(3):313-8.
- [61] Zhao YY, Zhao YD, Mirza MK, Huang JH, Potula HH, Vogel SM, Brovkovich V, Yuan JX, Wharton J, Malik AB. Persistent eNOS activation secondary to caveolin-1 deficiency induces pulmonary hypertension in mice and humans through PKG nitration. *J Clin Invest*. 2009 ;119(7):2009-18.
- [62] Kaneko FT, Arroliga AC, Dweik RA, Comhair SA, Laskowski D, Oppedisano R, Thomassen MJ, Erzurum SC. Biochemical reaction products of nitric oxide as quantita-

- tive markers of primary pulmonary hypertension. *Am J Respir Crit Care Med.* 1998 ; 158(3):917-23.
- [63] Malinowski A, Henrohn D, Eriksson A, Lundberg JO, Alving K, Wikström G. Increased plasma and salivary nitrite and decreased bronchial contribution to exhaled NO in pulmonary arterial hypertension. *Eur J Clin Invest.* 2011 ;41(8):889-97
- [64] Oka M, Hasunuma K, Webb SA, Stelzner TJ, Rodman DM, McMurtry IF. EDRF suppresses an unidentified vasoconstrictor mechanism in hypertensive rat lungs. *Am J Physiol.* 1993 ;264:L587-97.
- [65] Maruyama J, Maruyama K. Impaired nitric oxide-dependent responses and their recovery in hypertensive pulmonary arteries of rats. *Am J Physiol.* 1994 ;266(6 Pt 2):H2476-88.
- [66] Ferrario L, Amin HM, Sugimori K, Camporesi EM, Hakim TS. Site of action of endogenous nitric oxide on pulmonary vasculature in rats. *Pflugers Arch.* 1996 ;432(3):523-7.
- [67] Igari H, Tatsumi K, Sugito K, Kasahara Y, Saito M, Tani T, Kimura H, Kuriyama T. Role of EDRF in pulmonary circulation during sustained hypoxia. *J Cardiovasc Pharmacol.* 1998 ;31(2):299-305.
- [68] Bardou M, Goirand F, Marchand S, Rouget C, Devillier P, Dumas JP, Morcillo EJ, Rochette L, Dumas M. Hypoxic vasoconstriction of rat main pulmonary artery: role of endogenous nitric oxide, potassium channels, and phosphodiesterase inhibition. *J Cardiovasc Pharmacol.* 2001 Aug;38(2):325-34.
- [69] Stamler JS, Loh E, Roddy MA, Currie KE, Creager MA. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation.* 1994;89(5):2035-40.
- [70] Cooper CJ, Landzberg MJ, Anderson TJ, Charbonneau F, Creager MA, Ganz P, Selwyn AP. Role of nitric oxide in the local regulation of pulmonary vascular resistance in humans. *Circulation.* 1996 15;93(2):266-71.
- [71] Eddahibi S, Adnot S, Carville C, Blouquit Y, Raffestin B. L-arginine restores endothelium-dependent relaxation in pulmonary circulation of chronically hypoxic rats. *Am J Physiol.* 1992 ;263:L194-200.
- [72] Goret L, Tanguy S, Guiraud I, Dauzat M, Obert P. Acute administration of L-arginine restores nitric oxide-mediated relaxation in isolated pulmonary arteries from pulmonary hypertensive exercise trained rats. *Eur J Pharmacol.* 2008 26;581(1-2):148-56.
- [73] Shaul PW, Wells LB, Horning KM. Acute and prolonged hypoxia attenuate endothelial nitric oxide production in rat pulmonary arteries by different mechanisms. *J Cardiovasc Pharmacol.* 1993;22(6):819-27.
- [74] Oka M. Phosphodiesterase 5 inhibition restores impaired ACh relaxation in hypertensive conduit pulmonary arteries. *Am J Physiol Lung Cell Mol Physiol.* 2001;280(3):L432-5.



- [75] Elmedal B, de Dam MY, Mulvany MJ, Simonsen U. The superoxide dismutase mimetic, tempol, blunts right ventricular hypertrophy in chronic hypoxic rats. *Br J Pharmacol.* 2004;141(1):105-13.
- [76] Mam V, Tanbe AF, Vitali SH, Arons E, Christou HA, Khalil RA. Impaired vasoconstriction and nitric oxide-mediated relaxation in pulmonary arteries of hypoxia- and monocrotaline-induced pulmonary hypertensive rats. *J Pharmacol Exp Ther.* 2010;332(2):455-62.
- [77] MacLean MR, McCulloch KM. Influence of applied tension and nitric oxide on responses to endothelins in rat pulmonary resistance arteries: effect of chronic hypoxia. *Br J Pharmacol.* 1998;123(5):991-9.
- [78] Tian L, Lammers SR, Kao PH, Reusser M, Stenmark KR, Hunter KS, Qi HJ, Shandas R. Linked opening angle and histological and mechanical aspects of the proximal pulmonary arteries of healthy and pulmonary hypertensive rats and calves. *Am J Physiol Heart Circ Physiol.* 2011;301(5):H1810-8.
- [79] Maruyama J, Yokochi A, Maruyama K, Nosaka S. Acetylcholine-induced endothelium-derived contracting factor in hypoxic pulmonary hypertensive rats. *J Appl Physiol.* 1999;86(5):1687-95.
- [80] Pidgeon GP, Tamosiuniene R, Chen G, Leonard I, Belton O, Bradford A, Fitzgerald DJ. Intravascular thrombosis after hypoxia-induced pulmonary hypertension: regulation by cyclooxygenase-2. *Circulation.* 2004 26;110(17):2701-7.
- [81] Mathew R, Zeballos GA, Tun H, Gewitz MH. Role of nitric oxide and endothelin-1 in monocrotaline-induced pulmonary hypertension in rats. *Cardiovasc Res.* 1995 ;30(5):739-46.
- [82] Fullerton DA, Hahn AR, McIntyre RC Jr. Mechanistic imbalance of pulmonary vasomotor control in progressive lung injury. *Surgery.* 1996;119(1):98-103.
- [83] Gout B, Quiniou MJ, Khandoudi N, Le Dantec C, Saïag B. Impaired endothelium-dependent relaxation by adrenomedullin in monocrotaline-treated rat arteries. *Eur J Pharmacol.* 1999 3;380(1):23-30.
- [84] Ozturk EI, Uma S. Effects of atorvastatin and L-arginine treatments on electrical field stimulation-mediated relaxations in pulmonary arterial rings of monocrotaline-induced pulmonary hypertensive rats. *J Cardiovasc Pharmacol.* 2010 ;56(5):498-505.
- [85] Baber SR, Deng W, Master RG, Bunnell BA, Taylor BK, Murthy SN, Hyman AL, Kadowitz PJ. Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction. *Am J Physiol Heart Circ Physiol.* 2007 ;292(2):H1120-8.
- [86] Prié S, Stewart DJ, Dupuis J. EndothelinA receptor blockade improves nitric oxide-mediated vasodilation in monocrotaline-induced pulmonary hypertension. *Circulation.* 1998 2;97(21):2169-74.

- [87] Demiryürek AT, Karamsetty MR, McPhaden AR, Wadsworth RM, Kane KA, MacLean MR. Accumulation of nitrotyrosine correlates with endothelial NO synthase in pulmonary resistance arteries during chronic hypoxia in the rat. *Pulm Pharmacol Ther.* 2000;13(4):157-65
- [88] Weerackody RP, Welsh DJ, Wadsworth RM, Peacock AJ. Inhibition of p38 MAPK reverses hypoxia-induced pulmonary artery endothelial dysfunction. *Am J Physiol Heart Circ Physiol.* 2009 May;296(5):H1312-20. Epub 2009 Feb 6.
- [89] Sawada H, Mitani Y, Maruyama J, Jiang BH, Ikeyama Y, Dida FA, Yamamoto H, Imanaka-Yoshida K, Shimpō H, Mizoguchi A, Maruyama K, Komada Y. A nuclear factor-kappaB inhibitor pyrrolidine dithiocarbamate ameliorates pulmonary hypertension in rats. *Chest.* 2007 Oct;132(4):1265-74
- [90] Brennan LA, Steinhorn RH, Wedgwood S, Mata-Greenwood E, Roark EA, Russell JA, Black SM. Increased superoxide generation is associated with pulmonary hypertension in fetal lambs: a role for NADPH oxidase. *Circ Res.* 2003 Apr 4;92(6):683-91. Epub 2003 Feb 27.
- [91] Grobe AC, Wells SM, Benavidez E, Oishi P, Azakie A, Fineman JR, Black SM. Increased oxidative stress in lambs with increased pulmonary blood flow and pulmonary hypertension: role of NADPH oxidase and endothelial NO synthase. *Am J Physiol Lung Cell Mol Physiol.* 2006 Jun;290(6):L1069-77.
- [92] Archer SL, Marsboom G, Kim GH, Zhang HJ, Toth PT, Svensson EC, Dyck JR, Gombert-Maitland M, Thébaud B, Husain AN, Cipriani N, Rehman J. Epigenetic attenuation of mitochondrial superoxide dismutase 2 in pulmonary arterial hypertension: a basis for excessive cell proliferation and a new therapeutic target. *Circulation.* 2010 Jun 22;121(24):2661-71. Epub 2010 Jun 7.
- [93] Bonnet S, Michelakis ED, Porter CJ, Andrade-Navarro MA, Thébaud B, Bonnet S, Haromy A, Harry G, Moudgil R, McMurtry MS, Weir EK, Archer SL. An abnormal mitochondrial-hypoxia inducible factor-1alpha-Kv channel pathway disrupts oxygen sensing and triggers pulmonary arterial hypertension in fawn hooded rats: similarities to human pulmonary arterial hypertension. *Circulation.* 2006 Jun 6;113(22):2630-41. Epub 2006 May 30
- [94] Pryor WA, Squadrito GL. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol.* 1995 May;268(5 Pt 1):L699-722.
- [95] Kaneko FT, Arroliga AC, Dweik RA, Comhair SA, Laskowski D, Oppedisano R, Thomassen MJ, Erzurum SC. Biochemical reaction products of nitric oxide as quantitative markers of primary pulmonary hypertension. *Am J Respir Crit Care Med.* 1998 Sep;158(3):917-23.
- [96] Sasaki S, Asano M, Ukai T, Nomura N, Maruyama K, Manabe T, Mishima A. Nitric oxide formation and plasma L-arginine levels in pulmonary hypertensive rats. *Respir Med.* 2004 Mar;98(3):205-12

- [97] Morris CR, Morris SM Jr, Hagar W, Van Warmerdam J, Claster S, Kepka-Lenhart D, Machado L, Kuypers FA, Vichinsky EP. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? *Am J Respir Crit Care Med*. 2003 Jul 1;168(1):63-9. Epub 2003 Mar 5
- [98] Pullamsetti S, Kiss L, Ghofrani HA, Voswinckel R, Haredza P, Klepetko W, Aigner C, Fink L, Muyal JP, Weissmann N, Grimminger F, Seeger W, Schermuly RT Increased levels and reduced catabolism of asymmetric and symmetric dimethylarginines in pulmonary hypertension. *FASEB J*. 2005 Jul;19(9):1175-7. Epub 2005 Apr 12
- [99] Antoniades C, Shirodaria C, Leeson P, Antonopoulos A, Warrick N, Van-Assche T, Cunningham C, Tousoulis D, Pillai R, Ratnatunga C, Stefanadis C, Channon KM. Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. *Eur Heart J*. 2009 May;30(9):1142-50. Epub 2009 Mar 18
- [100] Zweier JL, Talukder MA. Targeting dimethylarginine dimethylaminohydrolases in pulmonary arterial hypertension: a new approach to improve vascular dysfunction? *Circulation*. 2011 Mar 22;123(11):1156-8. Epub 2011 Mar 7.
- [101] Pei Y, Ma P, Wang X, Zhang W, Zhang X, Zheng P, Yan L, Xu Q, Dai G. Rosuvastatin attenuates monocrotaline-induced pulmonary hypertension via regulation of Akt/eNOS signaling and asymmetric dimethylarginine metabolism. *Eur J Pharmacol*. 2011 Sep;666(1-3):165-72. Epub 2011 May 30
- [102] Achan V, Tran CT, Arrigoni F, Whitley GS, Leiper JM, Vallance P. all-trans-Retinoic acid increases nitric oxide synthesis by endothelial cells:a role for the induction of dimethylarginine dimethylaminohydrolase. *Circ Res*. 2002 Apr 19;90(7):764-9.
- [103] Uruno A, Sugawara A, Kanatsuka H, Kagechika H, Saito A, Sato K, Kudo M, Takeuchi K, Ito S. Upregulation of nitric oxide production in vascular endothelial cells by all-trans retinoic acid through the phosphoinositide 3-kinase/Akt pathway. *Circulation*. 2005 Aug 2;112(5):727-36. Epub 2005 Jul 25.
- [104] Hirokawa K, O'Shaughnessy KM, Ramrakha P, Wilkins MR. Inhibition of nitric oxide synthesis in vascular smooth muscle by retinoids. *Br J Pharmacol*. 1994 Dec;113(4):1448-54
- [105] Hampl V, Bíbová J, Banasová A, Uhlík J, Míková D, Hnilicková O, Lachmanová V, Herget J. Pulmonary vascular iNOS induction participates in the onset of chronic hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2006 Jan;290(1):L11-20. Epub 2005 Aug 19
- [106] Crossno JT Jr, Garat CV, Reusch JE, Morris KG, Dempsey EC, McMurtry IF, Stenmark KR, Klemm DJ. Rosiglitazone attenuates hypoxia-induced pulmonary arterial remodeling. *Am J Physiol Lung Cell Mol Physiol*. 2007 Apr;292(4):L885-97. Epub 2006 Dec 22

- [107] Calnek DS, Mazzella L, Roser S, Roman J, Hart CM. Peroxisome proliferator-activated receptor gamma ligands increase release of nitric oxide from endothelial cells. *Arterioscler Thromb Vasc Biol*. 2003 Jan 1;23(1):52-7.
- [108] Qin Y, Zhou A, Ben X, Shen J, Liang Y, Li F. All-trans retinoic acid in pulmonary vascular structural remodeling in rats with pulmonary hypertension induced by monocrotaline. *Chin Med J (Engl)*. 2001 May;114(5):462-5.
- [109] Zhang E, Jiang B, Yokochi A, Maruyama J, Mitani Y, Ma N, Maruyama K. Effect of all-trans-retinoic acid on the development of chronic hypoxia-induced pulmonary hypertension. *Circ J*. 2010 Aug;74(8):1696-703. Epub 2010 Jul 1.
- [110] Rosenberg HC, Rabinovitch M. Endothelial injury and vascular reactivity in monocrotaline pulmonary hypertension. *Am J Physiol*. 1988 Dec;255(6 Pt 2):H1484-91
- [111] Rabinovitch M, Gamble W, Nadas AS, Miettinen OS, Reid L. Rat pulmonary circulation after chronic hypoxia: hemodynamic and structural features. *Am J Physiol*. 1979 Jun;236(6):H818-27
- [112] Gangopahyay A, Oran M, Bauer EM, Wertz JW, Comhair SA, Erzurum SC, Bauer PM. Bone morphogenetic protein receptor II is a novel mediator of endothelial nitric-oxide synthase activation. *J Biol Chem*. 2011 Sep 23;286(38):33134-40. Epub 2011 Aug 1
- [113] Hood JD, Meininger CJ, Ziche M, Granger HJ. VEGF upregulates eNOS message, protein, and NO production in human endothelial cells. *Am J Physiol*. 1998 Mar;274(3 Pt 2):H1054-8
- [114] Morbidelli L, Chang CH, Douglas JG, Granger HJ, Ledda F, Ziche M. Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. *Am J Physiol*. 1996 Jan;270(1 Pt 2):H411-5.
- [115] Feng Y, Venema VJ, Venema RC, Tsai N, Caldwell RB. VEGF induces nuclear translocation of Flk-1/KDR, endothelial nitric oxide synthase, and caveolin-1 in vascular endothelial cells. *Biochem Biophys Res Commun*. 1999 Mar 5;256(1):192-7.
- [116] Taraseviciene-Stewart L, Kasahara Y, Alger L, Hirth P, Mc Mahon G, Waltenberger J, Voelkel NF, Tudor RM Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. *FASEB J*. 2001 Feb;15(2):427-38.
- [117] Maruyama K, Ye CL, Woo M, Venkatacharya H, Lines LD, Silver MM, Rabinovitch M. Chronic hypoxic pulmonary hypertension in rats and increased elastolytic activity. *Am J Physiol*. 1991 Dec;261(6 Pt 2):H1716-26
- [118] Ilkiw R, Todorovich-Hunter L, Maruyama K, Shin J, Rabinovitch M. SC-39026, a serine elastase inhibitor, prevents muscularization of peripheral arteries, suggesting a mechanism of monocrotaline-induced pulmonary hypertension in rats. *Circ Res*. 1989 Apr;64(4):814-25.

- [119] Carp H, Janoff A. In vitro suppression of serum elastase-inhibitory capacity by reactive oxygen species generated by phagocytosing polymorphonuclear leukocytes. *J Clin Invest.* 1979 Apr;63(4):793-7
- [120] Mitani Y, Zaidi SH, Dufourcq P, Thompson K, Rabinovitch M. Nitric oxide reduces vascular smooth muscle cell elastase activity through cGMP-mediated suppression of ERK phosphorylation and AML1B nuclear partitioning. *FASEB J.* 2000 Apr;14(5):805-14
- [121] Do e Z, Fukumoto Y, Takaki A, Tawara S, Ohashi J, Nakano M, Tada T, Saji K, Sugimura K, Fujita H, Hoshikawa Y, Nawata J, Kondo T, Shimokawa H. Evidence for Rho-kinase activation in patients with pulmonary arterial hypertension. *Circ J.* 2009 Sep;73(9):1731-9. Epub 2009 Jul 9.
- [122] Abe K, Shimokawa H, Morikawa K, Uwatoku T, Oi K, Matsumoto Y, Hattori T, Nakashima Y, Kaibuchi K, Sueishi K, Takeshit A. Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats. *Circ Res.* 2004 Feb 20;94(3):385-93. Epub 2003 Dec 11
- [123] Nagaoka T, Morio Y, Casanova N, Bauer N, Gebb S, McMurtry I, Oka M. Rho/Rho kinase signaling mediates increased basal pulmonary vascular tone in chronically hypoxic rats. *Am J Physiol Lung Cell Mol Physiol.* 2004 Oct;287(4):L665-72. Epub 2003 Sep 5.
- [124] Sauzeau V, Le Jeune H, Cario-Toumaniantz C, Smolenski A, Lohmann SM, Bertoglio J, Chardin P, Pacaud P, Loirand G. Cyclic GMP-dependent protein kinase signaling pathway inhibits RhoA-induced Ca<sup>2+</sup> sensitization of contraction in vascular smooth muscle. *J Biol Chem.* 2000 Jul 14;275(28):21722-9.
- [125] Ming XF, Viswambharan H, Barandier C, Ruffieux J, Kaibuchi K, Rusconi S, Yang Z. Rho GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. *Mol Cell Biol.* 2002 Dec;22(24):8467-77.

IntechOpen



