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Dual role of the L-arginine–ADMA–NO pathway in systemic hypoxic vasodilation and pulmonary hypoxic vasoconstriction

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Abstract

In healthy vascular endothelium, nitric oxide acts as a vasodilator paracrine mediator on adjacent smooth muscle cells. By activating soluble guanylyl cyclase, nitric oxide stimulates cyclic guanosine monophosphate (cGMP) which causes relaxation of vascular smooth muscle (vasodilation) and inhibition of platelet aggregation. This mechanism is active in both, the systemic and pulmonary circulation. In the systemic circulation, hypoxia results in local vasodilation, which has been shown to be brought about by stabilization of hypoxia-inducible factor-1α (HIF1α) and concomitant upregulation of endothelial nitric oxide synthase. By contrast, the physiological response to hypoxia in the pulmonary circulation is vasoconstriction. Hypoxia in the lung primarily results from hypoventilation of circumscript areas of the lung, e.g. by bronchial tree obstruction or inflammatory infiltration. Therefore, hypoxic pulmonary vasoconstriction is a mechanism preventing distribution of blood to hypoventilated areas of the lungs, thereby maintaining maximal oxygenation of blood. The exact molecular mechanism of hypoxic pulmonary vasoconstriction is less well understood than hypoxic vasodilation in the systemic circulation. While alveolar epithelial cells may be key in sensing low oxygen concentration, and pulmonary vascular smooth muscle cells obviously are the effectors of vasoconstriction, the pulmonary vascular endothelium plays a crucial role as an intermediate between these cell types. Indeed, dysfunctional endothelial nitric oxide release was observed in humans exposed to acute hypoxia, and animal studies suggest that hypoxic pulmonary vasoconstriction is enhanced by nitric oxide synthase inhibition. This may be caused, in part, by elevation of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthesis. High asymmetric dimethylarginine levels are associated with endothelial dysfunction, vascular disease, and hypertension.

Keywords

endothelium-dependent vasodilation, nitric oxide, hypoxic pulmonary vasoconstriction

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Introduction

Hypoxia is a deadly threat not only to every cell but also to the organism as a whole. Physiological mechanism helps the organism to adapt to conditions of low oxygen supply. The response to a mismatch between oxygen demand and oxygen supply in organs of the systemic circulation is an increase in blood flow.[1] Hypoxia in the systemic circulation may result from local vascular occlusion, low oxygen delivery with the blood stream, or reduced perfusion volume. In each case, compensatory mechanisms that increase local blood flow are activated to minimize ischemic tissue damage.

By contrast, hypoxia in the lung is most frequently a result of blocked airflow through the bronchial tree into the alveoli. In the lung, the vascular system responds to hypoxia with vasoconstriction rather than vasodilation like in the systemic circulation. This obvious difference between hypoxic vasodilation in the systemic circulation and hypoxic pulmonary vasoconstriction has aroused intense research

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interest for many decades ever since it was first described in the middle of the 20th century by the Swedish physiologists, von Euler and Liljestrand.[2] However, the exact molecular mechanisms underlying this phenomenon have remained elusive to this date.

This review aims to summarize our current understanding of the role of the endothelial L-arginine–ADMA–nitric oxide pathway in the responses of the systemic and pulmonary circulation to hypoxia.

The endothelial L-arginine–ADMA–nitric oxide pathway

Nitric oxide (NO) is a critically important mediator of vasodilation under a variety of physiological and pathophysiological conditions. The generation of NO is performed by enzymatic activities of NO synthases (NOSs), of which at least three major isoforms with distinct functions, tissue distribution, and regulation have been described. NOS I was first isolated from rat cerebellum and was therefore named neuronal NOS (nNOS). In the nerve system, it plays an important role in modulating memory formation and pain transduction, but nNOS has also been shown to be expressed in other tissues like epithelia. NOS II is an isoform that is found in macrophages and other immune cells upon cytokine stimulation; it has therefore been called inducible NOS (iNOS). A third isoform, NOS III, was first isolated from endothelial cells and named endothelial NOS (eNOS) thereafter. There are a number of important functional and regulatory differences between these three major isoforms, which are summarized in Table 1. The differences in expression, function, and tissue distribution of the three NOS isoforms have been extensively reviewed elsewhere.[3,4]

The most important isoform in the context of vasodilation and vasoconstriction during hypoxia evidently is eNOS. This isoform, although originally classified as a constitutively expressed enzyme, is regulated by multiple transcriptional and posttranscriptional mechanisms. For example, estrogens and resveratrol have been demonstrated to upregulate eNOS mRNA expression,[5,6] and part of the protective action of the cholesterol-lowering drugs, statins, have been attributed to their ability to upregulate eNOS transcription.[7]

On a posttranscriptional level, the amino acid sequence of the eNOS protein contains several phosphorylation sites.[8,9] Phosphorylation of each of these sites either upregulates or downregulates NOS protein activity; numerous kinases and phosphatases have been shown to contribute to this regulation.[9,10] Myristoylation of eNOS was demonstrated to enhance its anchoring in the plasma membrane and its linking to caveolin, a membrane protein exclusively found in invaginations—caveolae—of the endothelial cell plasma membrane. This process generates a microenvironment of close proximity of eNOS with the L-arginine membrane transporter that helps to control eNOS activity.[11,12]

On the level of eNOS catalytic activity, methylarginines are endogenous inhibitors competing with L-arginine for binding to the catalytic site of NOS (N^G-monomethyl-L-arginine—L-NMMA and asymmetric dimethylarginine—ADMA)[13] or to binding to the L-arginine membrane uptake carrier (ADMA and symmetric dimethylarginine—SDMA).[14] Methylarginines have been recognized in recent years as major regulators of NOS catalytic function (see below).

Finally, once released from the endothelial cell, the extremely reactive radical NO can rapidly react with other radicals, such as superoxide radicals (O_2^-). Reaction of NO with O_2^- yields peroxinitrite ($ONOO^-$), an extremely cytotoxic molecule that has been involved in protein nitrosylation in various pathophysiological cardiovascular scenarios[15] as well as in unspecific host defense against bacterial pathogens.[16] Another aspect of similar importance is that the reaction with superoxide leads to inactivation of the biological

Table 1. Biochemical and physiological characteristics of the three isoforms of NOS.

	NOS I	NOS II	NOS III
Alternative name	Neuronal NOS	Inducible NOS	Endothelial NOS
Abbreviation	nNOS	iNOS	eNOS
Tissue distribution	Central and peripheral neuronal cells, epithelial cells, macula densa cells, pancreatic islet cells	Macrophages[a], lymphocytes[a], neurophils[a], eosinophils[a], endothelial cells[a], Kupffer cells[a], and mast cells[a]	Endothelial cells
Intracellular localization	Cytoplasmatic (partly particulate)	Cytoplasmatic	Particulate (>90% membrane-associated)
Molecular size	160 kD	130 kD	135 kD
Expression	Constitutive	Inducible (LPS, IFN-γ)	Constitutive[b]
Activity	Low (Ca^{2+}-dependent)	High (Ca^{2+}-independent)	Low (Ca^{2+}-dependent)

Note: NOS: nitric oxide synthase; kD: kilo Daltons; Ca^{2+}: calcium; IFN: interferon; LPS: bacterial lipopolysaccharide.
[a]Immunohistochemical detection of iNOS was performed in these cell types after incubation of cells with various cytokines and/or bacterial lipopolysaccharide.
[b]eNOS was shown to be transcriptionally upregulated by a variety of stimuli like estrogen, resveratrol, statins, and HIF-1α.

functions of NO—indeed, postsecretory oxidative inactivation of NO is believed by some to be the major mechanism underlying dysfunction NO-mediated vasodilation in atherosclerosis.[17] Fig. 1 gives a schematic overview of the various levels of regulation of eNOS mRNA expression, posttranslational protein modification, and catalytic function.

Biomedical role of ADMA as a regulator of NO function

ADMA is a competitive inhibitor of eNOS. While ADMA was first isolated in 1970 from rat urine,[18] its functional role of decreasing NO production has only been discovered in 1992.[19] ADMA was shown to inhibit in a concentration-dependent manner the conversion of L-[$^{15}N_2$]-arginine to $^{15}NO_2^-$ and $^{15}NO_3^-$ in cultured human endothelial cells in vitro[13] and in rabbits in vivo.[20] Human subjects with elevated serum cholesterol and high ADMA plasma concentration have impaired endothelium-dependent, flow-mediated vasodilation, a validated surrogate for NO function in vivo.[21] This vascular dysfunction is promptly reversible upon administration of excess L-arginine.

In prospective clinical studies, ADMA has been characterized extensively as a cardiovascular risk factor. Patients with end-stage renal failure undergoing hemodialysis treatment have excessively high ADMA plasma levels secondary to impaired renal elimination of this amino acid. However, even within this group of patients, those with the highest ADMA levels have the highest probability of experiencing a major adverse cardiovascular event or die of it, while those in the lowest quartile of the distribution of ADMA levels have the best prognosis.[22] Interestingly, in the context of this review, erythrocyte ADMA accumulation in an animal model of chronic kidney disease contributes to impaired erythropoietin response via suppression of erythropoietin receptor expression.[23]

Subsequent studies have established ADMA as a risk factor in cohorts with other cardiovascular and metabolic diseases and in the general population (for review, cf., Böger et al.[24]). Large, population-based cohorts have uniformly revealed an increase in mortality risk by some 21% for every 0.13 µmol/l increase in plasma ADMA concentration.[25,26] The magnitude of this risk association is therefore comparable with the risk increase associates with a four

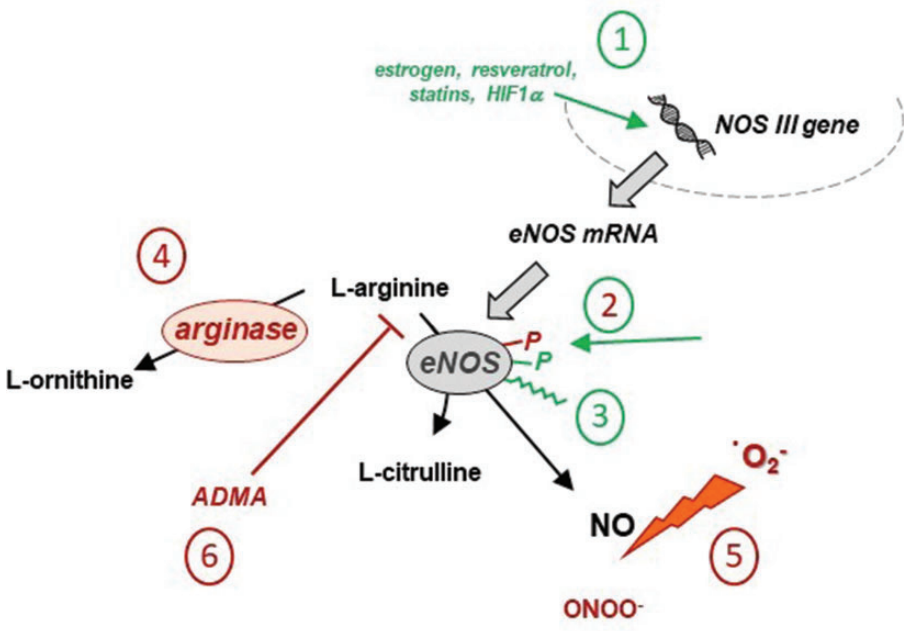

Fig. 1. The L-arginine–ADMA–nitric oxide pathway: points of regulation. ① Transcription of the NOS III gene is upregulated by various endogenous and exogenous agents, like estrogen, HIF-1α, resveratrol, and statin drugs. ② The eNOS protein contains a number of serine and threonine sites that are subject to phosphorylation, resulting in up- or downregulation of enzymatic activity. ③ Myristoylation of the eNOS protein increases its ability to anchor in the plasma membrane, in the proximity of caveolae which regulate eNOS activity. ④ L-arginine, the substrate of NOS, is also a substrate of arginases, which convert L-arginine into L-ornithine and urea—at the same time withdrawing substrate from the NOS enzyme. ⑤ Once NO is released, it is a highly reactive radical that easily reacts with other compounds presenting a single free electron; the major radical of this kind is superoxide radical. Reaction of NO with superoxide generates the highly cytotoxic peroxinitrite ($ONOO^-$) but at the same time inactivates the biological function of NO. ⑥ The catalytic activity of NOS is inhibited, in a competitive manner, by ADMA, an endogenous methylated L-arginine derivative. The tissue and plasma concentrations of ADMA itself are subject to complex mechanisms of regulation. ADMA: asymmetric dimethylarginine; HIFα: hypoxia-inducible factor-1α; eNOS: endothelial NOS; NOS: NO synthase; NO: nitric oxide.

year-increase in age and signifies that ADMA is a major contributor to overall risk.

Biosynthesis and metabolism of dimethylarginines

L-Arginine methylation has not been shown to occur for the free amino acid, but for protein-bound L-arginine residues, through the action of protein arginine N-methyltransferases (PRMTs).[27] Arginine methylation has turned out in recent years to be a tightly regulated process during which specific proteins are methylated by specific subtypes of PRMTs. The family of enzymes by now comprises at least nine isozymes belonging to four families with different enzymatic characteristics: Type 1 PRMTs lead to asymmetric arginine dimethylation of proteins, type 2 PRMTs cause symmetric dimethylation, type 3 PRMTs stop the reaction when arginine residues are monomethylated (monomethylarginine also is an intermediate of type 1 and type 2 PRMT enzymatic activity and can be released from all three reaction types), and type 4 PRMTs have not yet been clearly characterized. Comprehensive reviews of the complex biology of protein arginine methylation have been given by Bedford and Clarke[28] and Fulton et al.[29] During physiological protein breakdown, free mono- and dimethylarginines are released.

While in their initial observation dating back to 1992, Vallance et al. identified and purified dimethylarginines from urine,[19] we now know that enzymatic cleavage is the main mechanism of degradation for dimethylarginines. Dimethylarginine dimethylaminohydrolase (DDAH) cleaves NMMA and ADMA to L-citrulline and dimethylamine and monomethylamine, respectively, whereas SDMA is not a substrate for DDAH. Two isoforms of DDAH with differing tissue distribution have been identified and named DDAH1 (the major isoform in the kidneys and liver) and DDAH2 (the major isoform in vascular and neuronal tissues).[30] Although early studies using siRNA-mediated knockdown of DDAH1 and DDAH2 suggested that DDAH1 is a major regulator of plasma ADMA concentration but not endothelium-dependent vasodilation, while DDAH2 modulates endothelium-dependent vasodilation but not circulating ADMA levels,[31] we later used an endothelium-specific DDAH1 knockout mouse line to demonstrate that DDAH1 is the major DDAH isoform regulating ADMA plasma concentration, vascular function, and blood pressure.[32]

An alternative metabolic pathway for both, ADMA and SDMA, may be mediated by alanine glyoxylate aminotransferase 2 (AGXT2). AGXT2 utilizes ADMA and SDMA as alternative substrates, converting them in the presence of glyoxylate to α-keto-dimethylguanidinovaleric acid and glycine.[33] However, ADMA- or SDMA-metabolizing activity of AGXT2 has so far mostly been shown when the enzyme was overexpressed.[34,35] We found in a genome-wide association study of dimethylarginines that SDMA plasma concentration was linked at a genome-wide level of significance to single-nucleotide polymorphisms in the AGXT2 gene, and that overexpression of AGXT2 conveyed SDMA-metabolic activity.[34] Fig. 2 depicts the interaction of the enzymes involved in methylarginine biosynthesis and metabolism.

Dysregulation of the activity and/or expression of enzymes regulating ADMA concentration may thus contribute to impaired NO generation, endothelial dysfunction, vasospasm, and elevated vascular resistance, both in the systemic and pulmonary circulation. Knockout and transgenic mouse models corroborate this hypothesis: Mice lacking endothelial NOS were first generated and characterized in the mid-1990s. Their major phenotype, besides dysfunctional endothelium-dependent vasodilation, is a gene-dose-related elevation of systemic blood pressure.[36] A few years later, Fagan et al. reported that homozygous eNOS knockout mice showed hyperresponsiveness of the pulmonary circulation to mild hypoxia.[37] More recently generated mouse models show that genetic engineering of genes modulating ADMA concentration has similar hemodynamic consequences: Mice overexpressing the human DDAH1 gene show circulating ADMA levels that are about 40% of those measured in wild-type littermates. These hDDAH1-tg mice have significantly lower systemic mean arterial pressure and lower systemic vascular resistance.[38] The opposite model, DDAH1 knockout mice, is characterized by elevated ADMA concentration, endothelial dysfunction, and higher systemic and pulmonary blood pressures.[39]

Vasodilation as a physiological response to hypoxia in the systemic circulation

Reactive vasodilation in hypoxia in the systemic circulation may result from two major causes: (a) Local vascular occlusion by thromboembolic events or by atherosclerotic arterial stenosis may lead to local hypoxia and ischemia in the affected vascular bed. Blood flow velocity is increased at the site of stenosis in a manner dependent on the degree of lumen reduction, and mechanoreceptors in the luminal membrane of the endothelium respond to this stimulus by activating eNOS activity, which causes poststenotic vasodilation.[40] (b) Low oxygen delivery with the blood stream may be caused by reduced oxygenation of blood in the lungs, by reduced oxygen transport capacity of the red blood cells like in anemia, or by reduced perfusion volume like in congestive heart failure. Systemic hypoxia leads to activation of HIF1α, which is translocated to the nucleus and controls mRNA expression of genes that may counteract the impact of hypoxia. The discovery of the molecular mechanism of HIF1α activation has been awarded with the 2019 Nobel Prize in Physiology or Medicine; it has been reviewed in depth by the three Nobel laureates.[41–43] In each case, compensatory mechanisms that increase local blood flow are activated to minimize ischemic tissue damage. Increased endothelial NO formation has been shown to regulate coronary blood flow during myocardial ischemia,[44] to improve the myocardial adaptation to

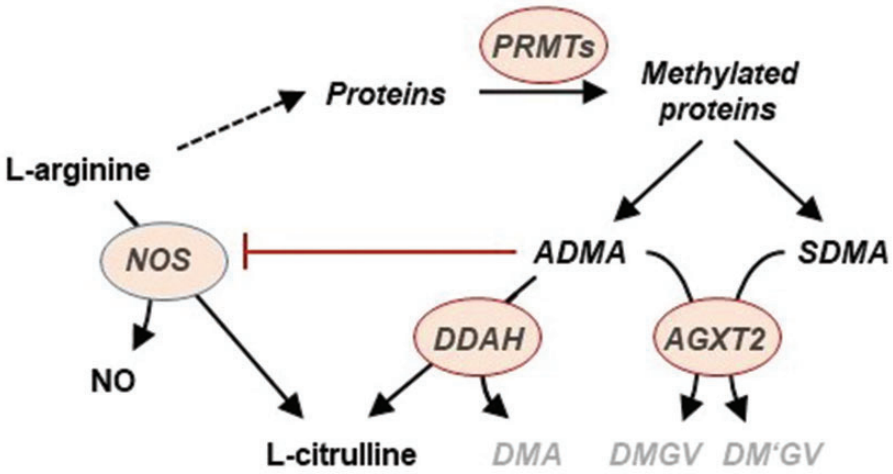

Fig. 2. Biosynthesis and metabolism of asymmetric and symmetric dimethylarginine. L-arginine residues within specific proteins are subject to methylation by PRMTs. Arginine methylation of proteins modulates their function in a posttranslational manner. When dimethylated proteins are degraded during physiological protein turnover, ADMA and SDMA are released. ADMA is a competitive inhibitor of NOSs. ADMA, but not SDMA, is degraded by DDAH into L-citrulline and DMA. Both dimethylarginines may be cleaved by an alternative pathway through AGXT2, resulting in the formation of symmetric or asymmetric dimethylguanidinovaleric acid (DMGV and DM'GV). PRMT: protein arginine N-methyltransferase; ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine; NOS: NO synthase; NO: nitric oxide; DDAH: dimethylarginine dimethylaminohydrolase; DMA: dimethylamine; AGXT2: alanine glyoxylate aminotransferase 2.

ischemic episodes,[45] and to improve the adaptation to hypoxia,[46] both during ischemia/reperfusion in the heart[47] and during altitude exposure in the cerebral circulation.[48]

The physiology and pathophysiology of hypoxic pulmonary vasoconstriction

The main physiological function of the lung is to deliver fully oxygenated blood into the systemic circulation. A blocked part of the bronchial tree that impairs airflow to a circumscript area of the lung would impede optimal oxygenation of blood, if it were not for the pulmonary circulation to respond with local vasoconstriction, thereby re-distributing blood to better oxygenated areas of the lungs. This physiological function for hypoxic pulmonary vasoconstriction was first observed by the Swedish physiologists von Euler and Liljestrand in cats and soon after confirmed to exist in humans, too.[2,49] It was later named after their discoverers, and—under the name of ''Euler-Liljestrand mechanism''—it is now integral part of every textbook of physiology.

Hypoxic vasoconstriction in the lungs may become pathological when an individual is exposed to systemic hypoxia over a prolonged period of time. Generalized vasoconstriction throughout the lungs may lead to increased vascular resistance in pulmonary arteries and, consequently, in elevated pulmonary arterial pressure. If not resolved, this eventually leads to right ventricular hypertrophy and failure. A common example for this is chronic or chronic-intermittent hypobaric hypoxia at high altitude, which is associated with a considerable increase in the prevalence of pulmonary hypertension. Chronic-intermittent hypoxia differs from chronic hypoxia in that exposure to high-altitude-associated hypoxia is permanent in chronic hypoxia, while it is interrupted by recurring short periods of normoxia in chronic-intermittent hypoxia. Thus, chronic-intermittent hypoxia bears the additional health issue of recurrent acute adaptation to high altitude.[50] For example, we recently reported the prevalence of mean pulmonary arterial pressures above 30 mm Hg (the stricter cut-off of 30 mm Hg is used for altitude residents as compared to 25 mm Hg for lowlanders, to account for the general, modest increase in pulmonary arterial pressure upon altitude exposure even in healthy individuals) to be as high as 9% in Chilean mining workers commuting weekly between the mining areas in the Andes at altitudes of about 4500 m and weekend off-time at sea level, a prevalence which compares to a mean 0.0015% for Western Europeans permanently living at sea level or low altitudes.[50]

Molecular mechanisms of hypoxic pulmonary vasoconstriction

Hypoxic pulmonary vasoconstriction is mediated by a contractile response of the pulmonary vascular smooth muscle cells (PVSMCs). Smooth muscle cell contraction is highly dependent on elevated cytosolic calcium concentration within the PVSMC; therefore, the effector mechanisms responsible for hypoxic pulmonary vasoconstriction likely involve modulation of PVSMC calcium handling. L-type calcium channels, non-specific calcium channels, and potassium channels, the latter by affecting membrane depolarization, have been shown to be involved in PVSMC calcium elevation.[51–53] In addition, endothelium-derived

vasoconstrictor and vasodilator mediators appear to impact on pulmonary arterial smooth muscle cell contraction. Endothelium-derived vasoconstrictor molecules have since long been discussed to be involved in pulmonary hypoxic vasoconstriction. Endothelin-1, superoxide radicals, and arachidonic acid-derived endoperoxides and/or thromboxane A_2 are the major factors generated in the endothelium that exert vasoconstrictor activity.[54–56] Endothelium-derived vasoconstrictor and vasodilator molecules physiologically are finely tuned to maintain a subtle balance of vascular tone and to adapt local blood flow to the varying needs of oxygen and nutrient demands.

Evidence for a role of NO in modulating the pulmonary hypoxic vasoconstrictor response

Like in other arteries in the systemic circulation, vascular tone is determined largely by endothelial NO release in healthy pulmonary arteries under normoxia. Acute and chronic hypobaric hypoxia at high altitude result in endothelial dysfunction, a situation defined by impaired endothelium-dependent, NO-mediated vasodilation in response to brief phases of ischemia in the forearm or in response to local infusion of acetylcholine. Endothelium-dependent vasodilation is acutely impaired in lowlanders after arrival to high-altitude hypoxia[57] as well as in Tibetan inhabitants of the Himalaya region, despite the good genetic adaptation of this population to chronic hypobaric hypoxia.[58] Inhabitants of the Andean high-altitude region also showed distinct endothelial dysfunction, which was more pronounced in individuals with cardiovascular risk factors or disease than in controls.[59]

In an early study, Archer et al. studied the effect of L-NMMA on what at that time was called endothelium-dependent relaxing factor (EDRF)-induced modulation of vascular tone in hypoxic-isolated porcine pulmonary arterioles.[60] These authors reported that L-NMMA markedly potentiated the hypoxia-induced increase in pulmonary arterial pressure. After pre-incubation with L-arginine, the vasoconstrictor effect was inverted into vasodilation, suggesting that L-arginine availability was involved in the competitive inhibition of EDRF (i.e., NO) release by L-NMMA. These results suggest that inhibition of NO by L-NMMA unmasked the effects of vasoconstrictor stimuli that are released in the hypoxic lung circulation. Another group of investigators followed 15 healthy mountaineers during a 19-day trek into Nepal.[61] As these individuals ascended to an altitude of about 5000 m, markers of NO production like salivary nitrite, S-nitroso-hemoglobin, and urinary nitrate excretion all significantly and transiently increased. Furthermore, Beall et al.[62] performed a meta-analysis of published studies on the acute and chronic effects of hypoxia on exhaled NO. Exhaled NO is supposed to be a marker of pulmonary NO release.[63] They reported that Tibetans and Bolivian Aymara natives of the Andean high plateau—populations well adapted during evolution to life at high altitude[64,65]—exhaled significantly more NO than US lowland inhabitants. In addition, among reports of humans exposed to high altitude, those with a prior history of high-altitude pulmonary edema (HAPE) showed significantly less NO levels in the exhaled air than HAPE-free visitors to high altitude. Again, these data suggest a compensatory role of NO during acute and chronic high-altitude adaptation.

Giaid and Saleh provided evidence of markedly decreased eNOS gene expression in the endothelium of patients with pulmonary hypertension.[66] However, subsequent studies found pulmonary expression of eNOS unchanged in pulmonary hypertension,[67] and some studies reported increased expression of eNOS and/or the inducible isoform of NO synthase.[68]

In summary, NOS gene expression in hypoxia may not always be equal to actual NO production, as NOS enzymatic activity may be influenced by a variety of factors relevant to pulmonary hypoxia. ADMA may be a primary candidate molecule as modulators of pulmonary vascular tone in hypoxia.

Conclusions

The endothelial L-arginine–ADMA–NO pathway is highly regulated under physiological and pathophysiological conditions to maintain the delicate balance between vasoconstriction and vasodilation that ensures appropriate distribution of blood flow according to metabolic and physiological needs. Under hypoxic conditions, the systemic circulation responds by local vasodilation, which is in part caused by increased NOS activity secondary to activation of mechanoceptors by increased blood flow at sites of arterial stenosis. Another, more sustained mechanism of increased NO-mediated vasodilation is upregulation of eNOS gene expression by HIF-1α. The pulmonary circulation, however, responds by vasoconstriction to hypoxic challenge. NO appears to attenuate hypoxic vasoconstriction, suggesting that individuals with impaired NO production may be more susceptible to develop hypoxic pulmonary arterial hypertension. Future studies will have to determine whether biomarkers of NO generation or function may serve as risk markers and whether intervention aimed at increasing NO production may prevent hypoxia-associated pulmonary hypertension.

Conflict of interest

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