Review Article

Role of nitric oxide in the control of the pulmonary circulation: physiological, pathophysiological, and therapeutic implications*

Papel do óxido nítrico na regulação da circulação pulmonar: implicações fisiológicas, fisiopatológicas e terapêuticas

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Abstract

Nitric oxide (NO) is an endogenous vasoactive compound that contributes to pulmonary vascular homeostasis and is produced by three nitric oxide synthase (NOS) isoforms—neuronal NOS (nNOS); inducible NOS (iNOS); and endothelial NOS (eNOS)—all three of which are present in the lung. Studies using pharmacological inhibitors or knockout mice have shown that eNOS-derived NO plays an important role in modulating pulmonary vascular tone and attenuating pulmonary hypertension. However, studies focusing on the role of iNOS have shown that this isoform contributes to the pathophysiology of acute lung injury and acute respiratory distress syndrome. This review aimed at outlining the role played by NO in the control of the pulmonary circulation, both under physiological and pathophysiological conditions. In addition, we review the evidence that the L-arginine-NO-cyclic guanosine monophosphate pathway is a major pharmacological target in the treatment of pulmonary vascular diseases.

Keywords: Nitric oxide; Arginine; Nitric oxide synthase; Cyclic GMP; Pulmonary circulation.

Resumo

O *nitric oxide* (NO, óxido nítrico) é um mediador endógeno vasoativo que contribui para a homeostase vascular pulmonar. O NO é produzido por três isoformas das *nitric oxide synthases* (NOS, óxido nítrico sintases)-NOS neuronial (nNOS); NOS induzida (iNOS); e NOS endotelial (eNOS)-estando as três presentes no pulmão. Estudos que utilizaram inibidores farmacológicos ou camundongos *knockout* têm demonstrado que o NO derivado da eNOS desempenha importantes papéis ao modular o tônus vascular pulmonar e atenuar a hipertensão pulmonar. Por outro lado, estudos focados no papel da iNOS têm mostrado que essa isoforma contribui para a fisiopatologia da lesão pulmonar aguda e da síndrome do desconforto respiratório agudo. Esta revisão objetivou delinear o papel desempenhado pelo NO no controle da circulação pulmonar, tanto em condições fisiológicas como fisiopatológicas. Além disso, revisamos as evidências de que a via L-arginina-NO-guanosina monofosfato cíclico seja um importante alvo farmacológico para a terapia de doenças vasculares pulmonares.

Descritores: Óxido nítrico; Arginina; Óxido nítrico sintase; GMP cíclico; Circulação pulmonar.

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Introduction

The chemical identification of the endothelium-derived relaxing factor nitric oxide (NO) has enabled better understanding of important physiological processes, especially cardiovascular system processes, such as the regulation of vascular tone and platelet function. In the body, the nitric oxide synthase (NOS) family of enzymes synthesize NO form the amino acid L-arginine. The principal effects of NO are related to the activation of guanylate cyclase, leading to increased formation of intracellular cyclic guanosine monophosphate (cGMP).[1]

The normal pulmonary circulation is a low-pressure and low-resistance system, which can accommodate the entire cardiac output, since it is completely dilated.[2] This physiological peculiarity observed in the pulmonary vascular bed is consistent with the observation that hardly any alteration is observed in pulmonary artery pressures under increasing pulmonary blood flow. In addition, no significant alterations in pulmonary arterial pressure occur, even after the administration of vasodilators in sufficient doses to cause significant reduction of systemic pressures.[3]

Details on the mechanisms involved in the physiological regulation of the pulmonary vascular tone have yet to be clarified. However, when NO was discovered to be an endogenous vasodilator, it was postulated that this molecule could contribute to maintaining low pulmonary vascular tone. However, the results of studies carried out to date are inconsistent in this respect, especially when comparing different types of findings. In addition, it has been observed that altered bioavailability of NO is common in lung diseases, with increased concentration in diseases accompanied by inflammation, for example, acute respiratory distress syndrome (ARDS)[4] or reduced synthesis of NO in diseases characterized by increased pulmonary arterial pressure, such as pulmonary arterial hypertension.[5]

In this article, we review the principal findings on the role played by NO in the physiology, pathophysiology and pharmacology of the pulmonary circulation.

Discovery of NO and its synthesis in the lungs

In the late 1980s, Furchgott and Zawadzki proposed that the vascular smooth muscle relaxation in rabbit aorta was secondary to the release of a vasodilating substance by the endothelium.[6] Subsequently, they discovered that this vasodilating substance was NO.[7]

Endogenous NO is formed from the amino acid L-arginine through a catalyzed reaction by the NOS family of enzymes, which convert L-arginine into NO and L-citrulline, requiring two cosubstrates: oxygen and reduced nicotinamide adenine dinucleotide.[1]

The three known isoforms of NOS were identified in the lung, two of them being more intensely and constitutively expressed in neuron bodies (nNOS) or in the endothelial cells of pulmonary vessels (eNOS). An inducible isoform (iNOS) is found in alveolar macrophages, and its expression is regulated by inflammatory mediators.[8,9]

The constitutive NOS isoforms (nNOS and eNOS) are regulated by calcium and calmodulin ions, releasing NO in small (nanomolar) quantities in short-duration peaks. Despite this quite limited NO production by constitutive NOS, the existing levels are sufficient to keep the extremely vasodilated baseline systemic vascular tone stable, inhibit the interaction of leukocytes with the endothelium, inhibit platelet aggregation and control cell proliferation.[10]

When the eNOS expression and, consequently, NO baseline levels decrease,[11] or in situations that lead to the consumption of NO by reactive oxygen species (situations of increased oxidative stress),[12] a phenomenon known as endothelial dysfunction occurs. When it affects pulmonary vessels, a series of alterations occur, which are present in certain pathological conditions, one of which is pulmonary arterial hypertension.[13,14]

The iNOS isoform can be induced by inflammatory stimuli.[4]

It is an isoform that is independent of calcium and calmodulin ions, which promotes the release of greater (micromolar) amounts of NO for as long as the aggressive stimulus is present.[1,9] Recent evidence has revealed that NO production is increased in lung diseases accompanied by intense inflammation, such

as ARDS,[15] and that NO derived from iNOS might aggravate such diseases.[16]

NO and the normal pulmonary circulation

The evaluation of the role played by NO in regulating the pulmonary circulation results from studies that used two approaches: the pharmacological inhibition of NOS[1] and the use of knockout animals for each of the three isoforms of NOS.[2]

The isoforms of NOS can be pharmacologically inhibited by L-arginine analogs such as NG-monomethyl-L-arginine (L-NMMA) and NG-nitro-L-arginine-methyl-ester (L-NAME). These analogs act as competitive inhibitors of NOS.[17] Most of the studies that evaluated the effects of these drugs were carried out with animals and led to the conclusion that endogenous NO production does not play an important role in keeping the low tone of pulmonary circulation in basal conditions.[18,19] In the studies cited, the authors showed that, regardless of the route of administration (oral or intravenous) or duration of exposure to NOS inhibitors (acute or chronic), the animals presented significant systemic arterial hypertension, confirming the role of NO in maintaining vascular system homeostasis. However, NOS inhibitors had little effect on pulmonary artery pressures under the same conditions.[18,19] In contrast, a study in humans revealed a significant increase of pulmonary arterial pressure after infusion with a NOS inhibitor.[20] It is of note that this pulmonary hypertensive effect was only observed with very high doses, whereas smaller doses of the same drug produced systemic hypertension with no major effects on the pulmonary circulation.[18,20,21]

In order to evaluate the contribution of each NOS isoform on the control of pulmonary vascular tone and avoid the problem of limited drug selection, the strategy of using animals knocked out for the nNOS, iNOS and eNOS genes was devised.[22] Although this type of approach also presents limitations, studies have shown that only eNOS knockout animals present limited increases in pulmonary artery pressures.[23,24] However, when eNOS knockout animals were submitted to hypoxic conditions, in which pulmonary vessels respond with vasoconstriction, pulmonary hypertension was significantly higher than in control animals. These data suggest that NO is not the only element involved in maintaining low pulmonary vascular tone. However, NO is important in modulating the vasoconstrictive response associated with hypoxia.[22]

Since a complete loss of eNOS activity in pulmonary arterial hypertension is unlikely in humans, the effects of the loss of only one of the eNOS alleles ($eNOS^{+/-}$) was studied. A 50% reduction in eNOS expression in control animals did not have any effect on pulmonary arterial pressure. However, the $eNOS^{+/-}$ animals presented greater increases in hypoxia-induced pulmonary arterial pressure than did control animals.[25] This finding confirms the protective role of NO during pulmonary hypertension.

Therefore, we can conclude that, under normal conditions, NO production in the pulmonary circulation contributes little to maintaining the low pressures observed in this vascular bed. However, the opposite is true under pathophysiological conditions, especially when there is pulmonary arterial hypertension.

Reduced NO synthesis and the pathophysiology of pulmonary arterial hypertension

In patients with pulmonary arterial hypertension, reduced bioavailability of NO has been observed.[24] Some clinical studies have associated pulmonary arterial hypertension with reduced pulmonary levels of eNOS, which would result in decreased synthesis of NO.[11,24] However, other studies have shown unaltered or even increased eNOS expression in the lungs of patients with pulmonary arterial hypertension.[25,26]Nevertheless, this apparent controversy can be explained by methodological differences, such as the selection of patients presenting different stages/severity of pulmonary arterial hypertension. Another possibility is increased activity of arginase II, an enzyme that competes with NOS for the L-arginine substrate, leading to a decrease in NO production in patients with pulmonary arterial hypertension.[24] A decrease in plasma L-arginine levels in patients with pulmonary arterial hypertension is also possible and is likely to contribute to the decreased synthesis of NO in such patients.[27] In addition, plasma concentrations of the endogenous L-arginine analog, asymmetric dimethylarginine, which inhibits both

the transport of L-arginine and the synthesis of NO, seems to be high in patients with idiopathic pulmonary hypertension, correlating positively with mortality.[28] In addition to decreased bioavailability of NO, the expression of phosphodiesterase 5, the principal enzyme responsible for the degradation of cGMP in the pulmonary vascular smooth muscle,[29] has been shown to be increased in patients with pulmonary arterial hypertension.[30] Experimental evidence from various models indicates that the inhibition of NO synthesis caused by NOS inhibiting drugs or occurring in NOS knockout animals promotes more severe pulmonary arterial hypertension than that seen in controls.[17,18] This indicates that endogenous NO production plays a protective role in experimental models of pulmonary arterial hypertension.[17,18] We should bear in mind that the available NOS inhibitors are not selective for each of the NOS isoforms.[9] However, it has been suggested that NO production by eNOS is at least partially responsible for attenuating the intense pulmonary vasoconstriction observed in experimental models of pulmonary arterial hypertension.[17] In addition, when exposed to hypoxia, eNOS knockout animals develop significantly greater pulmonary arterial hypertension than do animals knocked out for the remaining NOS isoforms ($nNOS^{-/-}$ and $iNOS^{-/-}$) or control (wild type) animals.[20,21] These findings suggest that the reduction in NO bioavailability could be a pharmacological target of therapeutic measures aimed at the treatment of pulmonary arterial hypertension.

Paradoxically, the excessive synthesis of NO participates in the pathophysiology of certain lung diseases

Recent experimental data suggest that NO production in greater quantity by iNOS can have harmful effects on the pulmonary circulation under pathological conditions accompanied by intense inflammation, such as ARDS,[14] and under other conditions, such as acute lung injury.[31] Under such conditions, inflammation of the pulmonary vascular endothelium occurs, resulting in noncardiogenic pulmonary edema, pulmonary arterial hypertension, hypoxemia and decreased lung compliance.[32,33] Various studies have implicated excessive iNOS-derived NO production in the worsening of these important clinical conditions.[34] For example, it has been experimentally demonstrated that the pronounced increase of NO concentrations in the pulmonary vessels, in parallel with the increase in concentrations of pulmonary peroxynitrite anion, contribute to the development of pulmonary arterial hypertension during hypoxia.[35] The use of selective iNOS pharmacological inhibitors produce significant reductions in the pulmonary arterial pressure in this model.[31] In addition, in studies of acute lung injury, iNOS knockout animals have been shown to present less oxidative stress and less pulmonary edema when compared to control mice.[36,37] One possible explanation for these findings is that the chemical combination of excessive NO production and the superoxide anion forms peroxynitrite, which is a highly reactive molecule that causes various types of cellular damage.[38] Peroxynitrite reacts with protein tyrosine residues, leading to the formation of 3-nitrotyrosine, which can cause severe functional impairment of lung epithelial cells,[39] as well as promoting the development of pulmonary arterial hypertension.[40] In support of these experimental findings, it has been observed that patients with ARDS present elevated lung concentrations of NO metabolites (nitrite and nitrate) and 3-nitrotyrosine.[14,41] In accordance with these clinical findings, it has also been observed that increased iNOS expression is associated with increased 3-nitrotyrosine concentrations in the lungs in experimental models of acute lung injury.[36] In conjunction, these observations suggest that selective iNOS inhibition can have beneficial effects in the treatment of lung diseases accompanied by inflammation and pulmonary arterial hypertension. However, this idea has not yet been clinically validated.

Therapeutic strategies aimed at avoiding the reduced NO bioavailability observed under conditions of pulmonary arterial hypertension

Although not all relevant mechanisms of the pathophysiology of pulmonary arterial hypertension have yet been clarified, it is known that increased activity of vasoconstrictive substances, remodeling of the pulmonary vascular wall, inflam-

mation and thrombosis play significant roles.[42] Evidence suggests that the subsequent endothelial dysfunction and cellular proliferative stimulus are fundamental to the pathogenesis of pulmonary arterial hypertension.[13] Endothelial dysfunction seems to be associated with the deficient local production of vasodilating substances (especially NO and prostacyclin), as well as with increased release of vasoconstrictors, such as thromboxane A_2 and endothelin.[13,42] Therefore, strategies that provide increased bioavailability of NO or higher intracellular levels of cGMP have great potential as therapeutic alternatives. Dietary supplementation with L-arginine, for example, seems to cause significant reductions in pulmonary arterial pressure in patients with pulmonary hypertension.[27] These effects are quite probably due to the fact that this amino acid is the substrate of NO production by NOS isoforms. In fact, the increased production of endogenous NO can be demonstrated through the increased plasma levels of L-citrulline, one of the final products of the enzymatic NOS reaction.[43] In addition, the acute infusion of L-arginine decreases pulmonary vascular resistance in patients with pulmonary arterial hypertension, which is associated with endogenous NO production.[43] Even in a single-dose regimen, the oral administration of L-arginine caused reductions in pulmonary vascular resistance and mean pulmonary artery pressure in patients with pulmonary arterial hypertension. In addition, its use for a week resulted in significant improvement in physical exercise capacity.[44] Another alternative for the treatment of pulmonary arterial hypertension is inhaled NO, which produces selective and acute vasodilation of the hypertensive pulmonary circulation.[30,45] Inhaled NO relaxes pulmonary vascular smooth muscle, reducing pulmonary vascular resistance and pulmonary arterial pressure.[30] Its chronic use, for approximately nine months, in patients with idiopathic pulmonary arterial hypertension, has also been shown to improve hemodynamics and pulmonary function.[46] In addition, patients who respond to the use of inhaled NO present better five-year survival than do those who do not respond.[47] However, the beneficial effects presented by some authors have not been consistently reproduced in other studies. It is believed that the two principal adverse effects of the use of inhaled NO are the formation of nitric dioxide and the phenomenon of "acute rebound", which occurs after inhalation is interrupted.[35] The mechanisms responsible for the latter effect remain unknown. However, there is evidence that NO has a negative feedback effect when it inhibits eNOS.[48] Another clinically useful therapeutic possibility is treatment with sildenafil. This agent is a potent selective phosphodiesterase 5 inhibitor, which can preserve cGMP pulmonary levels and thereby relax the pulmonary vascular smooth muscle.[49] Recent experimental evidence has shown that sildenafil is a potent and selective pulmonary vasodilator. In addition, it also seems to inhibit vascular remodeling associated with chronic hypoxia.[30] Patients with primary pulmonary hypertension and treated chronically with sildenafil present a reduction in pulmonary arterial pressure.[50] In addition, healthy volunteers with induced hypoxia or exposed to altitude sickness who received oral sildenafil presented less pulmonary hypoxic vasoconstriction than did those who received a placebo. This effect was accompanied by higher cGMP plasma concentrations.[51,52] Supporting these findings, a multicenter study demonstrated the safety and efficacy of this drug when used in patients with symptomatic pulmonary arterial hypertension. A total of 278 patients treated for 12 weeks presented improved pulmonary hemodynamics, decreased pulmonary arterial pressure and improved functional capacity.[53] The effects of inhaled NO can be potentiated when it is used in combination with sildenafil, since both drugs increase cGMP concentrations. It has been recently shown that combined treatment with inhaled NO and sildenafil increases the duration and the magnitude of the inhaled NO-induced pulmonary vasodilation in patients with ventricular insufficiency and pulmonary arterial hypertension.[54]

Perspectives and conclusion

In addition to the drugs mentioned, others have been tested in animal experimentation aiming at avoiding some limitations in the effect of the drugs currently available in the treatment of pulmonary vascular diseases. For example, one compound (BAY 41-2272) was found to stimulate the soluble guanylate cyclase enzyme, independent of NO, and to increase cGMP levels in pulmonary vessels.[55] Administration of BAY 41-2272 has reduced pulmonary arterial hypertension in various experimental models.[55] The limitation of its clin-

ical use is related to the lack of selective effect in the pulmonary vascular bed. In addition, systemic arterial hypotension has been reported in previous studies of this drug. One possible solution to this problem has been recently suggested: the administration of BAY 41-2272 via inhalation, resulting in selective pulmonary vasodilating effects.[56] In addition to the stimulation of soluble guanylate cyclase with BAY 41-2272, sodium nitrite has also demonstrated a vasodilating effect on the hypertensive pulmonary circulation. This seems to be due to the reduction of nitrite to NO under pathophysiological conditions accompanied by acidosis and hypoxemia.[57] Recent experimental studies have shown that the administration of nitrite attenuated pulmonary hypertension, and that these effects were associated with increased NO formation.[58-60] In conclusion, it is evident that NO is a molecule of great importance in the cardiovascular system, including the pulmonary circulation. Various studies have indicated that, under certain pathological conditions, NO pathway signaling in the pulmonary vascular bed is impaired either by its decreased bioavailability or by an exacerbated increase in its synthesis. Therefore, therapeutic strategies that partially activate the L-arginine-NO-cGMP pathway (L-arginine, inhaled NO and sildenafil) have been shown to have positive effects not only in various experimental models but also in clinical studies. Nevertheless, although inhibition of the excessive production of NO by iNOS under certain pathophysiological conditions has been shown to have beneficial effects in experimental studies, it has yet to be clinically validated.

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