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SHORT REVIEW

Endothelial modulation of pulmonary vascular tone

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ABSTRACT: Pulmonary endothelial cells normally synthesize prostacyclin (PGI_2) and nitric oxide (NO), which are both potent vasodilators. Although PGI_2 is largely used to treat patients with severe pulmonary hypertension, its role in the physiology and pathophysiology of the pulmonary circulation is still debated. NO, which is now considered as the endogenous nitrovasodilator, is perhaps more involved than PGI_2 in the mechanisms that modulate pulmonary vascular tone in health and disease. There is evidence to suggest that background release of NO contributes to the normally low pulmonary vascular tone in normoxia. Although there are theoretical grounds to hypothesize that hypoxia reduces the synthesis of NO, lack of the latter does not seem to account for the acute hypoxic pulmonary vasoconstriction. Instead, there is evidence to suggest that NO activity is increased in order to modulate the pulmonary vasopressor response to acute alveolar hypoxia. However, more consistent, concerning the role of NO, are data gathered from studies performed in chronic hypoxic conditions. Both experimental data and studies performed in man demonstrate impairment of NO synthesis and/or release in chronic hypoxic pulmonary hypertension. The impaired NO production, whilst reducing the ability of the pulmonary vasculature to relax, also favours the occurrence of excessive pulmonary vasoconstriction. Lack of NO synthesis might also permit mitogenesis and proliferation of various cell types within the vascular wall.

We hypothesize that functional alterations of pulmonary endothelium are likely to affect both reactivity and growth of pulmonary vessels. In this respect, NO probably has a pivotal role in modulating pulmonary vascular tone and controlling pulmonary vascular remodelling in health and disease.
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Pulmonary hypertension often occurs in end-stage chronic obstructive lung disease (COLD), thereby worsening the prognosis of this condition. Indeed, the higher the pulmonary arterial pressure, the poorer the prognosis [1]. Although prolonged alveolar hy-poxia is certainly a major contributing factor for pulmonary hypertension to develop in COLD patients, the underlying mechanisms of the increase in pulmonary arterial pressure still remain uncertain [2]. What is known, however, from detailed histopathological studies [3–5], is that the intima of pulmonary vessels is seldom unscathed by chronic alveolar hypoxia. It is also known that the intima and its main cell type - the endothelium - can no longer be considered as just a simple layer of cells that interposes a physical barrier between the underlying vascular smooth muscle and the circulating blood [6]. Indeed, since the discovery of prostacyclin (PGI_2) by MONCADA *et al.* [7], and the so-called endothelium-derived relaxing factor (EDRF) by FURCHGOTT and ZAWADZKI [8], there is increasing evidence to suggest a fundamental role of endothelium in the modulation of vasomotor tone in health and disease.

Endogenous vasodilators synthesized by the pulmonary endothelium

Prostacyclin

PGI_2 is a powerful pulmonary vasodilator and is currently used to treat patients with severe pulmonary hypertension, especially those with primary pulmonary hypertension [9]. The role of PGI_2 in the pathophysiology of chronic pulmonary hypertension remains, however, unclear. Pulmonary hypertension induced by chronic hypoxia in neonatal calves is associated with reduced pulmonary artery production of PGI_2 [10]. By contrast, PGI_2 production is increased in the endothelium and vascular smooth muscle of pulmonary arteries from rats with chronic hypoxic pulmonary hypertension [11]. Whether these contradictory results are species-dependent (rats *versus* calves) or age-related (neonates *versus* adult animals) requires further investigation. Nevertheless, it seems unlikely that PGI_2 has a major role in the mechanisms that maintain a low pulmonary resistance during exercise, a major physiological adaptation process of the pulmonary circulation in response to increased blood flow [12].

Nitric oxide

Increased blood flow, through the shear stress it generates on the luminal surface of endothelial cells, is precisely one of the factors causing the release of the second major endogenous vasodilator synthesized by the endothelium, namely EDRF [13]. Discovered in 1980 by FURCHGOTT and ZAWADZKI [8], EDRF is now identified with either the free radical nitric oxide (NO) [14], or a nitroso compound which ultimately releases NO [15]. The molecular target of either form is the soluble enzyme guanylate cyclase. Stimulation of the latter by NO increases the level of the second messenger cyclic guanosine monophosphate (cGMP) within vascular smooth muscle, thereby causing vasorelaxation [16] (fig. 1). The nitrogen atom of NO derives from the N-guanidino terminal of the amino acid, L-arginine [17], whereas, recent evidence suggests, that the oxygen atom is provided by molecular oxygen (O_2) [18] (fig. 1). NO is synthesized from these two precursors by the enzyme NO synthase [19], which exists in at least two isoforms, a constitutive and an inducible one [20]. The constitutive NO synthase is thought to be important in the modulation of vascular

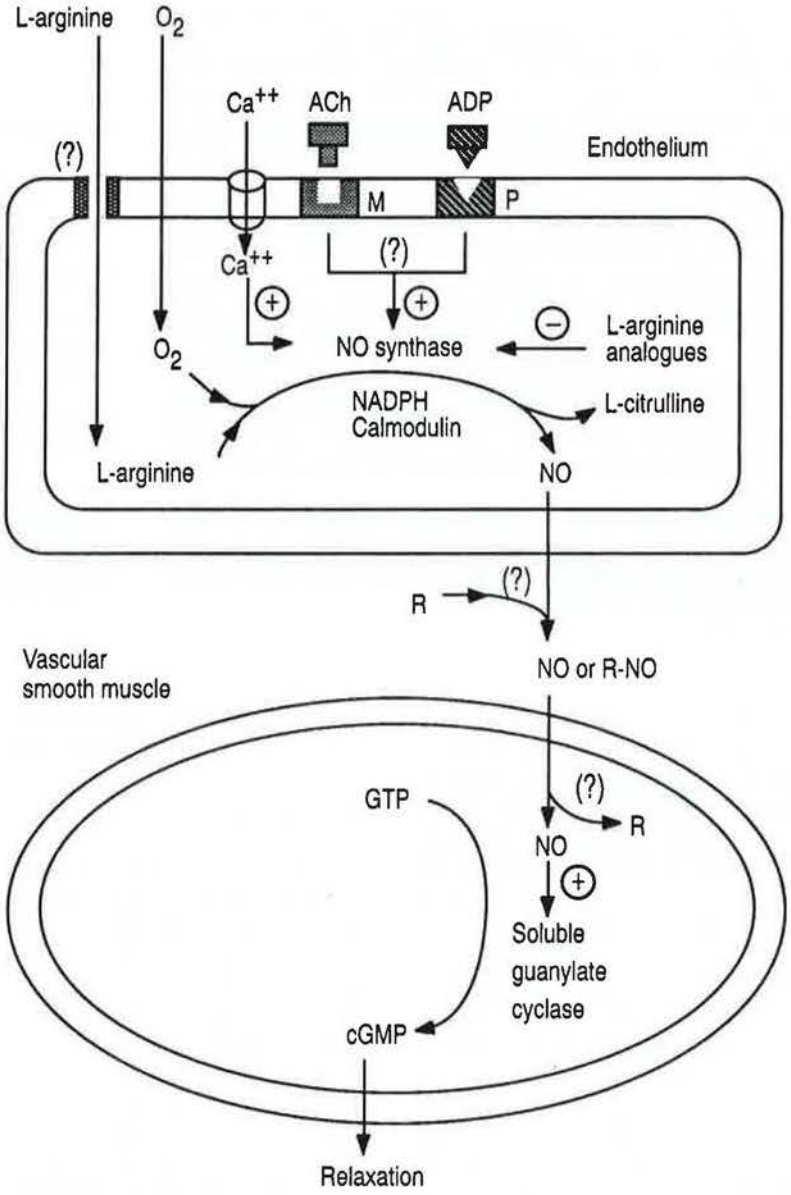

Fig. 1. – Endothelial biosynthetic pathway and action of NO on vascular smooth muscle. Stimulation of specific endothelial receptors, *e.g.* muscarinic (M) and purinergic (P) receptors, activates the endothelial enzyme, NO synthase. The latter could also be activated by a rise in cytosolic calcium (Ca^{++}). NO synthase forms NO and L-citrulline from L-arginine and molecular oxygen (O_2). This synthesis requires the presence of co-factors (NADPH, calmodulin), and is competitively inhibited by L-arginine analogues. NO is released from endothelium either as a free radical or combined to a putative carrier molecule (R). The free radical NO activates the vascular smooth muscle soluble enzyme, guanylate cyclase, increasing cGMP level and thereby causing relaxation. ACh: acetylcholine; ADP: adenosine diphosphate; GTP: guanosine triphosphate; cGMP: cyclic guanosine monophosphate; NADPH: reduced nicotinamide adenine dinucleotide phosphate. The question marks (?) reflect mechanisms which are, as yet, uncertain.

tone [20], whereas the inducible enzyme is involved in the cytotoxic activity of the macrophage [20]. The synthesis of NO is stereospecifically inhibited by various L-arginine analogues, which act as competitive inhibitors of different forms of the NO synthase [20] (fig. 1).

As compared with the systemic circulation [21], studies of endothelium-dependent relaxation mediated by NO in the pulmonary vascular bed are relatively scarce [22]. Nevertheless, sufficient evidence has emerged in recent years to allow a preliminary assessment of the role of NO in the modulation of pulmonary vascular tone in health and disease. Endothelium-dependent relaxation resulting from EDRF (NO) release has been found in isolated pulmonary arteries from most mammalian species [23–26], including humans [27–30]. In patients without pulmonary hypertension who undergo lobectomy for lung carcinoma, release of NO in response to various endothelium-dependent vasodilators is, in most cases, sufficient to fully relax precontracted pulmonary vascular rings [27–30]. These *in vitro* potent pulmonary vasorelaxant effects of endothelium-dependent vasodilators, such as acetylcholine, are consistent with *in vivo* studies [31], and provide a cellular and molecular basis for their well-known pulmonary vasodilator action, which was first described more than 30 yrs ago [32].

Physiology and pathophysiology of nitric oxide in the pulmonary circulation

Once sufficient evidence suggesting that NO is a potent endogenous pulmonary vasorelaxant paracrine substance is gathered, three questions rapidly emerge as to its role in health and pulmonary vascular disease. Firstly, is background release of NO involved in the mechanisms that maintain a normally low pulmonary vascular tone at rest? Secondly, is acute hypoxic pulmonary vasoconstriction due to impairment of this release? Thirdly, is NO synthesis and/or release impaired in chronic hypoxic pulmonary hypertension?

Nitric oxide and basal normoxic pulmonary vascular tone

To date, contradictory results make it difficult to definitely answer the first question. In isolated vascular rings from various species, of different sizes ranging from resistance to conduit pulmonary arteries, removal of NO production, either by mechanical means (by rubbing the intimal surface) [33, 34] or biochemical means (by pretreating with L-arginine analogues) [30, 35], consistently results in a significantly greater response to vasoconstrictor stimuli [30, 33–35]. This suggests the existence of a braking mechanism, which readily triggers the release of NO to counteract any rise in pulmonary vascular tone. Whether background release of NO also prevails *in vivo* to account for the low pulmonary vascular tone at rest is still unclear. In isolated perfused lungs of rats, inhibition of NO activity by methylene blue [36] or the L-arginine analogue, N^G-monomethyl-L-arginine [37], has no effect on resting perfusion pressure in normoxic conditions. By contrast, methylene blue [38] and the L-arginine analogue, N^ω-nitro-L-arginine [39], significantly increase pulmonary vascular resistance in perfused lungs from humans [38] and rabbits [39], respectively. Despite these apparent contradictory results, it is tempting to speculate that baseline release of NO is probably important to maintain a low pulmonary vascular tone in man [38], an effect prevailing in some [39], but not all, mammalian species [36, 37].

Nitric oxide and acute hypoxic pulmonary vasoconstriction

Better consensus seems to exist as to the answer to the second question, evaluating the role of NO in acute hypoxic pulmonary vasoconstriction. Indeed, irrespective of the species or type of inhibitors [36, 37, 39–42], it is consistently found that inhibition of NO synthesis markedly enhances the pulmonary pressor response to acute hypoxic challenges [36, 37, 39–42]. These results not only rule out the hypothesis of a blunted NO release as the direct cause of hypoxic pulmonary vasoconstriction, they also suggest that NO activity is in fact increased during acute hypoxia. This increased activity probably represents an important physiological defence mechanism, enabling the pulmonary vascular bed to limit excessive vasoconstriction during hypoxia. Instead, these observations suggest that NO probably modulates pulmonary vascular tone at rest and during acute alveolar hypoxia.

Nitric oxide and chronic hypoxic pulmonary hypertension

A step which naturally follows brings us to the third question concerning NO synthesis and/or release during chronic hypoxic pulmonary hypertension, especially in conditions associated with chronic alveolar hypoxia. Endothelium-dependent relaxation to acetylcholine of either isolated pulmonary arterial rings [43], or perfused lungs [44], is markedly reduced in rats with chronic hypoxic pulmonary hypertension as compared with normoxic animals. This altered vasoreactivity is specifically due to endothelial dysfunction as the pulmonary vasorelaxant response to sodium nitroprusside, a vasodilator acting directly on vascular smooth muscle, is not affected by chronic hypoxia [43, 44]. These experimental results are consistent with those from a series of studies using human tissues [34, 35, 45, 46]. Indeed, endothelium-dependent relaxation is markedly impaired in isolated pulmonary arterial rings from patients undergoing heart-lung transplantation for end-stage COLD [34, 35, 45, 46] as compared with control patients. It is likely that this impaired relaxation is due to reduced NO synthesis and/or release and that the

latter results from impaired activity of the constitutive enzyme, NO synthase, during hypoxia. Firstly, this stems from the observations that animals and humans *in vivo* exhale NO and that this NO excretion is reduced during hypoxia [47]. Secondly, *in vitro* NO synthase activity is markedly decreased by hypoxia [48] which, in turn, results in reduced endothelium-dependent relaxation.

on the other, it is unlikely that thickening of the intima and/or the media directly alters the vascular response to NO. Rather, on the basis of recent evidence suggesting that NO and NO-generating vasodilators exert an inhibitory effect on mitogenesis and cell proliferation [49], it is tempting to speculate that reduced NO production has a dual, and parallel, effect on pulmonary vasoreactivity and vascular remodelling. Lack of NO

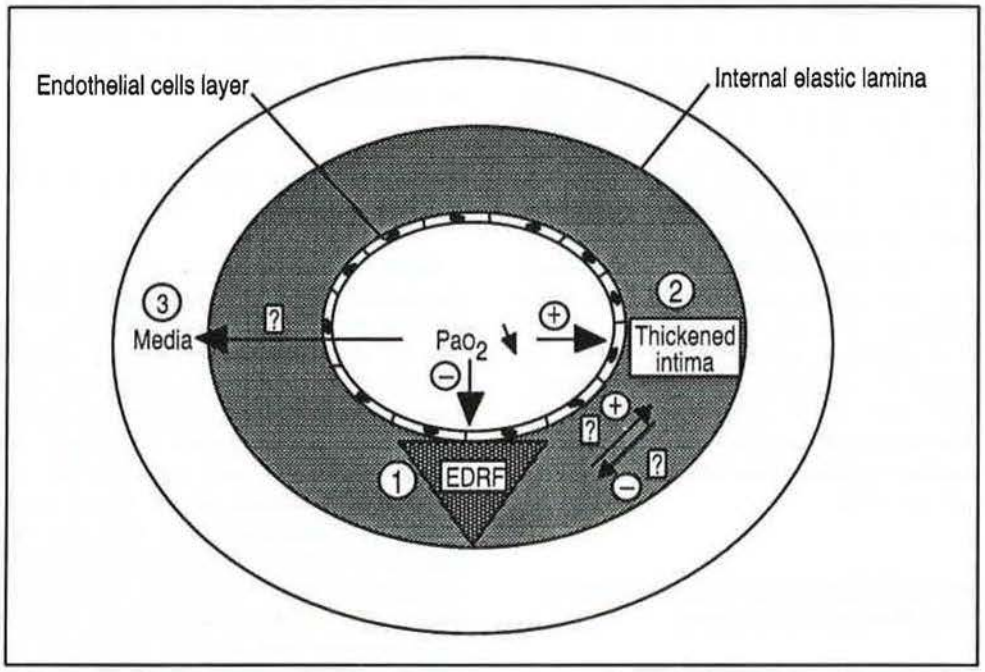

Fig. 2. – Putative actions of chronic alveolar hypoxia causing hypoxaemia on reactivity and structural changes of pulmonary vessels. Low levels of arterial oxygen tension (Pao_2) decrease the synthesis and/or release of EDRF (NO) from the endothelium (1). This in turn causes a rise in pulmonary vascular tone and thickening of the intima (2) through, as yet, undefined mechanisms (small arrows and question marks). At a later stage, proliferation and phenotypic changes of smooth muscle cells in the media might occur as a result of chronic defect of EDRF (NO) (3). EDRF: endothelium-derived relaxing factor.

The impaired relaxation is associated with an exaggerated contractile response of rings from COLD patients to the alpha-adrenergic agonist phenylephrine [34, 35]. Removal of endothelial production of NO eliminates this difference, increasing the tension in control rings but not in rings from COLD patients [34, 35]. This suggests that the normal release of NO to brake the vasoconstrictor effects of phenylephrine, whilst being effective in reducing the rise in tension in control rings, is lacking in rings from COLD patients, thus explaining the greater response to phenylephrine in the latter as compared with the former [34, 35]. This further indicates that reduced NO production not only impairs relaxation but also leads to the occurrence of excessive vasoconstriction in the pulmonary vascular bed of COLD patients.

The reduced endothelium-dependent relaxation is related to structural changes affecting the intima and the media of pulmonary vascular rings from COLD patients [34]. In other words, the less the endothelium-dependent relaxation, the more the structural alterations. As the vasorelaxant response to sodium nitroprusside is normal on the one hand, and NO is highly diffusible

causes a rise in pulmonary vascular tone through mechanisms which are already discussed, and favours remodelling of pulmonary vessels by facilitating proliferation and phenotypic changes of cells of the media and intima [50] (fig. 2).

Future prospects

That NO is a potent endogenous nitrovasodilator of the systemic circulation is certainly no longer questionable [20, 21]. Interestingly, NO possibly also reduces smooth muscle tone in the bronchial wall where soluble guanylate cyclase is present [51]. Several laboratories are now making an effort to redefine the role of NO in the pulmonary circulation [30, 33, 34, 37, 39, 40, 43, 44]. For the future, there are perhaps two different, but equally fascinating, directions for investigators to pursue. Firstly, to search for the cause(s) of impaired NO synthesis. This will require increasing use of molecular biological techniques applied to studies of human disease in preference to those of experimental conditions. Secondly, to better define the role of NO

as a therapeutic means on the basis of preliminary results suggesting that inhaled NO is both an effective and selective pulmonary vasodilator [52, 53]. This will demand from physicians a more thorough knowledge about the toxicity and long-term tolerance effect of this single, but far from simple, free radical.

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