

Endothelial modulation of pulmonary vascular tone

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ABSTRACT: Pulmonary endothelial cells normally synthesize prostacyclin (PGI₂) and nitric oxide (NO), which are both potent vasodilators. Although PGI₂ 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 PGI₂ 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 hypoxia 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 (PGI₂) 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

PGI₂ 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 PGI₂ 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 PGI₂ [10]. By contrast, PGI₂ 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 PGI₂ 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].

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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

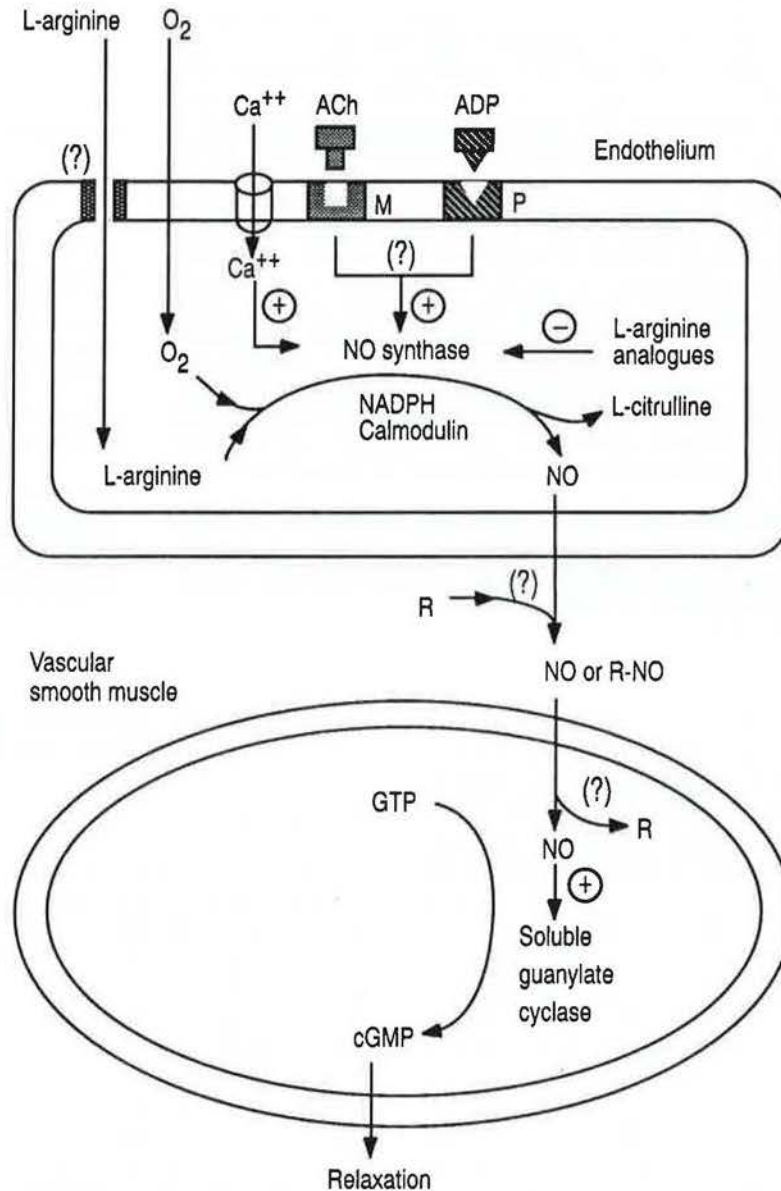


Fig. 1. - Endothelial biosynthetic pathway and action of NO on vascular smooth muscle. Stimulation of specific endothelial receptors, e.g. muscarinic (M) and purinergic (P) receptors, activates the endothelial enzyme, NO synthase. The latter could also be activated by a rise in cytosolic calcium (Ca^{++}). 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

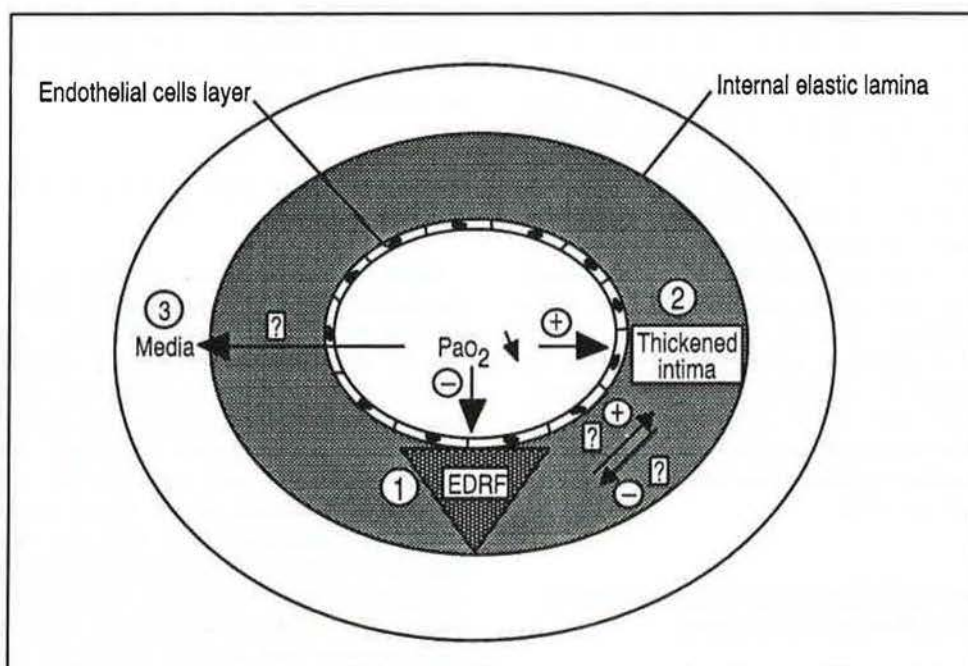


Fig. 2. - Putative actions of chronic alveolar hypoxia causing hypoxaemia on reactivity and structural changes of pulmonary vessels. Low levels of arterial oxygen tension (P_{aO_2}) decrease the synthesis and/or release of EDRF (NO) from the endothelium (1). This in turn causes a rise in pulmonary vascular 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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References

- Weitzenblum E, Hirth C, Ducolone A, Mirhom R, Rasaholinjanahary J, Ehrhart M. - Prognostic value of pulmonary artery pressure in chronic obstructive pulmonary disease. *Thorax*, 1981; 36: 752-758.
- Reeves JT, Voelkel NF. - Mechanisms of chronic pulmonary hypertension: basic considerations. In: Wagenvoort CA, Denolin H, eds. *Pulmonary circulation: advances and controversies*. Amsterdam, Elsevier, 1989; pp. 27-39.
- Meyrick B, Reid L. - The effect of continued hypoxia on rat pulmonary arterial circulation: an ultrastructural study. *Lab Invest*, 1978; 38: 188-200.
- Magee F, Wright JL, Wiggs BR, Paré PD, Hogg JC. - Pulmonary vascular structure and function in chronic obstructive pulmonary disease. *Thorax*, 1988; 43: 183-189.
- Wilkinson W, Langhorne CA, Heath D, Barer GR, Howard P. - A pathophysiological study of 10 cases of hypoxic cor pulmonale. *Q J Med*, 1988; 66: 68-85.
- Dinh-Xuan AT, Higenbottam TW. - Non-prostanoid endothelium-derived vasoactive factors. *J Int Med Res*, 1989; 17: 305-315.
- Moncada S, Glycowski R, Bunting S, Vane JR. - An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*, 1976; 263: 663-665.
- Furchgott RF, Zawadzki JV. - The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, 1980; 288: 373-376.
- Dinh-Xuan AT, Higenbottam TW, Scott JP, Wallwork J. - Primary pulmonary hypertension: diagnosis, medical and surgical treatment. *Respir Med*, 1990; 84: 189-197.
- Badesch DB, Orton EC, Zapp LM, Westcott JY, Hester J, Voelkel NF, Stenmark KR. - Decreased arterial wall prostaglandin production in neonatal calves with severe chronic pulmonary hypertension. *Am J Respir Cell Mol Biol*, 1989; 1: 489-498.
- Shaul PW, Kinane B, Farrar MA, Buja M, Magness RR. - Prostacyclin production and mediation of adenylate cyclase activity in the pulmonary artery: alterations after prolonged hypoxia in the rat. *J Clin Invest*, 1991; 88: 447-455.
- Lindenfield J, Reeves JT, Horwitz LD. - Low exercise pulmonary resistance is not dependent on vasodilator prostaglandins. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1983; 55: 558-561.
- Rubanyi GM, Romero JC, Vanhoutte PM. - Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol*, 1986; 250: H1145-H1149.
- Palmer RMJ, Ferrige AG, Moncada S. - Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, 1987; 327: 524-526.
- Myers PR, Minor RL Jr, Guerra R Jr, Bates JN, Harrison DG. - Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature*, 1990; 345: 161-163.
- Murad F. - Cyclic guanosine monophosphate as a mediator of vasodilation. *J Clin Invest*, 1986; 78: 1-5.
- Palmer RMJ, Ashton DS, Moncada S. - Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, 1988; 333: 664-666.
- Kwon NS, Nathan CF, Gilker C, Griffith OW, Mathews DE, Stuehr DJ. - L-citrulline production from L-arginine by macrophage nitric oxide synthase: the ureido oxygen derives from dioxygen. *J Biol Chem*, 1990; 265: 13442-13445.
- Palmer RMJ, Moncada S. - A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem Biophys Res Commun*, 1989; 158: 348-352.
- Moncada S, Palmer RMJ, Higgs EA. - Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev*, 1991; 43: 109-142.
- Vane JR, Ånggård EE, Botting RM. - Regulatory functions of the vascular endothelium. *N Engl J Med*, 1990; 323: 27-36.
- Cremona G, Dinh-Xuan AT, Higenbottam TW. - Endothelium-derived relaxing factor and the pulmonary circulation. *Lung*, 1991; 169: 185-202.
- Chand N, Altura BM. - Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: role in lung vascular diseases. *Science*, 1981; 213: 1376-1379.
- Ignarro LJ, Burke TM, Wood KS, Wolin MS, Kadowitz PJ. - Association between cyclic GMP accumulation and acetylcholine-elicited relaxation of bovine intrapulmonary artery. *J Pharmacol Exp Ther*, 1984; 228: 682-690.
- Satoh H, Inui J. - Endothelial cell-dependent relaxation and contraction induced by histamine in the isolated guinea-pig pulmonary artery. *Eur J Pharmacol*, 1984; 97: 321-324.
- Tanaka DT, Grunstein MM. - Vasoactive effects of substance P on isolated rabbit pulmonary artery. *J Appl Physiol*, 1985; 58: 1291-1297.
- Thom S, Hughes A, Martin G, Sever PS. - Endothelium-dependent relaxation of isolated human arteries and veins. *Clin Sci*, 1987; 73: 547-552.
- Greenberg B, Rhoden K, Barnes PJ. - Endothelium-dependent relaxation of human pulmonary arteries. *Am J Physiol*, 1987; 252: H434-H438.
- Dinh-Xuan AT, Higenbottam TW, Clelland CA, Pepke-Zaba J, Wells FC, Wallwork J. - Acetylcholine and adenosine diphosphate cause endothelium-dependent relaxation of isolated human pulmonary arteries. *Eur Respir J*, 1990; 3: 633-638.
- Crawley DE, Liu SF, Evans TW, Barnes PJ. - Inhibitory role of endothelium-derived relaxing factor in rat and human pulmonary arteries. *Br J Pharmacol*, 1990; 101: 166-170.
- Harris P, Heath D. - Pharmacology of the pulmonary circulation. In: Harris P, Heath D, eds. *The human pulmonary circulation: its form and function in health and disease*. Third edn. Edinburgh, Churchill Livingstone, 1986; pp. 183-209.
- Fritts HW Jr, Harris P, Clauss RH, Odell JE, Courmand A. - The effect of acetylcholine on the human pulmonary circulation under normal and hypoxic conditions. *J Clin Invest*, 1958; 37: 99-108.
- Yamaguchi T, Rodman DM, O'Brien RF, McMurtry IF. - Modulation of pulmonary artery contraction by

- endothelium-derived relaxing factor. *Eur J Pharmacol*, 1989; 161: 259-262.
34. Dinh-Xuan AT, Higenbottam TW, Clelland CA, Pepke-Zaba J, Cremona G, AY Butt, Large SR, Wells FC, Wallwork J. - Impairment of endothelium-dependent pulmonary-artery relaxation in chronic obstructive lung disease. *N Engl J Med*, 1991; 324: 1539-1547.
35. Dinh-Xuan AT, Pepke-Zaba J, Higenbottam TW. - Impairment of nitric oxide production in pulmonary arteries from patients with chronic hypoxic pulmonary hypertension: putative mechanisms and pathophysiological implications. In: Moncada S, Marletta MA, Hibbs JB Jr, Higgs EA, eds. *Biology of nitric oxide*. London, Portland Press, 1992; (in press).
36. Mazmanian GM, Baudet B, Brink C, Cerrina J, Kirkiacharian S, Weiss M. - Methylene blue potentiates vascular reactivity in isolated rat lungs. *J Appl Physiol*, 1989; 66: 1040-1045.
37. Archer SL, Tolins JP, Raji L, Weir EK. - Hypoxic pulmonary vasoconstriction is enhanced by inhibition of the synthesis of an endothelium-derived relaxing factor. *Biochem Biophys Res Commun*, 1989; 164: 1198-1205.
38. Cremona G, Higenbottam TW, Dinh-Xuan AT, Wells FC, Large SR, Stewart S, Wallwork J. - Inhibitors of endothelium-derived relaxing factor increase pulmonary vascular resistance in isolated perfused human lungs. *Eur Respir J*, 1991; 4 (Suppl. 14): 336s, (Abstract).
39. Persson MG, Gustafsson LE, Wiklund NP, Moncada S, Hedqvist P. - Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response *in vivo*. *Acta Physiol Scand*, 1990; 140: 449-457.
40. Robertson BE, Warren JB, Nye PC. - Inhibition of nitric oxide synthesis potentiates hypoxic vasoconstriction in isolated rat lungs. *Exp Physiol*, 1990; 75: 255-257.
41. Brashers VL, Peach MJ, Rose CE Jr. - Augmentation of hypoxic pulmonary vasoconstriction in the isolated perfused rat lung by *in vitro* antagonists of endothelium-dependent relaxation. *J Clin Invest*, 1988; 82: 1495-1502.
42. Liu SF, Crawley DE, Barnes PJ, Evans TW. - Endothelium-derived relaxing factor inhibits hypoxic pulmonary vasoconstriction in rats. *Am Rev Respir Dis*, 1991; 143: 32-37.
43. Leach RM, Twort CHC, Ward JPT, Cameron IR. - Endothelial dysfunction in isolated pulmonary arteries from chronically hypoxic rats. *Thorax*, 1990; 45: 808, (Abstract).
44. Adnot S, Raffestin B, Eddahibi S, Braquet P, Chabrier PE. - Loss of endothelium-dependent relaxant activity in the pulmonary circulation of rats exposed to chronic hypoxia. *J Clin Invest*, 1991; 87: 155-162.
45. Dinh-Xuan AT, Higenbottam TW, Pepke-Zaba J, Clelland CA, Wallwork J. - Reduced endothelium-dependent relaxation of cystic fibrosis pulmonary arteries. *Eur J Pharmacol*, 1989; 163: 401-403.
46. Dinh-Xuan AT, Higenbottam TW, Wallwork J. - Relationship between chronic hypoxia and *in vitro* pulmonary relaxation mediated by endothelium-derived relaxing factors in human chronic obstructive lung disease. *Angiology*, 1992; 43: (in press).
47. Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. - Endogenous nitric oxide is present in the exhaled air of rabbits, guinea-pigs and humans. *Biochem Biophys Res Commun*, 1991; 181: 852-857.
48. Rengasamy A, Johns R. - Inhibition of EDRF/NO synthase by hypoxia. *FASEB J*, 1991; 5: A1418, (Abstract).
49. 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; 83: 1774-1777.
50. Dinh-Xuan AT, Cremona G, Higenbottam TW. - Endothelial dysfunction and remodelling of the pulmonary circulation in chronic hypoxic pulmonary hypertension. *Appl Cardiopulmon Pathophysiol*, 1992; 6: (in press).
51. Morrison KJ, Gao Y, Vanhoutte PM. - Epithelial modulation of airway smooth muscle. *Am J Physiol*, 1990; 258: L254-L262.
52. Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM. - Inhaled nitric oxide, a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation*, 1991; 83: 2038-2047.
53. Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J. - Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. *Lancet*, 1991; 338: 1173-1174.