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Impairment of pulmonary-artery endothelium-dependent relaxation in chronic obstructive lung disease is not due to dysfunction of endothelial cell membrane receptors nor to L-arginine deficiency

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1 Endothelium-dependent relaxation mediated by endothelium-derived relaxing factor (EDRF) or nitric oxide (NO), is impaired in pulmonary arteries (PA) of hypoxic patients with chronic obstructive lung disease (COLD). To determine the mechanisms responsible for this impairment, we compared the response of rings of isolated PA from 12 COLD patients and 8 controls to the endothelium-dependent vasodilators acetylcholine (ACh), adenosine diphosphate (ADP), and the calcium ionophore, A23187. The response of PA rings to the endothelium-independent nitro-vasodilator sodium nitroprusside (SNP) was also studied in both groups. The PA rings had been pre-contracted by the α-adrenoceptor agonist phenylephrine (PE).

2 Endothelium-dependent relaxation was significantly reduced in PA rings from COLD patients as compared with controls when tested with ACh (37.8 ± 8.8% vs 73.4 ± 7.9%), ADP (38.4 ± 6.7% vs 80 ± 5.6%), and the calcium ionophore, A23187 (35.8 ± 6.1% vs 87 ± 6.6%). Relaxation with SNP was, however, significantly greater in PA rings from COLD patients (99.4 ± 0.6% vs 90.3 ± 3.1%), as was the contractile response to PE (1.91 ± 0.21 g vs 1.33 ± 0.15 g). Pretreatment with the specific inhibitor of NO formation, N^G-monomethyl-L-arginine (L-NMMA; 10^{-4} M) significantly reduced the relaxation to ACh in all PA rings. This inhibition could be reversed by L-arginine (10^{-3} M), the substrate for NO synthesis. Pretreatment with L-arginine alone, however, did not restore the impaired endothelium-dependent relaxation of PA rings from COLD patients.

3 We conclude that EDRF (NO) production is impaired in PA rings from COLD patients and that this impairment is neither due to endothelial receptors dysfunction nor a defect of L-arginine availability and/or transport. Our hypothesis is that the abnormality must lie within the biosynthesis pathway of NO from L-arginine, possibly involving the endothelial enzyme cell, NO synthase, the normal function of which might be altered by chronic hypoxia.

Keywords: Nitric oxide; L-arginine; pulmonary hypertension; endothelial cell membrane receptors; nitrovasodilators

Introduction

Pulmonary hypertension often develops in patients with end-stage chronic obstructive lung disease (COLD) as a result of structural alterations of lung vessels and increased pulmonary vascular reactivity (Reeves & Voelkel, 1989). Remodelling of the intima is among the early markers of pulmonary vascular disease in COLD patients (Magee *et al.*, 1988). The endothelial cells, one of the main cell types of the intima, normally synthesize and release the powerful endothelium-derived relaxing factor (EDRF) (Furchgott & Zawadzki, 1980), now identified as nitric oxide (NO) (Palmer *et al.*, 1987). NO is formed from L-arginine (Palmer *et al.*, 1988), by a cytosolic enzyme, called NO synthase (NOS) (Palmer & Moncada, 1989). This enzyme exists in different isoforms including an inducible and a constitutive one (Moncada *et al.*, 1991). The latter is the predominant form in endothelial cells. The formation of NO can be stereospecifically inhibited by the L-arginine analogue, N^G-monomethyl-L-arginine (L-NMMA) (Rees *et al.*, 1989), which has been used to study the physiological roles of NO (Moncada *et al.*, 1991; Vane *et al.*, 1990).

NO modulates pulmonary vascular tone in man in both disease (Dinh-Xuan *et al.*, 1989; 1990a; 1991) and health (Dinh-Xuan *et al.*, 1990b). We found that relaxation of pulmonary arteries in response to the endothelium-dependent vasodilators, acetylcholine (ACh) and adenosine diphosphate (ADP), is impaired in patients with pulmonary hypertension in Eisenmenger's syndrome and COLD, including cystic fibrosis. This impairment could be due to altered function of muscarinic receptors and purinoceptors, or to endothelial cell dysfunction which is beyond the receptor-agonist coupling mechanism. Alternatively, it could result from a deficiency of L-arginine, a substrate for NO synthesis. To assess which mechanism is responsible, we report studies of the relaxation of isolated pulmonary arteries from COLD patients in response to various endothelium-dependent vasodilators, including the calcium ionophore, A23187. The latter is known to induce the release of NO non-specifically, without involving endothelial cell membrane receptors stimulation. We also report the effect of pretreatment *in vitro* with L-arginine on the impaired pulmonary endothelium-dependent relaxation in these patients. The aim of this study was to determine whether dysfunction of endothelial cell membrane receptors, substrate deficiency, or abnormal NOS activity, causes the impaired pulmonary endothelium-dependent relaxation in COLD patients.

Methods

Subjects

Isolated pulmonary arterial (PA) rings were studied from the explanted lungs of 12 patients undergoing lung transplantation for end-stage COLD (Higenbottam *et al.*, 1990). Control pulmonary arterial rings were obtained from 8 patients undergoing lobectomy or pneumonectomy for lung car-

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Table 1 Clinical characteristics and preoperative lung function and arterial blood gas values of the study groups

Diagnosis	*Age*	*Sex*	FEV_1*	*FVC** litres (% of predicted)	*RV**	Pa_{O_2}	Pa_{CO_2} kPa†
COLD patients							
Emphysema	47	F	0.5 (17)	2.8 (76)	5.3 (279)	8.7	7.1
Emphysema	56	F	0.2 (09)	0.8 (27)	8.5 (472)	NA*	NA
Cystic fibrosis	27	F	0.5 (18)	1.2 (37)	3.8 (345)	8.6	5.6
Cystic fibrosis	33	M	1.3 (33)	1.7 (35)	3.6 (212)	9.0	5.1
Cystic fibrosis	24	F	0.7 (21)	1.5 (41)	2.3 (209)	7.7	4.5
Cystic fibrosis	29	F	0.5 (16)	1.2 (33)	4.3 (307)	8.0	5.5
Bronchiectasis	48	M	0.3 (08)	1.3 (27)	7.2 (351)	6.1	6.6
Emphysema	56	M	0.2 (07)	1.3 (27)	7.8 (380)	3.5	6.6
Cystic fibrosis	30	F	0.6 (24)	1.6 (52)	2.6 (236)	9.6	5.7
Cystic fibrosis	28	M	0.6 (16)	1.3 (29)	4.3 (287)	6.8	7.5
Cystic fibrosis	41	M	0.5 (13)	2.4 (48)	6.1 (313)	6.6	5.2
Emphysema	54	M	0.4 (10)	4.0 (79)	7.4 (315)	7.8	6.1
Control patients							
§Lung carcinoma	62	M	2.7 (83)	3.3 (80)	2.7 (115)	11.7	5.2
§Lung carcinoma	53	F	2.2 (92)	2.9 (98)	2.0 (118)	NA	NA
§Lung carcinoma	55	M	2.3 (74)	3.9 (98)	3.0 (102)	11.4	5.1
§Lung carcinoma	50	M	2.9 (78)	3.7 (79)	2.2 (102)	10.9	5.6
Lung carcinoma	56	F	2.4 (98)	3.6 (118)	2.1 (117)	11.8	5.3
§Lung carcinoma	67	M	3.1 (102)	4.5 (115)	3.4 (136)	11.2	5.2
§Lung carcinoma	74	M	2.2 (81)	2.8 (80)	3.0 (115)	12.8	5.0
Lung carcinoma	63	F	2.2 (116)	3.1 (132)	2.2 (133)	12.3	5.3

*FEV_1 denotes forced expiratory volume in one second, FVC, forced vital capacity; RV, residual volume; NA, data not available.
†To convert values for Pa_{O_2} and Pa_{CO_2} to mmHg multiply by 7.5.
§Smoker ($\geq$10 pack years).

cinoma who did not have evidence of other chronic lung disease. Their individual diagnoses, anthropometric data, and pre-operative lung function tests together with blood gas measurements are listed in Table 1.

In vitro *study*

Tissue preparation Details of the preparation of PA rings have been described (Dinh-Xuan *et al.*, 1990b; 1991). In brief, the excised lung was immediately placed in Krebs-Ringer bicarbonate solution at 4°C which had been pregassed with 95% O_2 and 5% CO_2. Pairs of rings were obtained from lobar, segmental and subsegmental pulmonary arteries which had been carefully dissected and cleaned of excess connective tissue. The endothelium was carefully removed from some rings with a pipe cleaner. All pulmonary arterial rings obtained from the same patient were similar in size (range of external diameter, 1.3 to 2.2 mm), and were cut from adjacent parts of the middle of pulmonary arterial branches.

Cumulative dose-relaxation studies The PA rings were studied in 20 ml organ baths filled with Krebs-Ringer bicarbonate buffer, bubbled continuously with 95% O_2 and 5% CO_2, and kept at 37°C by an outer water bath warmed by a recirculating heater (Circulator C-400, Techne Ltd., Cambridge, U.K.). Changes in isometric tension were recorded in each ring preset at its optimal length for tension development by a force transducer (Category No. 52-9529, Harvard Bioscience, Massachussets, U.S.A.) connected to a chart drive recorder (PM 8252A, Philips, Eindhoven, The Netherlands). The rings were then allowed to equilibrate in the bath for at least 90 min during which time the fluid in the bath was changed every 15 min. After equilibration, all rings were preincubated for 30 min with indomethacin (10^{-5} M) which remained in the bath throughout the studies. All rings were submaximally precontracted with phenylephrine dichloride (PE) (10^{-6} M) to obtain a stable plateau of increased tension. Cumulative concentrations of the endothelium-dependent vasodilators, acetylcholine (ACh; 10^{-10} to 10^{-5} M), adenosine diphosphate (ADP; 10^{-10} to 10^{-5} M), and the calcium ionophore A23187 (10^{-8} to 10^{-3} M) were then added to the rings which were studied either with or without endothelium. Relaxation to cumulative doses of the endothelium-independent vasodilator, sodium nitroprusside (SNP; 10^{-8} to 10^{-4} M), was also studied in intact PA rings from both patient groups.

Inhibition of NO synthesis and pretreatment with L-arginine The optimal time of incubation (10 min), and the optimal dose of L-NMMA (10^{-4} M) were determined in preliminary studies on PA rings from 3 control and 3 COLD patients. Also, the endothelium-dependent rise in tension after adding L-NMMA was found to be maximal after submaximal contraction of the rings with PE. This led to a fixed protocol for the studies. To both rings with and without endothelium, L-NMMA (10^{-4} M) was added after PE, then after a delay of 10 min, the cumulative dose-response studies to ACh (10^{-10} to 10^{-5} M) were performed. To see whether L-arginine could reverse the inhibitory effects of L-NMMA, some rings were incubated for 10 min with both L-NMMA (10^{-4} M) and L-arginine. In addition, some rings were studied over 30 min (range, 30 to 45 min) incubation with L-arginine (10^{-3} M) alone to see whether *in vitro* supplementation of L-arginine could alter the endothelium-dependent relaxation to ACh in both diseased and control PA rings.

Drugs

All the drugs were purchased from Sigma Chemical (Poole, Dorset), except for L-NMMA which was donated by Wellcome Research Laboratories (Beckenham, Kent). The drugs were diluted in distilled water, except for indomethacin and the calcium ionophore, A23187, which were dissolved in 50% ethanol and dimethylsulphoxide, respectively. The final concentration of dimethylsulphoxide in the organ bath did not exceed 0.2% which, in itself, did not have any effect on the tissue, nor did 50% ethanol have any effect. All solutions were freshly prepared before use.

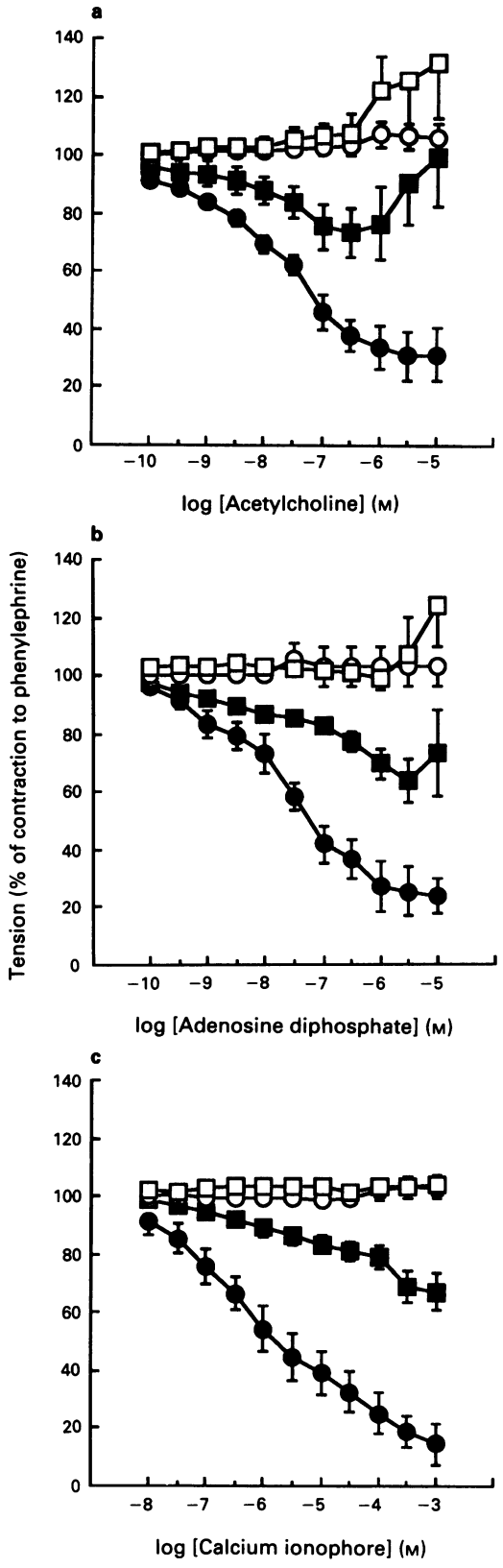

Figure 1 Endothelium-dependent relaxation in rings with (closed symbols) and without (open symbols) endothelium in response to cumulative doses of acetylcholine (a), adenosine diphosphate (b) and the calcium ionophore A23187 (c) in control (circles) and COLD (squares) patients. Results are expressed as means ± s.e.mean of 4 to 12 observations.

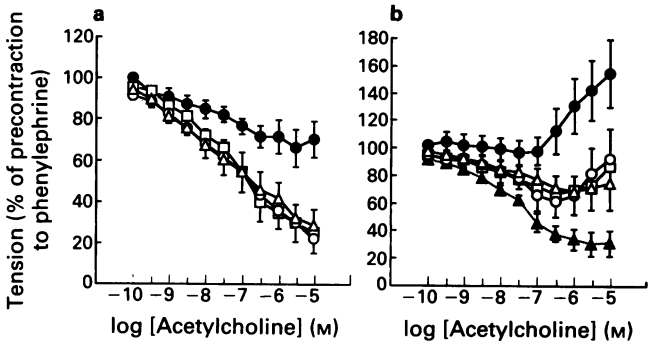

Figure 2 Dose-response curves in response to acetylcholine in rings from control (a) and COLD (b) patients. The rings are divided into 4 groups as follows; untreated (○), treated with N^G-monomethyl-L-arginine (L-NMMA, 10^{-4} M) (●), treated with L-NMMA (10^{-4} M) + L-arginine (10^{-3} M) (□), and treated with L-arginine (10^{-3} M) (△). In (b) results from untreated rings from controls (▲) are shown for comparison with L-arginine-treated rings from COLD patients (△). The results are expressed as means ± s.e.mean of 5–7 observations.

Statistical analysis

Student's *t* test for paired and unpaired values were used. When more than two groups of observations were compared, an analysis of variance was performed. This was followed by multiple comparisons using Scheffé's method to assess significant differences among group means. All data are expressed as mean ± s.e.mean. A *P* value of less than 0.05 was considered significant.

Results

All COLD patients had severe airflow obstruction FEV_1 = 16 ± 2% of predicted; range, 7–33%) and had arterial hypoxaemia (PaO_2 = 7.5 ± 0.5 kPa, range, 3.5–9.6 kPa) (Table 1). By contrast, none of the control patients had impaired lung function (FEV_1 = 90.5 ± 5% of predicted; range, 74–116%) and none had values of PaO_2 below 10.9 kPa (range, 10.9–12.8 kPa) (Table 1).

In both patient groups, ACh, ADP and the calcium ionophore, A23187, caused a concentration-dependent relaxation in PA rings where the endothelium was left intact, but not in those where the endothelium had been removed (Figure 1). Compared with controls there was a significant impairment of endothelium-dependent relaxation to ACh, ADP and the calcium ionophore A23187, in PA rings from COLD patients (Figure 1).

Pretreatment with L-NMMA (10^{-4} M) significantly inhibited endothelium-dependent relaxation to ACh in PA rings from both patient groups (Figure 2). However, this inhibition could be reversed by an excess of L-arginine (10^{-3} M) in all PA rings (Figure 2). Incubation of the rings with L-arginine (10^{-3} M) for over 30 min (range, 30 to 45 min) did not increase the endothelium-dependent relaxation to ACh of pulmonary arterial rings from either the control or the COLD patients (Figure 2). Impaired endothelium-dependent relaxation to ACh remained in the L-arginine-treated rings from COLD patients (maximal relaxation = 41.4 ± 9%) as compared with rings from control patients (maximal relaxation = 77.8 ± 7.2%) ($P < 0.05$) (Figure 2).

Rings of COLD patients developed a greater tension (1.91 ± 0.21 g) in response to phenylephrine (10^{-6} M) than rings from control patients (1.33 ± 0.15 g) ($P < 0.05$). However, this difference was eliminated by either mechanical removal of the endothelium or pretreatment with L-NMMA (10^{-4} M) (Figure 3). Both procedures resulted in a significantly greater contraction in response to phenylephrine (10^{-6} M) in control PA rings, but not in rings from COLD patients (Figure 3).

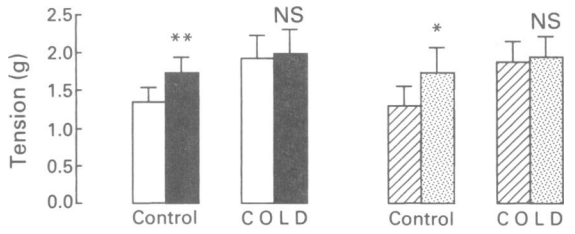

Figure 3 Absolute tension developed in response to phenylephrine (10^{-6} M) in rings with (open columns) and without (solid columns) endothelium, and rings treated (stippled columns) and untreated (hatched columns) with N^G-monomethyl-L-arginine (L-NMMA). Both removal of endothelium and L-NMMA pretreatment cause a significant rise in tension in rings from controls but not in COLD rings. $*P<0.05$; $**P<0.01$; NS (not significant). The results are expressed as means ± s.e.mean of 5–12 observations.

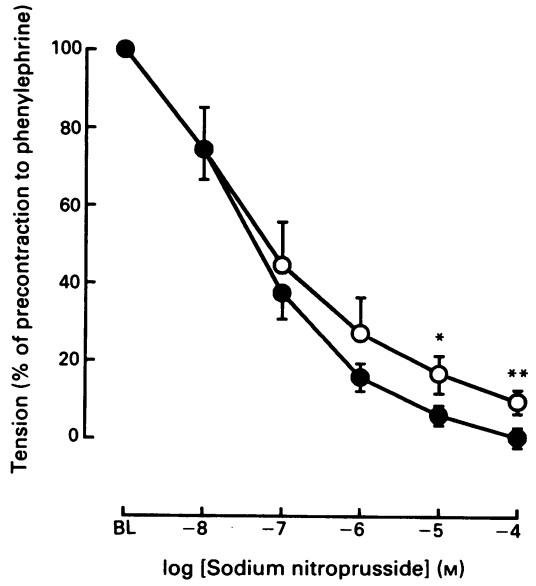

Figure 4 Endothelium-independent relaxation to sodium nitroprusside in rings with endothelium from control (○) and COLD (●) patients. Results are expressed as means ± s.e.mean of 6–8 observations. BL is baseline tension after precontraction to phenylephrine and before addition of sodium nitroprusside. $*P<0.05$; $**P<0.01$.

There was no significant difference between the concentration of SNP needed to achieve a 50% relaxation (EC_{50}) in PA rings from control patients ($-\log EC_{50} = 7.7 \pm 1.1$) and COLD rings ($-\log EC_{50} = 7.8 \pm 0.7$). However, there was a significantly greater degree of maximum relaxation in COLD rings compared with controls when tested with 10^{-5} M SNP ($94 \pm 1.5\%$ vs $83.3 \pm 4.9\%$; $P<0.05$) and with 10^{-4} M SNP ($99.4 \pm 0.6\%$ vs $90.3 \pm 3.1\%$; $P<0.01$) (Figure 4).

Discussion

These results confirm that endothelium-dependent relaxation in the PA of COLD patients is impaired (Dinh-Xuan *et al.*, 1991). They are consistent with the experimental observation that chronic hypoxia impairs pulmonary endothelium-dependent relaxation (Adnot *et al.*, 1991). The reduced EDRF (NO) release also accounts for the greater tension with PE in the PA rings from COLD patients. This effect is endothelium-dependent as mechanical removal or inhibition of NOS resulted in a similar rise in tension with PE in control rings but not those from COLD patients (Figure 3). Conversely, greater relaxation of PA rings from COLD patients occurred in response to SNP compared with controls (Figure 4). This is consistent with the view that a chronic reduction of endothelial NO production has occurred in COLD patients' pulmonary arteries.

A defective receptor-agonist coupling mechanism of the endothelial cell could account for the reduced EDRF (NO) release in the PA of COLD patients. Such a selective receptor dysfunction has been described in systemic arteries with atherosclerosis (Bossaller *et al.*, 1987) or after oxygen-derived free radical injury (Pieper & Gross, 1989). This was inferred from the impaired endothelium-dependent relaxation with both ACh and ADP, whilst normal relaxation occurred with the calcium ionophore, A23187. The calcium ionophore activates the endothelium NOS without requiring a specific membrane receptor, unlike ACh and ADP. By contrast, we observed comparable impaired endothelium-dependent relaxation not only with ACh and ADP but also calcium ionophore. This suggests an abnormality in the PA of COLD patients lies beyond the membrane receptor-agonist coupling mechanism.

The enzyme NOS has a recognition site for its substrate L-arginine and requires the dioxygen (O_2) molecule for the other substrate (Kwon *et al.*, 1990; Moncada *et al.*, 1991). A deficiency of L-arginine is unlikely to account for our results. Incubation with L-arginine can restore impaired endothelium-dependent relaxation in foetal pulmonary arteries (Abman *et al.*, 1991) and in cerebral vessels of hypercholesterolaemic rabbits (Rossitch *et al.*, 1991). Incubation with L-arginine however failed to improve the reduced endothelium-dependent relaxation in the PA of COLD patients or in normals.

The failure of incubation of the PA with excess L-arginine to restore relaxation is not due to defective transmembrane uptake of L-arginine into the endothelium. The inhibitory effects of L-NMMA, which utilizes a common transport system with L-arginine (Mann *et al.*, 1990), were readily reversed by an excess of L-arginine (10^{-3} M) in both normals and COLD patients PA (Figure 2).

Acute hypoxia can reduce EDRF release (Johns *et al.*, 1989; Warren *et al.*, 1989). This is putatively causing a substrate deficiency for the enzyme NOS. A similar explanation cannot account for our results from patients with chronic hypoxaemia. The PA rings were studied after 90 min equilibration under hyperoxic conditions in the organ baths, which resulted in values of PaO_2 in excess of 75 kPa (Pepke-Zaba *et al.*, 1992). Experimental chronic alveolar hypoxia causes a reversible impairment of pulmonary endothelium-dependent relaxation (Adnot *et al.*, 1991). In this study the COLD patients were all hypoxaemic. We have previously shown a significant correlation between the impaired endothelium-dependent relaxation in COLD patients with their degree of pre-operative chronic hypoxaemia (Dinh-Xuan *et al.*, 1991). It is possible that chronic hypoxia reduces the activity of pulmonary endothelial NOS although the mechanism requires elucidation.

In summary the pulmonary arteries, studied *in vitro*, from patients with COLD have evidence of impaired endothelium-dependent relaxation. This is not the result of substrate deficiency nor membrane receptor-agonist coupling dysfunction. It appears to reside within the cell as a reduction of activity of the pulmonary endothelium NOS activity.

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References

ABMAN, S.H., CHATFIELD, B.A., RODMAN, D.M., HALL, S.L. & MCMURTRY, I.F. (1991). Maturational changes in endothelium-derived relaxing factor activity of ovine pulmonary arteries in vitro. *Am. J. Physiol.*, **260**, L280–L285.

ADNOT, S., RAFFESTIN, B., EDDAHIBI, S., BRAQUET, P. & CHABRIER, P.-E. (1991). Loss of endothelium-dependent relaxant activity in the pulmonary circulation of rats exposed to chronic hypoxia. *J. Clin. Invest.*, **87**, 155–162.

BOSSALLER, C., HABIB, C.B., YAMAMOTO, H., WILLIAMS, C., WELLS, S. & HENRY, P.D. (1987). Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5'-monophosphate production in atherosclerotic human coronary artery and rabbit aorta. *J. Clin. Invest.*, **79**, 170–174.

DINH-XUAN, A.T., HIGENBOTTAM, T.W., CLELLAND, C.A., PEPKE-ZABA, J., CREMONA, G., BUTT, A.Y., LARGE, S.R., WELLS, F.C. & WALLWORK, J. (1991). Impairment of endothelium-dependent pulmonary-artery relaxation in chronic obstructive lung disease. *N. Engl. J. Med.*, **324**, 1539–1547.

DINH-XUAN, A.T., HIGENBOTTAM, T.W., CLELLAND, C.A., PEPKE-ZABA, J., CREMONA, G. & WALLWORK, J. (1990a). Impairment of pulmonary endothelium-dependent relaxation in patients with Eisenmenger's syndrome. *Br. J. Pharmacol.*, **99**, 9–10.

DINH-XUAN, A.T., HIGENBOTTAM, T.W., CLELLAND, C.A., PEPKE-ZABA, J., WELLS, F.C. & WALLWORK, J. (1990b). Acetylcholine and adenosine diphosphate cause endothelium-dependent relaxation of isolated human pulmonary arteries. *Eur. Respir. J.*, **3**, 633–638.

DINH-XUAN, A.T., HIGENBOTTAM, T.W., PEPKE-ZABA, J., CLELLAND, C.A. & WALLWORK, J. (1989). Reduced endothelium-dependent relaxation of cystic fibrosis pulmonary arteries. *Eur. J. Pharmacol.*, **163**, 401–403.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

HIGENBOTTAM, T.W., OTULANA, B.A. & WALLWORK, J. (1990). Transplantation of the lung. *Eur. Respir. J.*, **3**, 594–605.

JOHNS, R.A., LINDEN, J.M. & PEACH, M.J. (1989). Endothelium-dependent relaxation and cyclic GMP accumulation in rabbit pulmonary artery are selectively impaired by moderate hypoxia. *Circ. Res.*, **65**, 1508–1515.

KWON, N.S., NATHAN, C.F., GILKER, C., GRIFFITH, O.W., MATTHEWS, D.E. & STUEHR, D.J. (1990). L-citrulline production from L-arginine by macrophage nitric oxide synthase: the ureido oxygen derives from dioxygen. *J. Biol. Chem.*, **265**, 13442–13445.

MAGEE, F., WRIGHT, J.L., WIGGS, B.R. & HOGG, J.C. (1988). Pulmonary vascular structure and function in chronic obstructive pulmonary disease. *Thorax*, **43**, 183–189.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.

MANN, G.E., SHERIFF, C.-J. & PEARSON, J.D. (1990). In *Nitric Oxide from L-Arginine: A Bioregulatory System*. ed. Moncada, S. & Higgs, E.A. pp. 331–339. Amsterdam: Elsevier.

PALMER, R.M.J., ASHTON, D.S. & MONCADA, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **333**, 664–666.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

PALMER, R.M.J. & MONCADA, S. (1989). A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **158**, 348–352.

PEPKE-ZABA, J., HIGENBOTTAM, T.W., CREMONA, G., BUTT, A.Y. & DINH-XUAN, A.T. (1992). Ouabain inhibits hypoxia-induced relaxation of human conduit pulmonary arteries. *Am. Rev. Respir. Dis.*, **145**, A226.

PIEPER, G.M. & GROSS, G.J. (1989). Selective impairment of endothelium-dependent relaxation by oxygen-derived free radicals: distinction between receptor versus nonreceptor mediators. *Blood Vessels*, **26**, 44–47.

REES, D.D., PALMER, R.M.J., HODSON, H.F. & MONCADA, S. (1989). A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.*, **96**, 418–424.

REEVES, J.T. & VOELKEL, N.F. (1989). Mechanisms of chronic pulmonary hypertension: basic considerations. In *Pulmonary Circulation: Advances and Controversies*. ed. Wagenvoort, C.A. & Denolin, H. pp. 27–39. Amsterdam: Elsevier.

ROSSITCH, E. Jr., ALEXANDER III, E., BLACK, P.McL. & COOKE, J.P. (1991). L-arginine normalizes endothelial function in cerebral vessels from hypercholesterolemic rabbits. *J. Clin. Invest.*, **87**, 1295–1299.

VANE, J.R., ÄNGGÅRD, E.E. & BOTTING, R.M. (1990). Mechanisms of disease: regulatory functions of the vascular endothelium. *N. Engl. J. Med.*, **323**, 27–36.

WARREN, J.B., MALTBY, N.H., MACCORMACK, D. & BARNES, P.J. (1989). Pulmonary endothelium-derived relaxing factor is impaired in hypoxia. *Clin. Sci.*, **77**, 671–676.

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