Eur Respir J, 1995, 8, 513–514
DOI: 10.1183/09031936.95.08040513

European Respiratory Journal
ISSN 0903 - 1936

EDITORIAL

Nitric oxide and hypoxic pulmonary hypertension

M. Leeman*, R. Naeije

Within only a few years of the identification of nitric oxide (NO) as an endothelium-derived relaxant factor in 1987, there has been an explosion of literature showing that this simple gas is involved in the regulation of a wide range of functions, including vessel tone, cardiac contractility, platelet aggregation, neurotransmission, host defence and cytotoxicity [1]. These actions are mediated by the activation of soluble guanylate cyclase and the subsequent increase in the concentration of cyclic guanosine monophosphate (cGMP) in target cells.

Nitric oxide is formed from the amino acid L-arginine by the action of an enzyme NO synthase (NOS). This enzyme exists as calcium-calmodulin dependent constitutive forms (cNOS), which are basically expressed in endothelial cells, certain neurons, platelets, leucocytes and macrophages. Agonists, such as acetylcholine, bradykinin and calcium ionophore activate cNOS *via* a rapid increase in intracellular calcium concentration, resulting in the release of NO within seconds or minutes. In addition, calcium-calmodulin independent inducible forms of NOS (iNOS), that may be expressed after exposure to cytokines or endotoxin, have been described in macrophages, neutrophils, smooth muscle cells and endothelial cells. Induction of iNOs involves gene transcription, so that increased NO production occurs several hours after exposure. Corticosteroids inhibit the induction of iNOS but not that of cNOS. The release of large amounts of NO by the induction of iNOS contributes to the hyperdynamic circulatory states of patients with sepsis or decompensated liver cirrhosis.

A continuous release of NO from a tonic activation of endothelial cNOS is believed to be an important determinant of normal systemic blood pressure [1], but whether this mechanism contributes to maintain the normally low pulmonary vascular tone is still disputed. Studies on isolated pulmonary rings from many species suggest the existence of a braking mechanism, which triggers NO release to counteract any increase in pulmonary vascular tone; however, results from *in vitro* perfused lung studies have been contradictory [2]. In intact animals, discrepant results have also been reported. L-arginine analogues, which act as false substrate for NOS, and thereby block the formation of endogenous NO, did not affect pulmonary vascular pressure at controlled flow in dogs [3, 4], but increased pulmonary artery pressures (together with a decrease in cardiac output) at rest and during exercise in sheep [5]. In man, the L-arginine analogue N-monomethyl-L-arginine increased systemic arterial pressure but did not affect pulmonary artery pressure or pulmonary artery occluded pressure, and decreased cardiac output [6]. An unchanged pulmonary vascular pressure gradient in the presence of a reduction in cardiac output allows one to calculate an increased pulmonary vascular resistance, but whether this really corresponds to an increase in pulmonary vascular tone is doubtful [7]. It may be that baseline release of NO is important to maintain low pulmonary vascular tone in some but not all mammalian species [2, 8].

There has also been much speculation about a possible role for NO in the still unknown biochemical mediation of hypoxic pulmonary hypertension. It has been hypothesized that a decrease in synthesis or activity of NO could produce pulmonary hypertension, or, alternatively, that an increase in synthesis or activity of NO could attenuate pulmonary hypertension. The experimental data available support both possibilities, in acute as well as in chronic hypoxia.

Acute hypoxia. Exposure of pulmonary artery rings to hypoxia decreased their cGMP content and their endothelium-dependent relaxation [9], suggesting reduced NO synthesis or activity. On the other hand, inhibition of NO synthase (by nitro-L-arginine) or effect (by methylene blue) enhanced acute hypoxic vasoconstriction in intact dogs [4], suggesting that hypoxia increases NO synthesis.

Chronic hypoxia. The endothelium-dependent vasodilator response to acetylcholine and to calcium ionophore A-23187 in preconstricted isolated rat lungs was significantly reduced after 7 days of exposure to hypoxia and abolished after 3 weeks of hypoxia [10], suggesting that chronic hypoxic pulmonary hypertension could result from an endogenous NO deficiency. This study is in keeping with the report of a decreased endothelium-dependent relaxation in isolated pulmonary artery rings from patients with chronic obstructive lung disease [11]. However, other studies on isolated lungs from chronically hypoxic rats showed an enhanced vascular tone and hypoxic vasoconstriction after L-arginine analogues, rather suggesting that a sustained tonic endogenous release of NO limits hypoxic pulmonary hypertension [12–14]. How can such contradictions be reconciled?

Most recently, the expression and localization of NOS in the lungs from chronically hypoxic and normoxic rats were studied using immunohistochemistry and NADPH diaphorase staining techniques [15]. In normoxic and

Correspondence: M. Leeman, Dept of Intensive Care, Erasme University Hospital, 808 route de Lennik, B-1070 Brussels, Belgium.

hypoxic rats, NOS was detected by both methods in the endothelium of large pulmonary arteries. Expression of NOS was not observed in the endothelium of normoxic small pulmonary resistance vessels, but prominently detected in the endothelium of these vessels after chronic hypoxia. Chronic hypoxia also induced de novo NOS expression in the smooth muscle cells of small and large pulmonary arteries [15]. These data are compatible with the idea that endogenous NO does not contribute to the regulation of basal pulmonary vascular tone. They can also explain the discrepancies between studies on pulmonary artery rings, taken from large conduit arteries far upstream to the main site of pulmonary vascular resistance, and haemodynamic studies on perfused lungs and intact animals. Finally, they allow the speculation that chronic hypoxia downregulates endothelial cNOS, known to respond to acetylcholine stimulation, but upregulates endothelial and smooth muscle iNOS, which does not respond to agonists, but can be inhibited by L-arginine analogues.

The interesting study of HAMPL and co-workers [16] adds an important argument in favour of an increased pulmonary endothelial NO synthesis during acute hypoxia. Using a modified chemiluminescence assay, these authors measured higher accumulation of NO and its metabolite nitrite (NO_2^-) in the superfusate of cultured bovine pulmonary endothelial cells under hypoxic than under normoxic conditions. Hypoxia also potentiated bradykinin-induced NO release and transiently increased cytosolic calcium concentration, an event known to initiate NO synthesis. The originality of this study is that it provides direct measurement of NO production during hypoxia, exclusively in pulmonary endothelial cells and independently of other stimuli, such as shear stress and flow. Using the same chemiluminescence technique, the same group of investigators [12] recently reported higher concentrations of NO degradation products in the effluent of isolated lungs from rats with chronic hypoxic pulmonary hypertension than in control normoxic lungs.

Judicious application of new techniques of molecular and cell biology integrated into physiologically relevant intact organ and animal models will help to resolve the still elusive mechanisms of hypoxic pulmonary hypertension, and hopefully offer new therapeutic perspectives in the near future.

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