Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 7
Sofia • 2002

Vessel Wall Injury and NOS III Expression in Hyperoxic Pulmonary Hypertension

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In normal lung (NL), 40% of endothelial cells (EC) in large arteries, and between 13% and 18% in alveolar duct (AD) and alveolar wall (AW) vessels expressed NOS III. In the hyperoxic lung (HL) the relative number of cells expressing NOS III increased by day 28.

In both the normal and hyperoxic lung the smooth muscle cells (SMC) of large arteries also expressed protein selectively: in the HL alone, the SMC that developed in AD and AW vessels were also positive.

Type I epithelial cells showed weak or no immunopositivity in NL compared to HL where the majority of cells were labelled. In NL 74% of type 2 epithelial cells were positive, while in HL virtually all cells were labelled. We conclude that NOS III expression increases in EC of HL and in SMC of developing small vessels. In this model the greatest change in signal in epithelial cells appears distally in type I and type II cells.

Supported by: Fogarty International Research Collaboration Award RO3 TWO 0483.

Key words: rat, pulmonary hypertension, NOS III

Introduction

Only a few millimeters in length, but numerous, the microvessels of the lung (D<100 μm and adjacent to the capillary network) are a critical site of restriction to blood flow in pulmonary hypertension. The microvascular bed is restricted by vessel loss and lumen narrowing as smooth muscle cells develop within their wall. To analyse the cellular and molecular basis of this response we have developed a model of pulmonary hypertension in the rat [3, 4, 5].

NITRIC OXIDE (ENDOTHELIAL DRIVE RELEASING FACTOR) [NO EDRF] is involved in the regulation of various bodily functions as it is secreted by variety of cell types. A significant role of NITRIC OXIDE (NO) was suggested by the findings of NITRIC OXIDE SYNTHASE (NOS) activity in lung preparations (1). Different NOS isoforms have been found in endothelial as well as non-endothelial

cells in normal human and rat lungs and in different pulmonary pathologies [2, 6, 7, 8, 9, 10] supporting the varied role of NO in lung physiology. NO gas has been shown to reverse pulmonary hypertension and it is present in the exhaled air of various mammals.

Little is yet known of the heterogeneity of expression of EDRF/NO within and between lung cell populations, or of their significance in wall remodelling. The aim of the present study is to establish the cellular site of the constitutive endothelial cell NOS (NOS III, ec NOS) expression in the hypertensive lung in the rat breathing high oxygen.

Material and Methods

The cellular sources of NOS III (ec NOS) expression in a rat model of hyperoxic pulmonary hypertension were established (Rats breathed 21% O_2 (n=3) or 87% O_2 (n=15) for 1-28 days at normobaric pressure).

Cellular sites of NOS III protein were identified in thick (5mm), thin (90hm) sections by the Streptavidin-Biotin peroxidase or Protein-A gold technique using a polyclonal (rabbit antihuman) antibody with appropriate controls.

Results

After 28 days of hyperoxia the relatively thin wall of microvessels (Fig. 1— *a*) is abnormally thick with a complete coat of new smooth muscle cells (Fig. 1 — *b*).

In normal lung 40% of endothelial cells in large arteries are positive to NOS III and 13% and 18% of endothelial cells in alveolar duct and alveolar wall vessel expressed NOS III.

In contrast in the hyperoxic lung the relative number of endothelial cell expressing NOS III increased by day 28 (Fig. 2, 3) 80%, 42% and 50% of endothelial cells expressed NOS III at these sites. The distribution of NOS III — positive endothelial cells in different vessel types in normal and hypertensive lung is summarised in Fig. 8 (PA — Pulmonary artery, ADV — Alveolar duct vessel, AWV — Alveolar wall vessel).

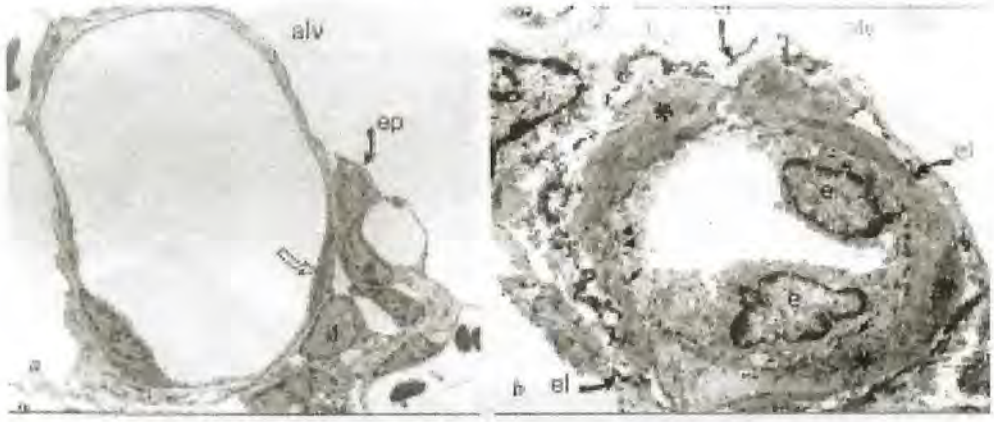

Fig. 1. Alveolar wall vessels: *a* — normal lung: non muscular vessel (× 2824); *b* — hyperoxic lung: e — endothelial cell, alv — alveolus, (× 5838) * — subendothelial contractile cells, ep-type 2 epithelial cell

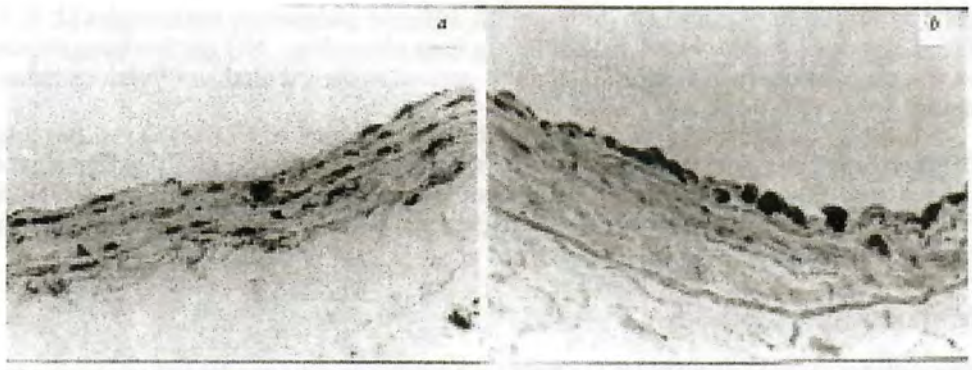

Fig. 2. NOS III immunoreactivity in the PA wall of control rat (*a*) (× 200) and after 28D of hyperoxia (*b*) (× 200)

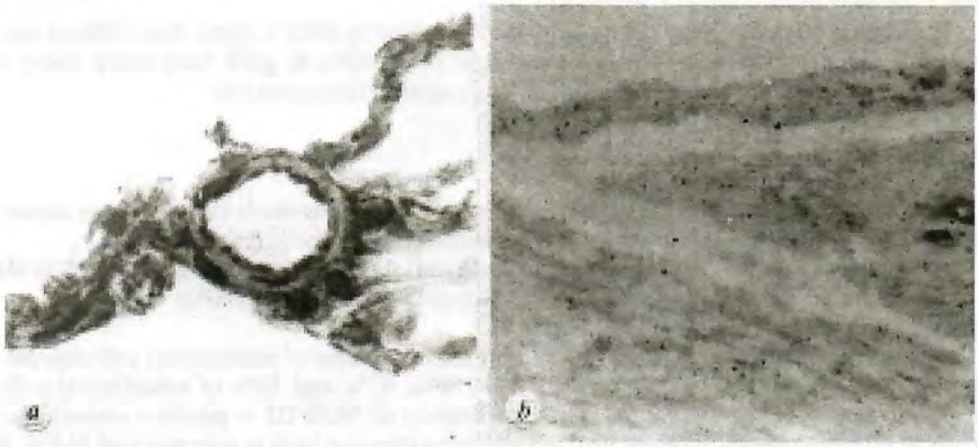

Fig. 3. Immunoreactivity in the lung: *a* — hyperoxic lung — AWV (× 400); *b* — NOS positive EC and SMC (× 30 000)

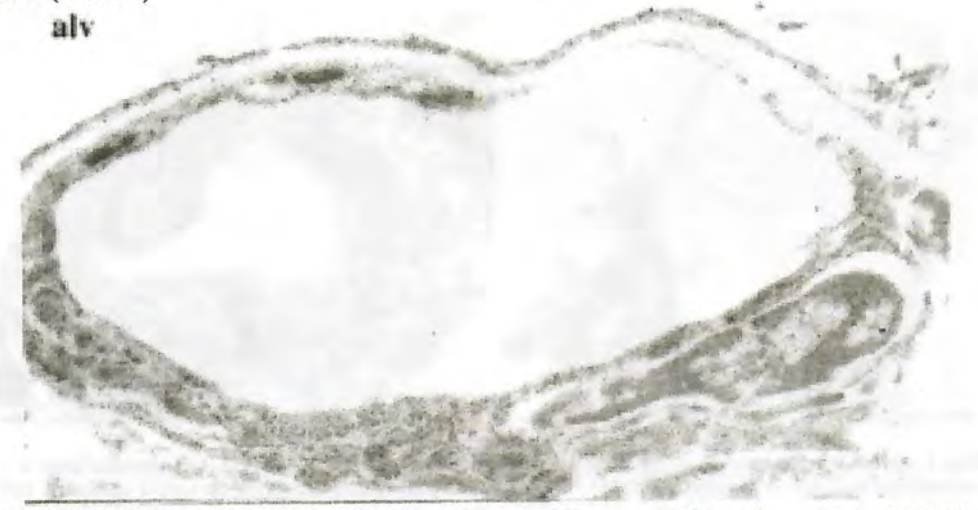

Fig. 4. NOS III immunoreactivity in EC of a capillary and Type 1 epithelium, alv — alveolus (× 7 000)

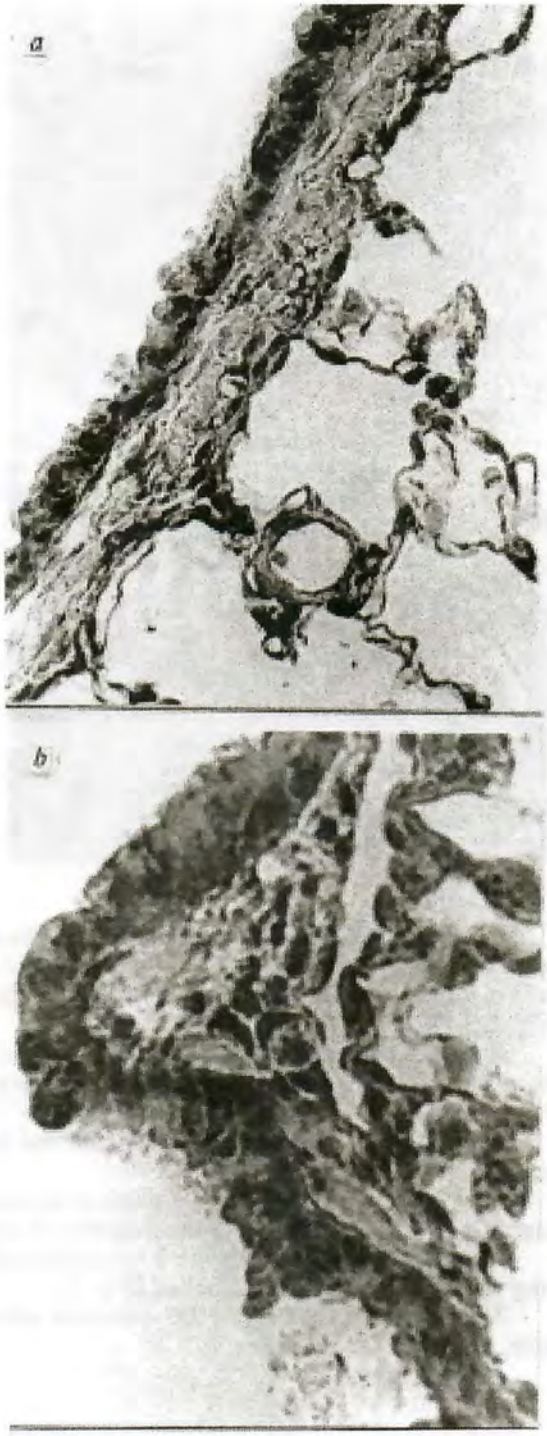

Fig. 5. Clara cells, ec NOS immunoreactivity (× 400)

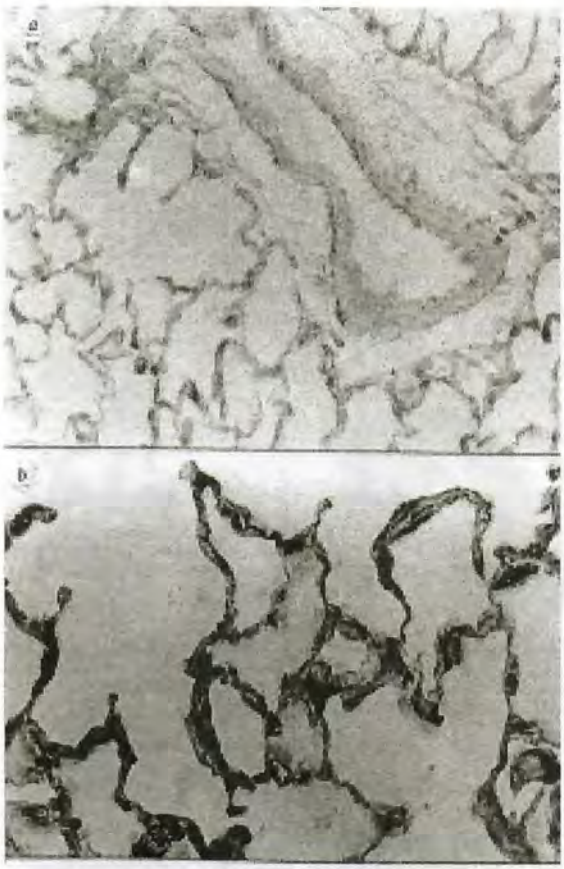

Fig. 6. NOS III in the alveolar epithelium *a* — control (× 200); *b* — 28 days after hyperoxia (× 400)

In both the normal and hyperoxic lung, the smooth muscle cells of large arteries expressed NOS III selectively: in the hyperoxic lung alone, the new smooth muscle cells that developed in alveolar duct vessels and alveolar wall vessels were also positive (Fig. 2, 3).

In all levels bronchiolar non-ciliated (Clara) cells were highly positive in both the normal and hyperoxic lung, the signal increasing distally in the hyperoxic lung (Fig. 5).

In ciliated cells the intensity of signal was lower in hyperoxic lung than normal lung.

Type 1 epithelial cells showed weak or no immunopositivity, in normal lung compared to hyperoxic lung where the majority of cells were labelled (Fig. 4, 6).

In normal lung 74% of type 2 epithelial cells were positive, while in hyperoxic lung virtually all cells were labelled (Fig. 7).

The results of the other NOS-possitive cells in the lung are summarised in the table.

Other resident cells in lung				
	UC	D28	UC	D28
Bronchiolar Epithelium	ciliated cells		Clara cells	
(D 600 - 1000 μm) (D 100 - 600 μm) (D < 100 μm)	++ + +	+- +- +-	++ ++ ++	++ +++ +++
Alveolar epithelium	type 1 cells		type 2 cells	
	single cells	all cells	74%	>98%

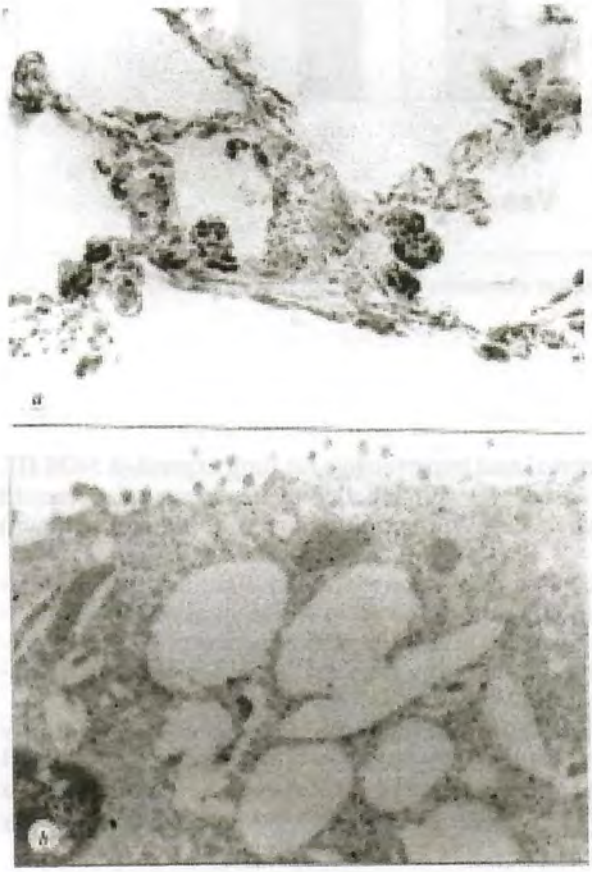

Fig. 7. Immunoreactivity in epithelial cells Type 2 — epithelial cells, ec(NITRIC OXIDE SYNTHASE) immuno-reactivity
a — light microscopic (× 400); *b* — electronmicroscopic study (× 20 000)

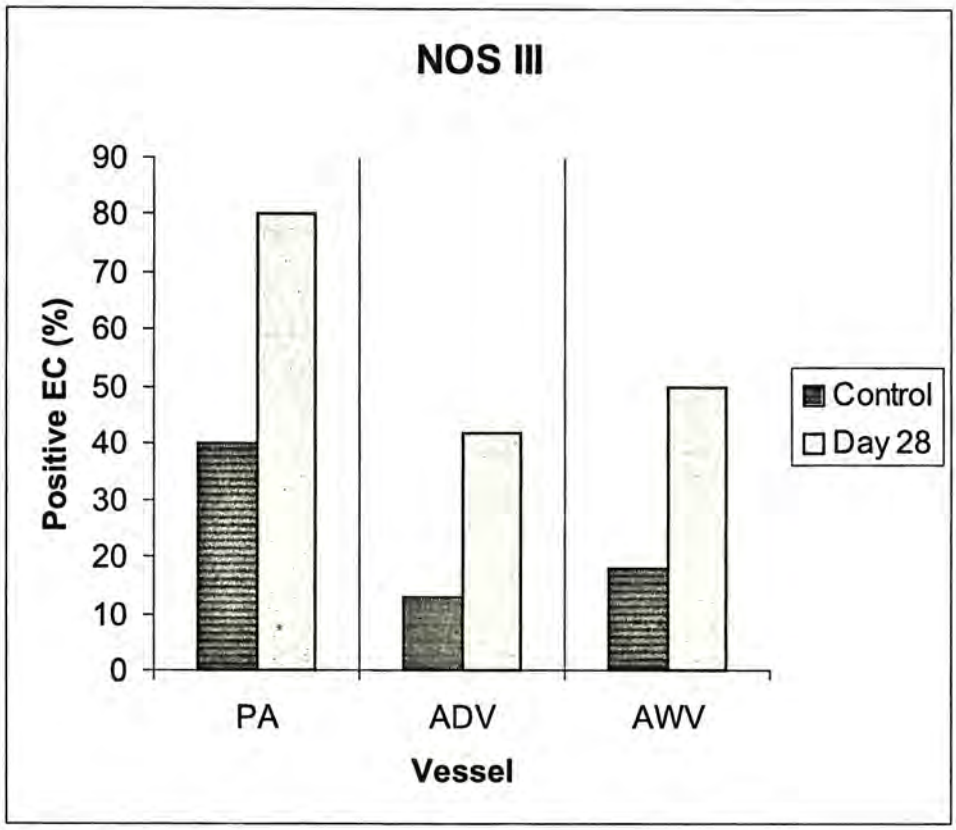

Fig. 8. Distribution of (%) NOS III positive endothelial cells in different pulmonary vessels

Conclusions

1. Each vessel population in the normal and hypertensive rat lung expressed NOS III. However, not all endothelial cells in a vessel were labelled. Selected vascular smooth muscle cells as well as cardiac muscle cells in the pulmonary vein (data not shown) express NOS III too.

2. NOS III protein immunoreactivity is increased in the pulmonary vascular endothelium of the rat hypertensive lung. It appears both in endothelial cells and developing smooth muscle cells of small vessels after 28 days of hyperoxia.

3. The greatest change in signal in the pulmonary epithelium in pulmonary hypertension occurs distally in Type 1 and Type 2 cells.

4. Recent evidence and our data show that the respiratory system is a relatively rich source of NO. The hypertensive rat lung appears to be a major source of NOS III and it is possible that this constitutive enzyme can be upregulated. This may have a beneficial effect on the potency of lung vessels as well as on blood flow in pulmonary hypertension.

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