HISTOCHEMICAL DETECTION OF NITRIC OXIDE SYNTHASE DURING THE DEVELOPMENT OF MURINE LUNG

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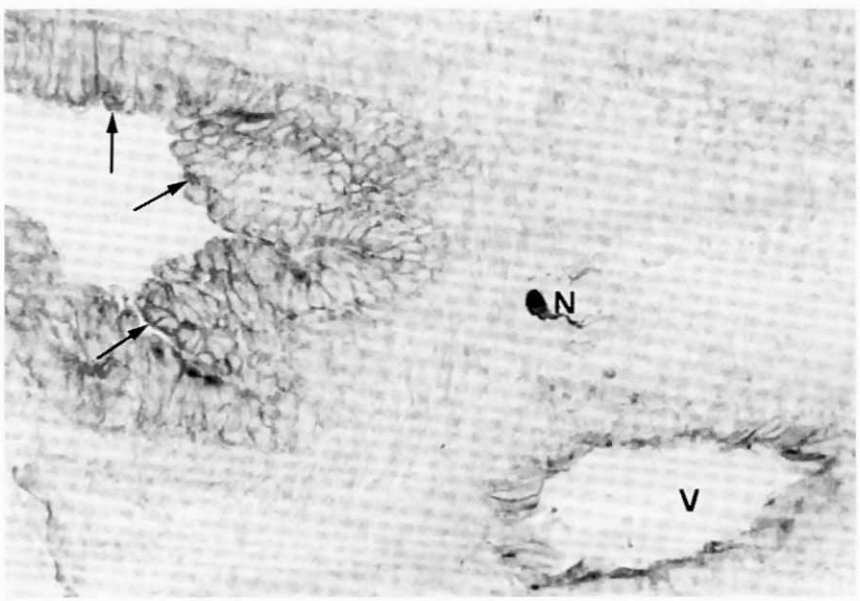
Aim. Pulmonary nitric oxide (NO) has been suggested to increase at the end of gestation and to play a role in the dramatic decrease in pulmonary vascular resistence that takes place at birth. In previous research we showed the presence of immunoreactive neurons for the synthase of NO (NOS) during the development of mouse lung. Since the amount of nervous structures seems to remain constant through gestational, neonatal and adult life, other cellular sources of endogenous NO have been investigated in the present paper.

Materials and Methods. The NADPH diaphorase technique (a marker for the presence of NOS) and the immunocytochemical method of avidin-biotin complexes using antisera raised against neuronal (n-NOS) and endothelial NOS (e-NOS) have been applied to paraffin-embedded and frozen lungs sections. Mice foetuses (E-gestational- days 13, 14, 15, 16, 17, 18, and 19), postnatal -P- (0, 1, 2, 6 days) and adult (ad) animals were studied.

Results. Both bronchiolar epithelial and endothelial lining gave immuno and/or enzymohistochemical reaction during development. Results are summarized in Table 1.

TABLE 1

	Bronchiolar epithelium		Endothelium	
	e-NOS	NADPH diaphorase	e-NOS	NADPH diaphorase
E-13	-	-	-	+
E-15	-	+	-	+
P-1	+	+	-	+
P-6	+	+	-	+/-
P-ad	+	+	-	+/-



In bronchioles, a faint diaphorase staining appears for the first time in the apical cytoplasm of the epithelium of E-15 animals (Fig. 1). As development takes place, the staining increases in intensity and extension through the cells: the label, remaining and being especially strong in the apical zone, reaches middle and basal cytoplasmic areas (Fig. 2). Although the immunolabeling appears later (P-1), the pattern of intensity and cellular distribution is similar to that of enzymohistochemical technique. The immunoreactive cells correspond to Clara cells.

Fig. 1. E-15 mouse foetal lung. NADPH-diaphorase label is detected in a neuron (N), the apical cytoplasm of bronchiolar epithelium (arrow), and the endothelium of the vessel (V). X 400.

In blood vessels, diaphorase activity has been obtained in the endothelium as soon as gestational day 13. Label is strong in all types of vessels (vascular outlines, arterioles, venules, macrovasculature) in foetuses (Fig. 1) and newborn animals. Later, endothelial staining decreases in the microvasculature (Fig. 2) and dissappears (P-6) in the macrovasculature. Although the antisera against e-NOS used in the present study have always rendered negative results in endothelial cells, the presence of diaphorase activity indicates the probable presence of a NOS.

Fig. 2. In the adult lung, a strong and NADPH-diaphorase label is found throughout the cytoplasm of most bronchiolar epithelial cells while only some endothelial cells of microvasculature are positive. X 400.

Conclusions.

1. Bronchiolar epithelium seems to be an important source of endogenous NO in murine lung. Since during foetal life the amount of NOS immunoreactivity/NADPH-diaphorase activity increases gradually, the epithelial NO seems to play a more important role in postnatal life, probably in relation with broncorelaxation.

2. On the contrary, since murine blood vessels display more diaphorase label in prenatal and neonatal life, endothelial NO -especially that of macrovasculature- may be a candidate for more specific gestational/perinatal roles.

References.

Guembe, L. and Villaro, A.C. (1994). Nitric oxide synthase-immunoreactivity in fetal and neonatal mouse lung. 17TH Conference of European Comparative Endocrinologists. Abstract nº 250.

Davidson, D., and Eldemerdash, A. (1990). Endothelium-derived relaxing factor: presence in pulmonary and systemic arteries of the newborn guinea pig. Pediatr Res. 27: 128-132.

Halbower, A.C., Tuder, R.M., Franklin,W.A., Pollock, J.S., Forstermann, U. and Abman, S.H. (1994). Maturation-related changes in endothelial nitric oxide synthase immunolocalization in developing ovine lung. Am J Physiol. L585-L591.

Hislop, A.A., Springall, D.R., Buttery, L.D.K., Pollock, J.S. and Haworth, S.G. (1995). Abundance of endothelial nitric oxide synthase in newborn intrapulmonary arteries. Archives of Disease in Childhood 73: F17-F21.

Perreault, T. and Demarte, I. (1993). Maturational changes in endothelium-derived relaxations in newborn piglet pulmonary circulation. Am J Physiol. 264: H302-H309.