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Nitric oxide synthase-immunoreactive neurons in human and porcine respiratory tract

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The presence of nitric oxide synthase (NO-synthase), the enzyme responsible for the production of nitric oxide (NO) from L-arginine, is shown immunocytochemically in the intrinsic neurons of the human and porcine respiratory tract. NO-synthase immunoreactivity is demonstrated in a subpopulation of neurons of the microganglia present in the wall of the extra- and intrapulmonary bronchi as well as in the hilar region of the lung in relation to blood vessels. The immunostaining was also found in some nerve fibers of the respiratory nervous system. Human and porcine lung gave similar results. The possible involvement of NO in the nonadrenergic noncholinergic (NANC) nervous regulation of the lung is discussed.

Nitric oxide (NO) has been shown to be an endogenous molecule with a role in transcellular communication. Some biological activities reported for NO are the relaxation of blood vessels, the prevention of blood clotting, as well as its action as a cytotoxic factor released by activated macrophages [17]. NO is also considered as a novel neural messenger [23]. Several recent studies give support to the hypothesis that NO acts as a neurotransmitter both in central [9] and peripheral [21] nervous system. In the mammalian gastrointestinal tract, extensive physiological and anatomical data suggest that NO could be the main nonadrenergic and noncholinergic (NANC) neurotransmitter, responsible for inhibitory neural regulation of the gut [21]. A significant morphological evidence is the demonstration of NO-synthase immunoreactivity in the intrinsic neurons of the gut of several mammals [1, 16]. In the present study, we were interested in knowing whether the neurons of the ganglionated plexus present in the mammalian respiratory tract are also immunoreactive for NO-synthase. In particular, the aim of the present work was a search for a morphological support of the physiological data recently reported concerning a possible role for NO in the pulmonary NANC relaxation both in human and pig respiratory tract [2, 7, 11].

Human pulmonary tissues were obtained from five autopsies (source: University Clinic of Navarra). All autopsies were performed within 5–10 h post mortem from patients that died either because of a traffic accident or from a non-pulmonary disease. Pieces of primary bronchus and hilar, medial and peripheral lung were taken. The tissues were fixed in Zamboni's formaldehyde-picric mixture [25] for 20 h at 4°C, washed in phosphate buffered saline 0.1 M, pH 7.3, dehydrated and embedded in paraffin. Three large-white piglets weighing 20–25 kg were anesthesized intravenously with sodium triamylal (Parke-Davis, New Jersey) (15 mg/kg) and killed by exsanguination. Pieces of primary bronchus and hilar, medial and peripheral lung were dissected and fixed as for human tissues.

Two antibodies against NO-synthase were used. One of them (NOS-3) was raised against the whole enzyme purified from an extract of rat brain. The second (#2385) was raised against a synthetic peptide from the deduced sequence of cloned neural NO-synthase [4]. The amino acid sequence is LPLLLQANGNDPELFQIPPELC. NOS-3 has been thorougly characterized by Western blot [24]. The specificity of #2385 was confirmed by preabsorption of the antiserum with the synthetic peptide. Sections were treated according to the avidin-biotin complex (ABC) immunocytochemical technique [10].

In histological sections of both human and porcine material, nervous ganglia and monofascicular nerve bundles were seen within the pulmonary hilus and in the wall of large bronchi. Ganglia and bundles of extrapulmonary bronchi were located both in the extrachondral

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