Cell Tissue Res (1992) 268:9–15

Cell & Tissue Research

© Springer-Verlag 1992

CGRP-immunoreactive endocrine cell proliferation in normal and hypoxic rat lung studied by immunocytochemical detection of incorporation of 5′-bromodeoxyuridine

L.M. Montuenga[1], D.R. Springall[1], J. Gaer[2], R.J.D. Winter[3], L. Zhao[3], J.T. McBride[1], K.M. Taylor[2], G. Barer[4], and J.M. Polak[1]

[1] Department of Histochemistry, [2] Department of Surgery, and [3] Department of Respiratory Medicine, Royal Postgraduate Medical School, Du Cane Road, London W12 ONN, UK
[4] Section of Experimental Medicine, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK

Received April 15, 1991 / Accepted September 29, 1991

Summary. We have tested the suggestion that the reported increase, in hypoxic rats, in the number of lung endocrine cells immunoreactive for the regulatory peptide CGRP is caused by an accumulation of peptide within the cells which renders them more detectable, rather than by a real increase in proliferation. The incorporation of continuously infused 5′-bromodeoxyuridine (BrdU) into nuclei of CGRP-containing cells was studied by immunohistochemistry in the airway and respiratory epithelium of rats kept in a hypoxic (10% O_2), normobaric conditions for 7 days and in normoxic, normobaric controls. Some CGRP-immunoreactive cells could also be labelled for BrdU. However, the ratio of the number of cells labelled with both CGRP and BrdU to the number of cells labelled with CGRP alone did not differ significantly between hypoxic and normoxic rats (7.1 ± 0.7 and 6.1 ± 1.2, respectively; mean ± SEM; $P = 0.49$). These data strongly suggest that CGRP-containing endocrine cells or their precursors do proliferate in adult rat lung, but that the proliferation is not increased significantly in hypoxia.

Key words: Lung – Endocrine cells – Calcitonin gene-related peptide – Bromodeoxyuridine – Differentiation – Proliferation – Hypoxia – Rat (Wistar)

Endocrine cells in the intrapulmonary airway epithelium have been identified in man, several other mammalian species, reptiles, birds and amphibians. They are present as single cells or as clusters that are reported to be innervated (Lauweryns et al. 1972; Lauweryns and Cokelaere 1973; Hung et al. 1973; Hung 1980). These clusters have been termed neuroepithelial bodies (NEBs) (Lauweryns et al. 1972; Lauweryns and Peuskens 1972) and are made up of cylindrical or cuboidal non-ciliated epithelial cells.

To discriminate neuroendocrine cells from other epithelial cells in the respiratory tract, the Grimelius and other argyrophilic methods (Lauweryns et al. 1972; Hage 1974; Moosavi et al. 1973) as well as general neuroendocrine markers have been used. Antibodies raised against neuron-specific enolase (NSE) (Sheppard et al. 1983; Gosney et al. 1988), chromogranin (Lauweryns et al. 1987), synaptophysin (Lee et al. 1987) and protein-gene-product 9.5 (Springall et al. 1988) have been successful tools to demonstrate this cell type. The first biologically active secretory product to be reported in pulmonary endocrine cells was serotonin (Lauweryns and Cokelaere 1973; Lauweryns et al. 1986; Sundler et al. 1980). It was not until bombesin-like immunoreactivity was localized to some of these cells that peptide storage was unequivocally established (Wharton et al. 1978). Since then, many other biologically active peptides have been reported in lung endocrine cells, including calcitonin (Gosney and Sissons 1985; Becker et al. 1980; Cutz et al. 1981), leucine-enkephalin (Cutz et al. 1981), and calcitonin-gene-related-peptide (CGRP) (Cadieux et al. 1986). CGRP is unique in being present not only in many solitary endocrine cells and NEBs but also in the sensory innervation of the respiratory tract of several mammals (Cadieux et al. 1986; Lundberg et al. 1985; Uddman et al. 1985). The function of the endocrine cell type in the lung is still a matter of discussion. A trophic effect of its secretory peptides on the nearby tissues has been proposed (Cutz 1982), as well as a chemoreceptor role, e.g. to decreased oxygen concentration in the air, in a similar way to that of the carotid body sensing blood gases (Lauweryns and Cokelaere 1973; Lauweryns and van Lommel 1983). Although there are contradictory reports, several authors have shown that more numerous endocrine cells can be detected in the epithelium of the airways in various experimental conditions, for instance in hypoxia (Keith and Will 1982). Nevertheless, the data concerning the mitotic activity of the lung endocrine cells in hamsters seem to point to an apparent mitotic arrest for this cell type at some point during fetal development (Hoyt et al. 1990). A plausible explanation for the apparent contradiction of increased, non-dividing cells is that the studies reporting hyperplasia

Offprint requests to: D.R. Springall