

## Immunohistochemical Study of the Endocrine Cells in the Lung of the Ostrich (*Struthio camelus*)

<sup>1</sup>Berrin Gencer Tarakçı, <sup>1</sup>Mine Yaman, <sup>2</sup>Ihsan Yaman, <sup>1</sup>Ali Bayrakdar and <sup>1</sup>Tuba Parlak

<sup>1</sup>Department of Histology and Embryology, Faculty of Veterinary Medicine,  
Firat University, 23119, Elazığ, Turkey

<sup>2</sup>Poultry Breeding High School, Firat University, 23119, Elazığ, Turkey

**Abstract:** The presence of serotonin, calcitonin, somatostatin-14, cholecystokinin (CCK), calcitonin gene related peptide (CGRP) endocrine cells in the lung of the ostrich (*Struthio camelus*) was investigated by immunohistochemistry. CGRP and serotonin immunoreactivity were observed in the PNEC cell (pulmonary neuroendocrine cell) with intermediate and low frequencies, respectively. Somatostatin containing cells were scarcely observed. CCK and calcitonin were not detected. These results suggesting that CGRP, serotonin and somatostatin may be involved in the lung ontogeny.

**Key words:** Pulmonary neuroendocrine cell, lung, ostrich, immunohistochemistry

### INTRODUCTION

The pulmonary neuroendocrine cells (PNEC) system is composed of solitary cells and PNEC clusters, neuroepithelial bodies (NEBs), widely distributed in the airway mucosa of various vertebrates (Scheuermann, 1987; Adriaensen and Scheuermann, 1993; Cutz, 1997; Pan *et al.*, 2004).

These cells have many features in common with their APUD series and may therefore be expected to produce biogenic amines and polypeptide hormones (Pearse, 1969).

Several studies have reported serotonin and some neuropeptides in PNEC system of mammals (Lauweryns *et al.*, 1982; Lauweryns *et al.*, 1986; Cutz *et al.*, 1984; Bhatnagar *et al.*, 1988; Stahlman *et al.*, 1987; Bayrakdar and Tarakçı, 2006) and lower vertebrates such as reptiles, amphibians and fishes (Sorokin and Hoyt, 1989; Scheurman *et al.*, 1987; Pastor *et al.*, 1987; Zaccone *et al.*, 1989 a, b).

Existence of various hormone producing cells was demonstrated in the lung of avian species including chicken (Wasano and Yamamoto, 1979; Salvi, 1992), quail (Adriaensen *et al.*, 1994), domestic fowl (Lopez *et al.*, 1993) and pigeon (Lopez *et al.*, 1993) using immunohistochemistry. However, no reports show distribution of immunoreactive endocrine cells in the lung of ostrich (*Struthio camelus*). Ostrich belong to Struthionidae family and are largest living bird in the

world (Kumari and Kemp, 1998). Thus, the present study was attempted to determine the regulatory peptides present in the lung of ostrich.

### MATERIALS AND METHODS

**Animals and tissue samples:** Five adult male ostriches were used. Birds with body mass of 45-60 kg were anaesthetized by injecting pentobarbitone sodium (50 mg kg<sup>-1</sup>) into pectoral muscle. The left carotid artery was cannulated at the base of the neck and allowed to exsanguinate. Tissue samples were taken from lung and fixed in 4% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. Seven µm-thick sections were obtained and processed for immunohistochemical staining.

**Immunohistochemistry: PAP (Peroxidase-Anti-Peroxidase) method:** Immunohistochemical staining was carried out by using the peroxidase-antiperoxidase (PAP) method. The blocking of endogenous peroxidase was carried out with 0.008% hydrogen peroxidase (H<sub>2</sub>O<sub>2</sub>) in methanol for 5 min (Sternberger, 1986). In order to block unspecific binding, an incubation with normal goat serum in 0.1 M phosphate buffered saline (PBS), pH 7.2 (Dilution 1:10) was performed. Sections were incubated for 16-20 h at 4°C with rabbit IgG antibodies against serotonin (Zymed Lab., 18.0077), calcitonin (Zymed Lab., 18.0012), somatostatin-14 (Chemicon, AB1976), cholecystokinin

(Chemicon, AB1973), calcitonin gene-related peptide (Chemicon, AB5920). Antibodies were diluted to 1:50, 1:200, 1:200, 1:500 and 1:1000 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark) followed by rabbit peroxidase anti-peroxidase complex (Zymed Lab., 61.2003, San Francisco), both at dilution of 1:50 in PBS, for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation step and finally immersed in glucose oxidase-DAB-nickel ammonium sulphate substrate (Shu *et al.*, 1988) for 10 min. After washing in distilled water and counterstaining with eosin, sections were dehydrated and cover slips mounted with aqueous permanent mounting medium.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (1979) by using (including the replacement of) specific antiserum preincubated with its corresponding antigen. Sections were examined with Leitz Dialux 20 microscope and photographs were taken.

**RESULTS**

Serotonin, CGRP, somatostatin positive pulmonary endocrine cells were identified in ostrich lung whereas CCK and calcitonin were never detected. The distribution and the relative frequency of these immunoreactive cells in ostrich lung were shown in Table 1.

Serotonin immunoreactive cells were located in both crypts and the surface of the respiratory epithelium. The number of such cells was small. They appeared as solitary or occasionally as small clusters of 2 or 3 cells in bronchi and bronchioles (Fig. 1). They were generally round to spherical-shaped close type cells.

Somatostatin contained immunoreactive cells were usually located in NEB form in alveolar sacs (Fig. 2). Solitary neuroepithelial cells also showed immunoreactivity (Fig. 3). They were fewer in number than the serotonin positive cells. These cells were spindle in shape.

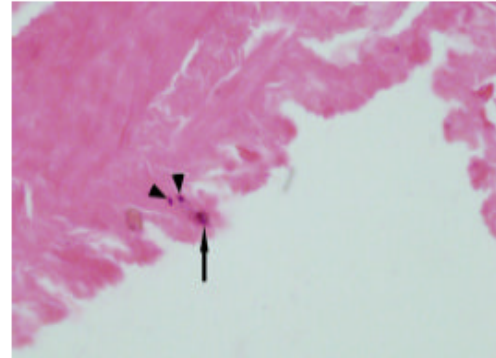
CGRP immunoreactive cells were found in NEB and solitary NEC forms in bronchi and bronchioles (Fig. 4).

**Table 1:** Distribution and relative frequency of serotonin, calcitonin, somatostatin-14, CCK and CGRP immunoreactive cells in the ostrich lung (n:5)

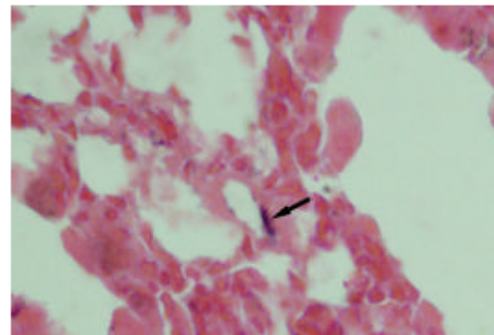
Lung	Bronchi and bronchioles	Alveolar sacs
Serotonin	++	-
Calcitonin	-	-
Somatostatin-14	-	+
CCK	-	-
CGRP	+++	-

Relative frequencies: +++: Numerous, ++: Moderate, +: Rare, -: Not detected, CCK: Cholecystokinin CGRP: Calcitonin gene related peptide

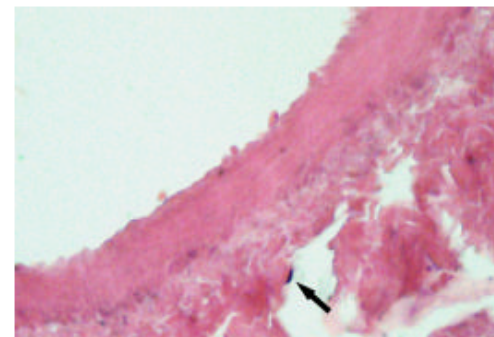
This immunoreactivity was also observed in nerve fibres around capillar walls (Fig. 5). CGRP positive spherical to spindle-shaped cells were seen more abundant than serotonin and somatostatin-immunoreactive cells.



**Fig. 1:** Serotonin immunoreactive cells were found in solitary NEC (arrow heads) and NEB form (arrow) in the lung of ostrich. X200



**Fig. 2:** NEB containing somatostatin in the alveolar sac of ostrich lung. X200



**Fig. 3:** Solitary NEC containing somatostatin in the alveolar sac of ostrich lung. X200

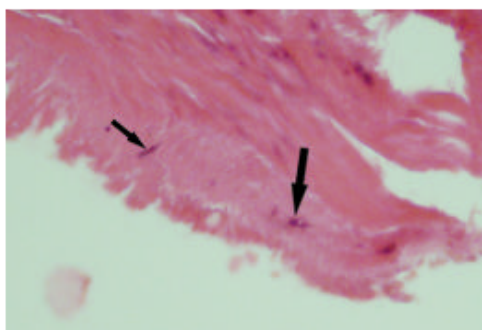


Fig. 4: CGRP immunoreactive cells were found in solitary NEC (small arrow) and NEB form (big arrow) in the lung of ostrich. X200

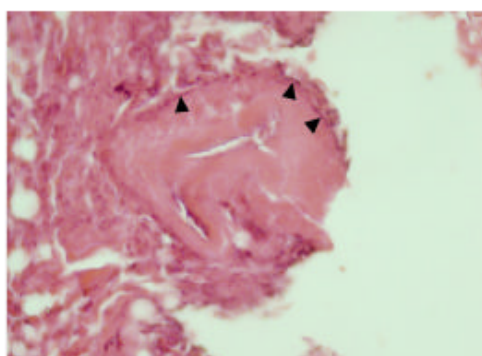


Fig. 5: CGRP immunoreactive nerve fibers around of blood vessel (arrowheads) of ostrich lung. X200

## DISCUSSION

Serotonin is the principal amine produced by PNEC system. The appreciable amount of serotonin in PNEC system of rabbit and rat (Cho *et al.*, 1989; Pan *et al.*, 2002; Wang *et al.*, 1999; Bayraktar *et al.*, 2006) were found mainly in foetal and neonatal lung (Cho *et al.*, 1989; Pan *et al.*, 2002; Wang *et al.*, 1999; Bayraktar and Tarakçı, 2006). In the present study, serotonin positive cells were found minimal in frequency in adult ostrich lung. Based on these observations, serotonin may play a regulatory, possibly tropic, role in lung maturation and differentiation in avian as demonstrated in mammals (Stahlman *et al.*, 1985; De Boich *et al.*, 1986; Hoyt *et al.*, 1991). Further studies requires at foetal and hatching ostrich to confirm these hypothesis. Furthermore, the fact that the number of serotonin immunoreactive pulmonary neuroendocrine cells significantly rose at hatching in chicken (Salvi and Renda, 1992) seem to also support the hypothesis that serotonin is released in reaction to air hypoxia, being responsible for the general vasoconstriction of the foetal lung (Lauweryns and Cokelaere, 1973).

Somatostatin is most known for its localization to pancreatic islet D cells (Bayraktar, 1996), but it has also been detected in pulmonary neuroendocrine cells and nerves of foetal rhesus monkey (Dayer *et al.*, 1985). In the present study, low somatostatin immunoreactivity was found in ostrich lung. Our findings are in agreement with the rare investigations previously made in some avian species (Adriaensen and Scheurman, 1993; Adriaensen *et al.*, 1994).

CGRP is a 37 amino-acide peptide coded by the calcitonin gene. In the respiratory tract, the presence of CGRP immunoreactivity was reported in guinea pig, human and mouse (Cadioux *et al.*, 1999; Verastegui *et al.*, 1997) and was localised either in nerve fibres or in neuroendocrine cells. Similar results were also observed in ostrich lung. Although the precise function of CGRP in the lung is largely speculative, CGRP shows both vasodilator and airways constrictor effects (Brain *et al.*, 1985; Palmer *et al.*, 1985).

In the present study, CCK and calcitonin immunoreactivity were not found.

In mammals serotonin and CGRP is considered to be prominent mediator in foetal and neonatal lungs and their role may be particularly important during lung development and neonatal adaptation (Bayraktar and Tarakçı, 2006). A similar regulatory function may also occur in the avian lung.

## REFERENCES

- Adriaensen, D. and D.W. Scheuermann, 1993. Neuroendocrine cells and nerves of the lung. *Anat. Rec.*, 236: 70-80.
- Adriaensen, D., D.W. Scheuermann, T. Gomi, A. Kimura, J.P. Timmermans and M.H.A. Groodt-Lasseel, 1994. The pulmonary neuroepithelial endocrine system in the quail, *Coturnix coturnix*. Light and electron microscopical immunocytochemistry and morphology. *Anat. Rec.*, 239: 65-74.
- Bayraktar, A. and B.G. Tarakçı, 2006. Serotonin, CGRP, Calcitonin, CCK, Somatostatin and VIP in the endocrine cells of developing rat lung. *Rev. Med. Vet.*, 157: 313-318.
- Bayraktar, M., 1996. Gastrointestinal system endokrinolojisi. *Temel ve Klinik Endokrinoloji*. Koloğlu S. Kozan offset.
- Bhatnagar, M., D.R. Springall, M.A. Ghatei, P.W.J. Burnet, Q. Hamid, A. Giaid, N.B.N. Ibrahim, F. Cuttitta, E.R. Spindel, R. Penketh, C. Rodek, S.R. Bloom and J.M. Polak, 1988. Localization of mRNA and co-expression and molecular forms of GRP gene products in endocrine cells of fetal human lung. *Histochem. Cell. Biol.*, 90: 299-307.

- Brain, S.D., T.J. Williams, J.R. Tippins, H.R. Morris and I. MacIntyre, 1985. Calcitonin gene-related peptide is a potent vasodilator. *Nature*, 313: 54-56.
- Cadieux, A., N.P. Monast, F. Pomerleau, A. Fournier and C. Lanoue, 1999. Bronchoprotector properties of calcitonin gene-related peptide in guinea pig and human airways. Effect of pulmonary inflammation. *Am. J. Respir. Crit. Care. Med.*, 159 (1): 235-243.
- Cho, T., W. Chan and E. Cutz, 1989. Distribution and frequency of neuroepithelial bodies in postnatal rabbit lung. *Cell. Tiss. Res.*, 255: 353-562.
- Cutz, E., J.E. Gillan and N.S. Track, 1984. Pulmonary Endocrine Cells in the Developing Human Lung and During Neonatal Adaptation. In: Becker, K.L. and A.F. Gazdar (Eds.). *The endocrine lung in health and disease*. Philadelphia, N.B. Saunders, pp: 210-231.
- Cutz, E., 1997. Introduction to pulmonary neuroendocrine cell system, structure-function correlations. *Microscop. Res. Technol.*, 37 (1): 1-3.
- Dayer, A.M., J. De Mey and J.A. Will, 1985. Localization of somatostatin, bombesin and serotonin-like immunoreactivity in the lung of the fetal rhesus monkey. *Cell. Tiss. Res.*, 239: 621-625.
- De Bock, V., K. Yoshizaki and S. Solomon, 1986. Serotonin content of rabbit lung and small intestine during perinatal development. *Life Sci.*, 38: 431-435.
- Hoyt, R.F., N.A. Mcnelly, M.E. Dowell and S.P. Sorokin, 1991. Neuroepithelial bodies stimulate proliferation of airway epithelium in fetal hamster lung. *Am. J. Physiol.*, 260 (Lung Cell Mol. Physiol. 4): L234-L240.
- Kumari, P. and S.J. Kemp, 1998. Polimorphic microsatellite markers in the ostrich (*Struthio camelus*). *Mol. Ecol.*, 7: 133-140.
- Lauweryns, J.M. and M. Cokelaere, 1973. Hypoxia-sensitive neuro-epithelial bodies: Intrapulmonary secretory neuroreceptors, modulated by the CNS. *Z. Zellforsch. Mikrusk. Anat.*, 145: 521-540.
- Lauweryns, J.M., V. De Boch, A.A. Verhofstad and H.W. Steinbusch, 1982. Immunohistochemical localization of serotonin in intrapulmonary neuroepithelial bodies. *Cell. Tiss. Res.*, 226 (1): 215-223.
- Lauweryns, J.M., L. Van Ranst, A.A.J. Verhofstad, 1986. Ultrastructural localization of serotonin in the intrapulmonary neuroepithelial bodies of neonatal rabbits by use of immuno-electron microscopy. *Cell. Tiss. Res.*, 243: 455-459.
- Lopez, J., M.A. Barrenechea, P. Burrell and P. Sesma, 1993. Immunocytochemical study of the lung of domestic fowl and pigeon: Endocrine cells and nerves. *Cell. Tiss. Res.*, 273: 89-95.
- Palmer, J.B.D., F.M.C. Cuss, M.A. Ghatei, D.R. Springall, A. Cadieux, S.R. Bloom, J.M. Polak and P.J. Barnes, 1985. Calcitonin gene-related peptide is localized to human airways nerves and potently constricts human airway smooth muscle. *Thorax*, 40: 713.
- Pan, J., H. Yeger and E. Cutz, 2002. Neuronal developmental marker *Forse-1* identifies a putative progenitor of the pulmonary neuroendocrine cell lineage during lung development. *J. Histochem. Cytochem.*, 50 (12): 1567-1578.
- Pan, J., H. Yeger and E. Cutz, 2004. Innervation of pulmonary neuroendocrine cells and neuroepithelial bodies in developing rabbit lung. *J. Histochem. Cytochem.*, 52 (3): 379-389.
- Pastor, L.M., J. Ballesta, R. Perez-Tomas and J.A. Martin, 1987. Immunocytochemical localization of serotonin in the reptilian lung. *Cell. Tiss. Res.*, 248: 713-715.
- Pearse, A.G.E., 1969. The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. *J. Histochem. Cytochem.*, 17: 303-313.
- Salvi, E. and T. Renda, 1992. An immunohistochemical study on neurons and paraneurons of the pre- and post-natal chicken lung. *Arch. Histol. Cytol.*, 55 (2): 125-135.
- Scheuermann, D.W., 1987. Morphology and cytochemistry of the endocrine epithelial system in lung. *Int. Rev. Cytol.*, 106: 35-88.
- Shu, S., G. Ju and L. Fan, 1988. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. *Neurosci. Lett.*, 85: 169-171.
- Sorokin, S.P. and R.F. Hoyt, 1989. Neuroepithelial Bodies and Small-Granule Cells. In: Massaro, D. (Ed.). *Lung Cell Biology. Lung biology in health and disease*, Dekker, New York, 41: 191-344.
- Stahlman, M.T., A.G. Kasselberg, D.N. Orth and M.D. Gray, 1985. Ontogeny of neuroendocrine cells in human fetal lung. II. An immunohistochemical study. *Lab. Invest.*, 52: 52-60.
- Stahlman, M.T., M. Jones, M.D. Gray, A.G. Kasselberg and W.K. Vaughn, 1987. Ontogeny of neuroendocrine cells in human fetal lung. III. An electron microscopic immunohistochemical study. *Lab. Invest.*, 56: 629-641.
- Sternberger, L.A., 1979. The Unlabelled Antibody Peroxidase-Antiperoxidase (PAP) Method. In: Sternberger L.A. (Ed.). *Immunocytochemistry*. J. Wiley, Sons, New York, pp: 104-169.
- Sternberger, L.A., 1986. *Immunocytochemistry*. 3rd Edn. John Wiley, New York.

- Verastegui, C., A. Prada Oliveira, J. Fernandez-Vivero, A. Romero and J.M. De Castro, 1997. Calcitonin gene-related peptide immunoreactivity in adult mouse lung. *Eur. J. Histochem.*, 41 (2): 119-126.
- Wang, D., M. Post and E. Cutz, 1999. Expression of serotonin receptor 2c in rat type II pneumocytes. *Am. J. Respir. Cell. Mol. Biol.*, 20 (6): 1175-1180.
- Wasano, K. and T. Yamamoto, 1979. APUD type receptor-secretory cells in the chicken lung. *Cell. Tiss. Res.*, 201: 197-205.
- Zaccone, G., L. Goniakowska-Witalinska, J.M. Lauweryns, S. Fasulo and G. Tagliafierro, 1989a. Fine structure and serotonin immunohistochemistry of the neuroendocrine cells in the lungs of the bichirs *Polypterus delhezi* and *P. orratipinnis*. *Basic Applied Histochem.*, 33: 277-289.
- Zaccone, G., G. Tagliafierro, L. Goniakowska-Witalinska, S. Fasulo, L. Ainis and A. Mauceri, 1989b. Serotonin-like immunoreactive cells in the pulmonary epithelium of ancient fish species. *Histochemistry*, 92: 61-63.