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

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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 thefore 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^{-1}) 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_2O_2) 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

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(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 oxidise-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 solitarily 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.

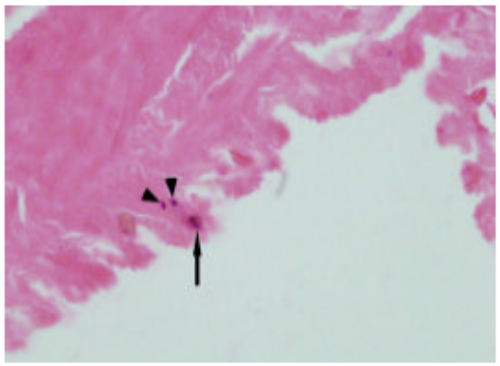

Fig. 1: Serotonin immunoreactive cells were found in solitary NEC (arrow heads) and NEB form (arrow) in the lung of ostrich. X200

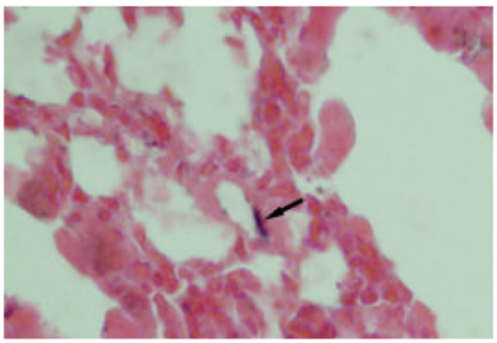

Fig. 2: NEB containing somatostatin in the alveolar sac of ostrich lung. X200

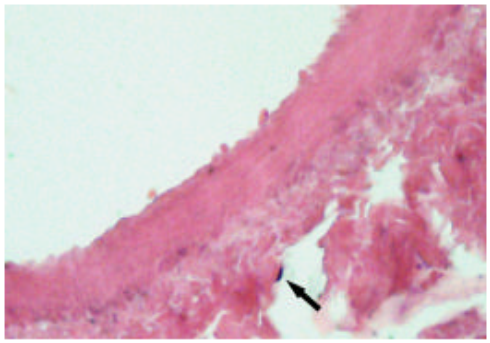

Fig. 3: Solitary NEC containing somatostatin in the alveolar sac of ostrich lung. X200

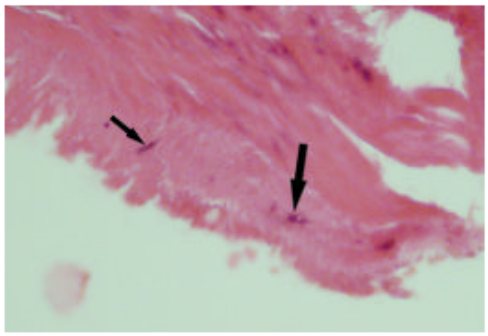

Fig. 4: CGRP immunoreactive cells were found in solitary NEC (small arrow) and NEB form (big arrow) in the lung of ostrich. X200

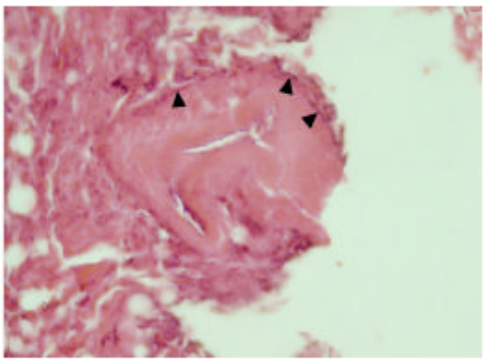

Fig. 5: CGRP immunoreactive nerve fibers around of blood vessel (arrowheads) of ostrich lung. X200

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; Bayrakdar 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 Boch *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 immunoreactivitiy was reported in guinea pig, human and mouse (Cadieux *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 (Bayrakdar and Tarakçı, 2006). A similar regulatory function may also occur in the avian lung.

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