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Coexistence of serotonin and cholecystokinin in paraneurons of the foetal sheep lung

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Summary. The coexistence of serotonin and cholecystokinin was studied in foetal sheep lungs at pseudoglandular stage of development by light microscopic immunohistochemistry. The coexistence was examined by staining consecutive sections with the different antibodies. Serotonin and cholecystokinin immunoreactivity was found within consecutive sections of most bronchopulmonary neuroepithelial bodies and in consecutive sections of the same intrapulmonary autonomic ganglia.

Key words: Coexistence, Serotonin, Cholecystokinin, Paraneurons, Sheep-Lung

Introduction

Bronchopulmonary paraneurons are present in airway mucosa as solitary neuroendocrine cells and neuroepithelial bodies and within intrapulmonary autonomic ganglia as small intensely fluorescent (SIF) cells. By immunohistochemistry, biogenic amines and several neuropeptides have been identified in different cells or in colocalization within the same cell (Scheuermann, 1987; Fujita et al., 1988; Luts, 1991).

In prior studies (Balaguer et al., 1991, 1992) we demonstrated the presence of the immunoreactivity to serotonin and cholecystokinin in solitary neuroendocrine cells and neuroepithelial bodies of foetal and neonatal sheep lung, with a similar distribution to that of other mammals. We also described serotonin and cholecystokinin immunoreactivity in intrapulmonary SIF cells of foetal sheep lungs at pseudoglandular stage. In the present paper, their possible coexistence was studied by immunohistochemical staining of alternate consecutive sections of foetal sheep lungs at this stage.

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Materials and methods

Six foetal sheep were obtained at a slaughterhouse, and were selected at pseudoglandular stage, gestational days 55-65, crown-rump 8-13 cm. The lungs were removed and 3-5 mm-thick samples were excised and rapidly fixed in cold (4 °C) Bouin's solution overnight and rinsed in cold (4 °C) 70% ethanol for 3 days. The tissues were further processed for embedding in paraffin and consecutive 3-4 µm sections of lungs were cut.

For immunoperoxidase staining, alternate sections were labelled with rabbit polyclonal antiserum to serotonin (Incstar Corp.) and cholecystokinin (Immunonuclear Corp.), using a commercially available avidin-biotin-peroxidase complex (ABC) immunostaining kit (Lipshaw Corp.). Primary antiserum was used at a dilution of 1/1000 in phosphate-buffered saline (pH 7.2) and incubation time was 18-22 hours at 4 °C in a moist chamber. Peroxidase activity was revealed by incubation in 100 ml 0.05 M Tris buffer (pH 7.6) containing 50 mg of 3,3'-diaminobenzidine tetrahydrochloride (Sigma) and 75 µl of 30% vol. hydrogen peroxide. After rinsing, sections were lightly counterstained with Carazzi's haematoxylin, dehydrated and mounted.

Negative staining controls were performed by replacing the primary antisera with non-immune rabbit serum or phosphate-buffered saline. As positive controls, sections of sheep duodenum were used. Additional positive controls included in the kit, Anti Kappa/Lambda, were also used.

Results

Serotonin and cholecystokinin immunoreactive cells were observed in airway mucosa and within intrapulmonary autonomic ganglia.

In airway epithelium they appeared individually or in clusters as neuroepithelial bodies, distributed throughout

all levels of the intrapulmonary airways. Small immunoreactive cells to both antibodies were detected in autonomic ganglia. In this location they also appeared as solitary cells or forming clusters.

Immunostaining of consecutive sections revealed the presence of serotonin and cholecystokinin within the same neuroepithelial bodies in most sections studied (Fig. 1a,b). Clusters of SIF cells also showed coexistence of serotonin and cholecystokinin within serial sections of the same pulmonary autonomic ganglia

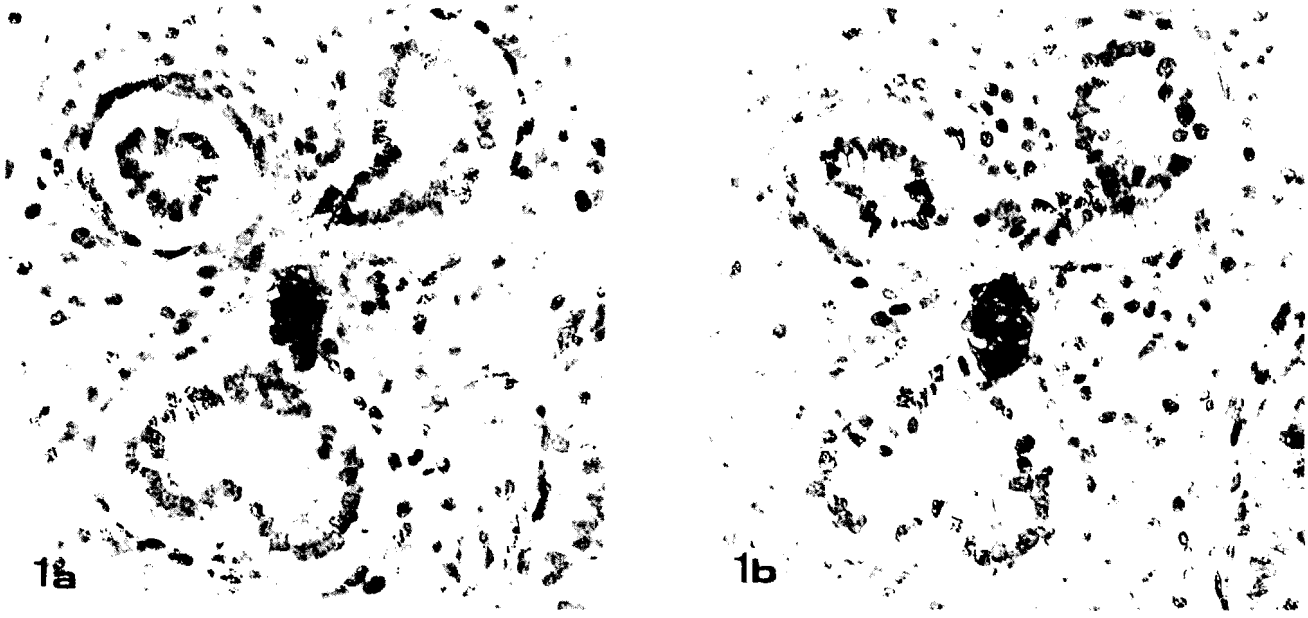

Fig. 1. Serial sections of lung from a sheep foetus. The neuroepithelial bodies show coexistence of serotonin **(a)** and cholecystokinin **(b)**. ABC-immunoperoxidase staining.

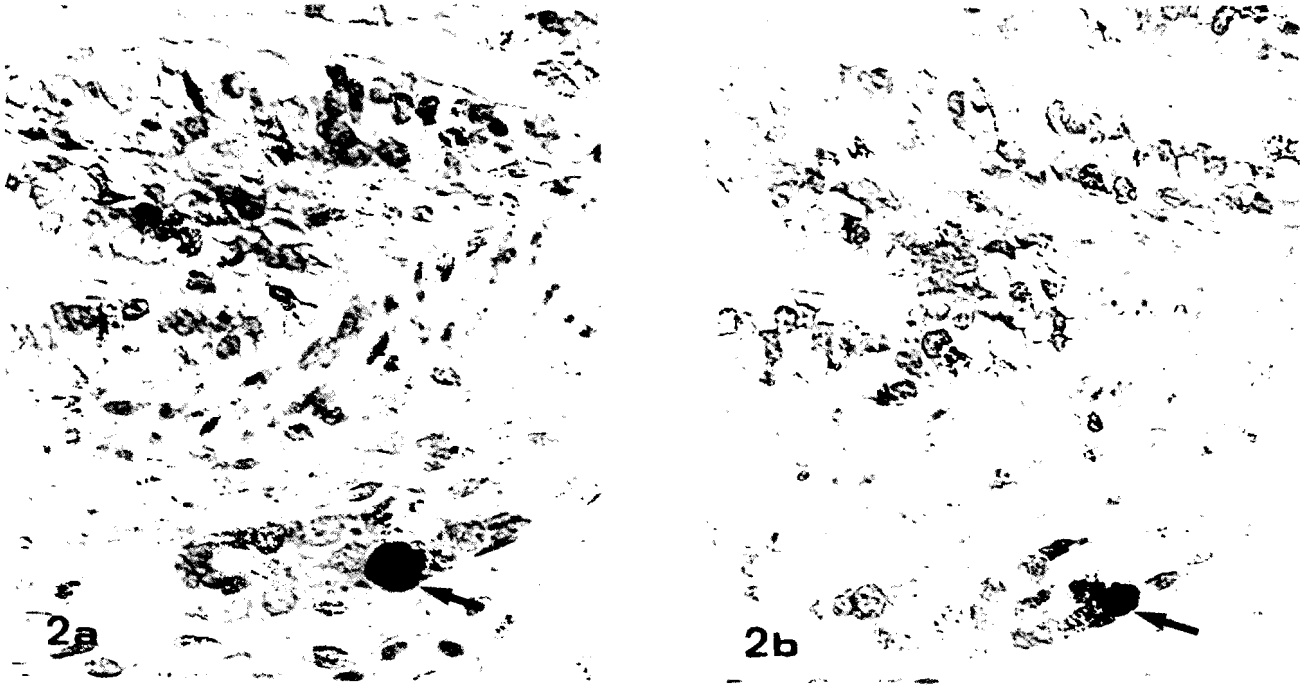

Fig. 2. Serial sections of lung from a sheep foetus. Serotonin **(a)** is colocalized with cholecystokinin **(b)** in immunoreactive SIF cells of autonomic ganglia (arrow). ABC-immunoperoxidase staining.

(Fig. 2a,b).

Discussion

In pulmonary intraepithelial paraneurons serotonin is found in coexistence with cholecystokinin, calcitonin gene-related peptide, gastrin-releasing peptide, enkephalin and somatostatin (Dayer et al., 1985; Will et al., 1985; Adriaensen et al., 1991). Serotonin immunoreactive SIF cells have been described in colocalization with enkephalin and substance P in other tissues (Kanagawa et al., 1986; Neel and Parsons, 1986). Although serotonin and cholecystokinin coexistence has been described previously in neurons of medulla oblongata of the rat (Mantyh and Hunt, 1984), to our knowledge, this study provides the first demonstration of the coexistence of serotonin and cholecystokinin in SIF cells.

The physiological role of biogenic amines that coexist with peptides in SIF cells is unknown. Owman et al. (1973) suggest that amines may be involved in the synthesis, storage or secretion of polypeptide hormones in endocrine cells. For Fujita et al. (1988), peptides and amines may act synergetically upon identical or related targets.

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