Localization of Neuropeptides in Endocrine Cells of the Chicken Thymus

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(Received 6 December 1996/Accepted 18 March 1997)

Abstract. Interactions between endocrine cells and epithelial cells, mediated by neurotensin, have been proposed in the chicken thymus. In this study, other neuropeptide candidates acting as mediators in the chicken thymus were examined immunohistochemically. Endocrine cells being oval, elongated or triangular in shape were immunoreactive with antibodies against methionine-enkephalin, neuropeptide Y, substance P, and vasoactive intestinal peptide. These findings suggest that 4 neuropeptides may be involved in cell-to-cell interactions in the chicken thymus. — Key words: immunohistochemistry, mediator, thymic microenvironment.

The mammalian thymus is physiologically under the control of the pituitary and hypothalamus. In addition to this control, intrathymic interactions via pituitary hormones, hypothalamic neuropeptides, and thymic hormones produced within the thymus exist between epithelial cells and thymocytes, as well as the in situ production of their respective receptors [3, 6–8]. Intrathymic interactions have been implicated in the control of the thymic microenvironment involving T-cell differentiation and maturation. In the avian thymus, intrathymic interactions via thymic hormones, avian thymic hormone and thymulin, are generally accepted, as they are in mammals [10]. The avian thymus contains endocrine cells as one of its major cellular components. In fact, endocrine cells in the chicken thymus were found to contain serotonin, somatostatin, and neurotensin at this time [4, 9, 12]. Of these, the neurotensin pathway from endocrine cells to epithelial cells within the thymus was suggested first [1, 2]. The route from endocrine cells to epithelial cells is different from the interaction pathways in the mammalian thymus. In this study, we examined whether other neuropeptides could be mediatory candidates for interactions among cells within the chicken thymus. An immunohistochemical technique was employed by the use of antibodies against 6 neuropeptides.

Eight adult female White Leghorn chickens (Gallus domesticus) (1.7–1.8 kg in weight and 16–18 months of age) were used in this study. They were anesthetized with sodium pentobarbital (25 mg/kg, i.v.) and perfused transcardially with Ringer’s solution followed by Bouin’s sodium pentobarbital (25 mg/kg, i.v.) and perfused transcardially with Ringer’s solution followed by Bouin’s solution (50 µm) sections were stained by the avidin-biotin-horseradish peroxidase complex (ABC) immunohistochemical method. The monoclonal or polyclonal antibodies used in this study were as follows: substance P (1:2,000), vasoactive intestinal peptide (1:5,000), galanin (1:10,000), methionine-enkephalin (1:12,000), neuropeptide Y (1:10,000), calcitonin gene-related peptide (1:10,000). Characteristics of the antibodies used are shown in Table 1. Dewaxed sections were blocked with 0.3% H2O2-methanol for 30 min, preincubated with 2% normal goat serum for 30 min, and incubated with the primary antiserum overnight at 4°C. The sections were incubated with biotinylated anti-rabbit IgG or anti-mouse IgG (1:400, Vector Lab., U.S.A.) for 30 min at room temperature and incubated with the ABC complex (ABC Elite Kit, Vector Lab.) for 30 min at room temperature. Peroxidase in the ABC complex was visualized by incubation in Tris-HCl buffer containing 3,3’-diaminobenzidine (20 mg/100 ml) and 0.003% H2O2. Frozen sections were immersed for 30 min in 0.3% H2O2 in PBS, and preincubated in 2% normal goat serum for 60 min at room temperature. Sections were then incubated overnight at 4°C in PBS containing the primary antiserum as described above, rinsed 3 times in PBS and incubated for 60 min at room temperature with biotinylated goat antibodies against rabbit or mouse IgG (1:400, Vector Lab.). After 3 washes, sections were incubated for 60 min at room temperature with the ABC complex. The peroxidase reaction was performed as described above for the paraffin sections. Then, preparations were washed in PBS, mounted on gelatin-coated glass slides, air-dried, dehydrated in a graded ethanol series, cleared in xylene, and covered with the coverslips. In the controls, the primary antiserum was

<table>
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<tr>
<th>Antibody</th>
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<tr>
<td>calcitonin gene-related peptide (CGRP)</td>
<td>synthetic rat CGRP</td>
<td>rabbit</td>
<td>Amersham Int.</td>
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<tr>
<td>galanin (Gal)</td>
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<td>rabbit</td>
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<tr>
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<td>Incstar</td>
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<tr>
<td>neuropeptide Y (NPY)</td>
<td>synthetic porcine NPY</td>
<td>rabbit</td>
<td>Amersham Int.</td>
<td>[11]</td>
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<td>substance P (SP)</td>
<td>SP</td>
<td>rat*</td>
<td>Chemicon Int.</td>
<td>[5]</td>
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<tr>
<td>vasoactive intestinal peptide (VIP)</td>
<td>synthetic VIP</td>
<td>rabbit</td>
<td>Bioproducts</td>
<td>not available</td>
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* Monoclonal IgG2a.
replaced by non-immune rabbit or mouse serum.

Immunoreactivity of 6 neuropeptides was examined in cryostat sections and paraffin sections. In both sections, endocrine cells were immunostained with antibodies against methionine-enkephalin, neuropeptide Y, substance P, and vasoactive intestinal peptide (Fig. 1a-d). They were oval, elongated or triangular in shape and were sparsely distributed in the medulla. In the paraffin sections in particular, immunoreactive fine granules were observed in the cytoplasm of endocrine cells (Fig. 1d). Calcitonin gene-related peptide and galanin did not show specific immunoreactivity in endocrine cells. In the control sections, no immunoreactivity was seen.

This study demonstrated the presence of methionine-enkephalin, neuropeptide Y, substance P, and vasoactive intestinal peptide in endocrine cells in the chicken thymus. The previous study suggested that neurotensin is a mediator in the pathway between endocrine cells and epithelial cells [1]. It is not known whether methionine-enkephalin, neuropeptide Y, substance P, and vasoactive intestinal peptide go to epithelial cells or thymocytes after endocrine cells excrete them. However, it is possible that the 4 neuropeptides are mediators in the chicken thymus and are involved in cell-to-cell interactions in the thymic microenvironment. Neuropeptides in the mammalian thymus are produced by epithelial cells and thymocytes.

However, no secretory granules are formed in their cytoplasm, which is called the cryptocrine [7]. Epithelial cells and thymocytes in the chicken thymus have not been stained with antibodies against neuropeptides so far. This does not immediately indicate the absence of cryptocrine in epithelial cells and thymocytes of the avian thymus.

The presence of 6 neuropeptides and serotonin has been elucidated in endocrine cells of the chicken thymus from this and previous studies [1, 4, 9, 12]. Håkanson et al. [9] ultrastructurally observed 2 types of endocrine cells from the size and shape of secretory granules in the chicken thymus. Thus, it is considered that each endocrine cell must produce more than one neuropeptide. We fully expect that neuropeptides co-localize in secretory granules. Further studies will determine what kind of neuropeptides secretory granules of endocrine cells contain.

REFERENCES