Substance P-Immunoreactive Endocrine Cells and Nerve Fibers in the Intestines of the Medaka, *Oryzias latipes*

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By use of an electron microscope, I discriminated five different types of enteroendocrine cells (Takahata, 1981), neurosecretory cells (Takahata, 1985) and their axons (Takahata, 1982) containing many secretory granules in the digestive tract of the medaka, *Oryzias latipes*. Such characteristic cells and axons were also observed in the digestive tracts of many other animals (Forssmann et al., 1969; Vassallo et al., 1969; Gabella, 1972; Fujita, 1973, 1975; Cook and Burnstock, 1976; Yamamoto, 1977; Yamada et al., 1978).

Recent immuno-electronmicroscopic studies have shown the presence of neuropeptides in the secretory granules of these cells and axons (Fukata et al., 1985; Uchida et al., 1985; Cetin, 1988). In this connection, it is assumed that the digestive tract of the medaka also contains neuropeptides in its enteroendocrine cells, and neurosecretory cells and their axons. It is however difficult to clarify this assumption because of the lack of immunohistochemical studies on the medaka.

I made an immunohistochemical examination in order to confirm the existence of neuropeptide in the digestive tract of the medaka. Substance P-immunoreactive cells and nerve fibers were also found when a light microscope was used. This paper reports on the results of this study.

Material and methods

Adult individuals of the medaka, *Oryzias latipes*, were purchased from an animal dealer. After decapitation, the intestines were removed, and divided into proximal, middle and distal parts. Each piece was fixed for 20 to 24 hrs in Bouin's fluid, or 4% paraformaldehyde, at 4℃.

The specimens were washed with 0.01 M phosphate buffered saline (PBS, pH 7.2) containing 10% sucrose for 2 to 3 hrs at 4℃, and then in PBS containing 15% sucrose for 2 to 3 hrs at 4℃, and finally in PBS containing 20% sucrose for 20 hrs at 4℃.

The washed specimens were embedded in an OCT compound and frozen in liquid nitrogen.

Sectionings and immunohistochemical reactions were carried out following the "simple method of cryo-sectioning and staining for immunohistochemistry" (Takahata, 1988). This method was devised in conformity with the ABC method (Hsu et al., 1981). Sections were cut into 20 to 25 μm thickness with a rotary microtome.

Free-floating sections were rinsed in PBS containing 0.3%Triton-X100. They were then stained as follows: 1) They were incubated with 3% H_2O_2 solution for 10 mins at 25℃; 2) Washed in PBS (0.01 M, pH 7.2) for 15 mins at 4℃; 3) incubated with normal goat serum for 30 mins at 25℃; 4) incubated with the substance P antiserum (AB 30, CRB Ltd.) in 1 : 1000 dilution for 24 to 48 hrs at 4℃; 5) washed in PBS for 15 mins at 4℃; 6) incubated with biotin-labelled anti-rabbit IgG* solution for 30 mins at 25℃; 7) washed in PBS for 15 mins at 4℃; 8) incubated with avidin-biotin-peroxidase complex solution (Vectastatin ABC kit PK-4001, Vector Laboratories, Inc.) for 30 mins at 25℃; 9) washed in PBS for 15 mins at 4℃; 10) incubate with 0.02% diaminobenzidine tetrahydrochloride (DAB) in 0.05 M Tris buffer (pH 7.2) containing 0.006% H_2O_2 for a few minutes at room temperature; 11) wahshed in distilled water for 5 mins; 12) mounted on glass slides and dry; 13) dehydrated in a graded series of ethanol, and mounted.

The following controls were carried out to test the specificity of the immunoreactions: 1) replacement of substance P antiserum with an unimmunized rabbit serum; 2) incubation of a mammalian tissue already known to contain substance P; 3) an immuno-absorption test conducted by preincubation of the substance P antiserum (1/1000 in PBS) in synthetic substance P (10–20 μg/ml, diluted antiserum) at 4℃ for 24 hrs prior to immunostaining.

Results and discussion

Substance P-immunoreactive cells were scattered among the absorptive cells of the mucous epithelium (Fig. 1A).

The basal part of the cells swelled and faced to the basement membrane of the mucous epithelium. The apical part of the cells projected slightly upward toward the digestive lumen (Fig. 1B).

Many substance P-immunoreactive cells were found throughout the intestines. However, no difference in the density of these cells was recognized in

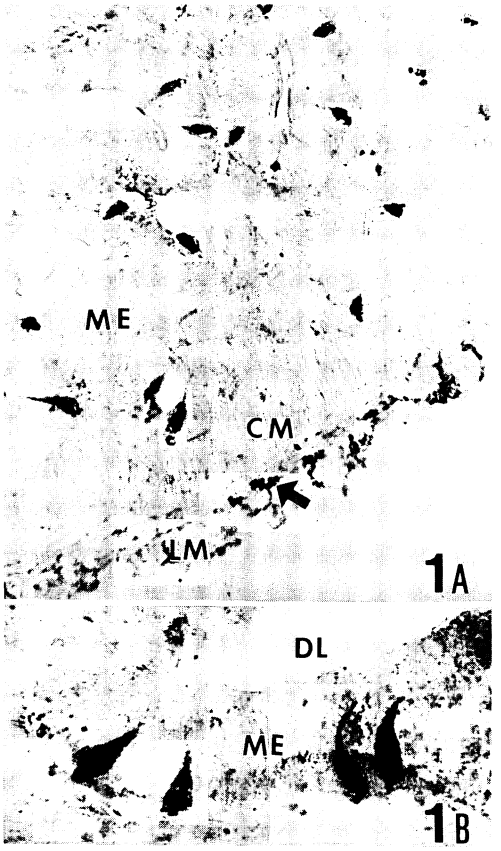

Fig. 1. Substance P-immunoreactive cells and the distal parts of nerve fibers (arrow) in the intestines of *Oryzias latipes*. A: Substance P-immunoreactive cells are scattered among the absorptive cells of the mucous epithelium. CM, circular muscle layer; LM, longitudinal muscle layer; ME, mucous epithelium. ×330. B: Substance P-immunoreactive cells at higher magnification. DL, digestive lumen; ME, mucous epithelium. ×660.

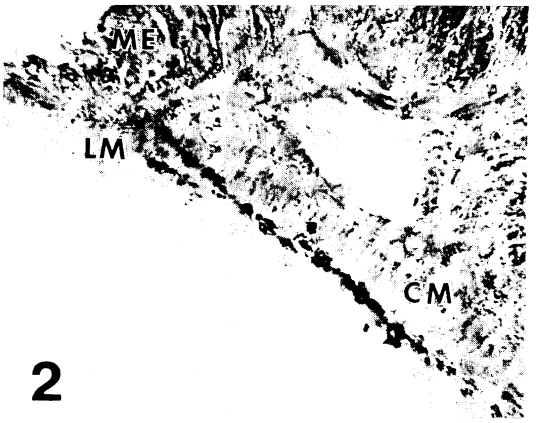

Fig. 2. Substance P-immunoreactive nerve fibers in the myenteric plexus. CM, circular muscle layer; LM, longitudinal muscle layer; ME, mucous epithelium. ×660.

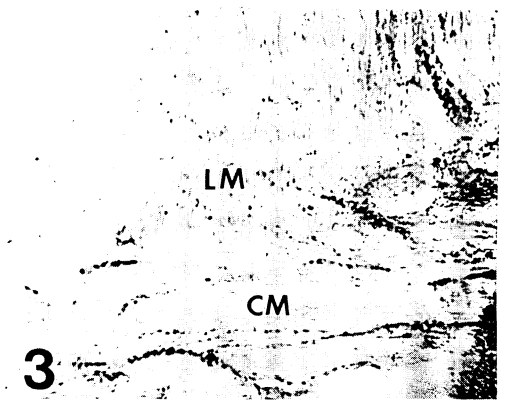

Fig. 3. Substance P-immunoreactive nerve fibers in the muscle layers. CM, circular muscle layer; LM, longitudinal muscle layer. ×330.

any of the three parts of the intestines.

It was considered that these cells were the endocrine cells previously described (Takahata, 1981) because of their morphological feature and distribution. In that report, the endocrine cells were divided into five types on the basis of the morphological feature of their secretory granules. In this study, however, it was difficult to confirm to which cell type the substance P-immunoreactive cells were equivalent.

Substance P-immunoreactive cells of the mucous epithelium in the digestive tract have already been demonstrated in the case of the teleosts *Pelmatochromis pulcher, Helostoma temmincki* (Langer et al., 1979), *Salmo gairdneri* (Holmgrem et al., 1982), and *Barbus conchonius* (Rombout and Reinecke, 1984). The distribution of these cells is different among species, but the cells of the medaka are similar to the fundamental morphological feature. Accordingly, it is assumed that these cells are homologous endocrine cells.

Substance P-immunoreactive nerve fibers were found, remarkably, in the myenteric plexus (Fig. 2). On the other hand, several substance P-immunoreactive nerve fibers were demonstrated in the circular and longitudinal muscle layers (Fig. 3). Substance P-immunoreactive nerve fibers contained small dot-like varicosities (Fig. 4) and formed a loose network

Fig. 4. Substance P-immunoreactive nerve fibers in the myenteric plexus. Nerve fibers possess small dot-like varicosities (arrowheads) and form a loose network. ×660.

in the myenteric plexus.

However, there was no substance P-immunoreactive nerve fiber in the submucosa and mucosa, and no substance P-immunoreactive nerve cell bodies were detected in any tissue.

On the basis of their distribution, substance P-immunoreactive nerve fibers are hypothesized to correspond to neurosecretory-like axons which were previously recognized (Takahata, 1982).

Substance P-immunoreactive nerve fibers and cell bodies in the digestive tract have already been demonstrated in mammals (Nilsson et al., 1975; Pearse and Polak, 1975; Jessen et al., 1980; Schultzberg et al., 1980; Costa et al., 1980, 1981; Leander et al., 1981; Kuwahara et al., 1983; Tange, 1983; Domoto et al., 1984; Keast et al., 1987), amphibians (Holmgren et al., 1985) and teleosts (Holmgren et al., 1982; Rombout and Reinecke, 1984).

The morphological features of substance P-immunoreactive nerve fibers of the medaka are similar to those of the mammals (Jessen et al., 1980; Costa et al., 1981; Leander et al., 1981; Keast et al., 1985, 1987) and amphibians (Holmgren et al., 1985), but when compared with these tetrapods they are small in number and distributed in limited tissues.

It would be interesting to clarify the ultrastructure of the substance P-immunoreactive cells and nerve fibers, especially the relations between substance P and secretory granules. This problem has not yet been examined, and I intend to make it the subject of some future study.

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メダカの腸管壁に観察された substance P 免疫反応陽性の内分泌細胞と神経線維

高畑悟郎

メダカの腸管壁において，substance P 免疫反応陽性の内分泌細胞と神経線維を，光顕的に観察した．substance P 免疫反応陽性の内分泌細胞は，粘膜上皮の吸収上皮細胞の間に散在していて，腸の全域を通じて観察された．腸の前部，中部，後部の間における分布状態に差異は認められなかった．細胞の形態的特徴は，すでに報告されている他の硬骨魚種の substance P 免疫反応陽性細胞と類似していた．substance P 免疫反応陽性神経線維は，おもに筋間神経叢に分布しているが，内輪筋層や，縦走筋層内にも観察された．substance P 免疫反応陽性神経線維は，小さな点状の varicosity を持ち，筋間神経叢では，ゆるい網目を形成していた．

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