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Occurrence of Neuropeptide Y (NPY)-like-Immunoreactive Cells in the Vitellointestinal Duct and Yolk Sac of Two Species of Elasmobranchs

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ABSTRACT—In order to extend our knowledge on the occurrence of neuropeptide Y (NPY)-like-immunoreactive cells in the vitellointestinal duct and yolk sac of oviparous elasmobranchs, we performed immunohistochemical examination on two species of elasmobranchs, *Cephaloscyllium isabellum* and *Raja kenojei*. In both species, NPY-positive cells were demonstrated in the epithelial layer of the vitellointestinal duct and internal yolk sac, as previously reported in *Scyliorhinus torazame*. In the *Cephaloscyllium* embryo, NPY-positive cells were sporadically encountered even in the endodermal layer of the external yolk sac proximal to the vitellointestinal duct. These findings support the view that the NPY-positive cell is a common endocrine component in the vitellointestinal duct of oviparous elasmobranchs.

INTRODUCTION

In our recent immunohistochemical study on the ontogenetic development of neuropeptide Y (NPY)-like-immunoreactive cells in the gastroenteropancreatic endocrine system of the cloudy dogfish, *Scyliorhinus torazame*, NPY-positive cells were unexpectedly demonstrated in the vitellointestinal duct and its derivative structure, i.e., the internal yolk sac [7]. These cells were polymorphic in shape, intercalated between the epithelial cells, and contained secretory granules that could be selectively immunostained with anti-porcine NPY antiserum. Apparently, their occurrence was transient, but their function remains unknown. On the other hand, from the viewpoints of comparative anatomy and zoology, the following question should be answered: Is the occurrence of such NPY-positive cells peculiar to *Scyliorhinus torazame*? In order to answer this question and to extend our knowledge on the NPY-positive cells in elasmobranchs, we conducted the present immunohistochemical examination on the embryos and juveniles of two species of Japanese elasmobranchs, *Cephaloscyllium isabellum* and *Raja kenojei*.

MATERIALS AND METHODS

One embryo (58 mm in total length) of the swell shark, *Cephaloscyllium isabellum*, and four embryos (42–88 mm) and eight juveniles (87–121 mm) of the skate, *Raja kenojei*, were used in this study. They were obtained by raising eggs freshly laid under laboratory conditions. The parent fish had been maintained for breeding in the aquaria of Sado Marine Biological Station of Niigata University, a facility located on the west coast of Sado Island in the Sea of Japan. In this study, embryos and juveniles were selectively examined. After having been anesthetized with 0.5% *m*-aminobenzoate-methanesulfonate (MS-222), the animals (except for the embryos) were perfused through the heart initially with physiological saline for elasmobranchs and subsequently with Bouin's solution without acetic acid. The embryos were also anesthetized and immersed in the fixative. The visceral organs or embryonal body with yolk sac were appropriately trimmed, dehydrated, and embedded in paraffin. Serial sections were cut at a thickness of 8-10 μm mainly in sagittal and transverse planes. NPY-expressing cells were immunohistochemically stained with a commercial kit (Nichirei, Tokyo) utilizing the streptavidin-biotin method. Polyclonal antibody (anti-synthetic porcine NPY antibody, UCB Bioproducts, Belgium) was used as the primary antibody, at a dilution of 1 : 3000. The peroxidase complex was visualized by the diaminobenzidine method.

The sections were washed, dehydrated, and mounted in Canada balsam. Some sections were counterstained with Mayer's hematoxylin. The NPY immunostaining was controlled by absorption of the primary antibody with NPY at concentrations of 1 and 10 μM diluted antibody for 48 hr at 4°C. Possible cross reactivity of the antibody with other peptides was checked by absorption of 10 μM primary antibody for 48 hr at 4°C with synthetic peptide including avian pancreatic polypeptide (APP), polypeptide YY (PYY), and molluscan cardioexcitatory tetrapeptide (FMRFamide). All these peptides were commercially obtained (Peninsula Laboratories, USA). The absorption test showed that the immunoreactivity of the NPY antiserum was abolished by NPY, but not by the other antigens employed.

RESULTS AND DISCUSSION

In the embryo of the swell shark, NPY-like-immunoreactive cells were demonstrated in the epithelial lining of the vitellointestinal duct and internal yolk sac, although they were less frequent there than in the pancreas or gut epithelium (Figs. 1 and 2). The labeled cells were sparsely distributed in the internal yolk sac as well as in the vitellointestinal

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duct. They were sporadically encountered even in the endodermal layer of the external yolk sac proximal to the vitellointestinal duct (Fig. 3). No labeled cell was seen in the rest of the endodermal layer of the external yolk sac. In the vitellointestinal duct, the immunostained cells appeared as cuboid, ovoid or fusiform in shape, and were apparently diagnosed as the "closed type" of the cells, i.e., no apical process projecting into the cavity of the duct could be found.

In the skate, NPY-positive cells could also been found in the epithelial layer of the vitellointestinal duct, pancreas, and midgut of the 42-mm embryo, the minimum size of the embryos examined in this study (Fig. 4). In the larger embryo, 58 mm in total length, the immunopositive cells tended to be increased in number and in intensity of immunostaining (Fig. 5). Their occurrence extended to the epithelial layer of the internal yolk sac, although none of the

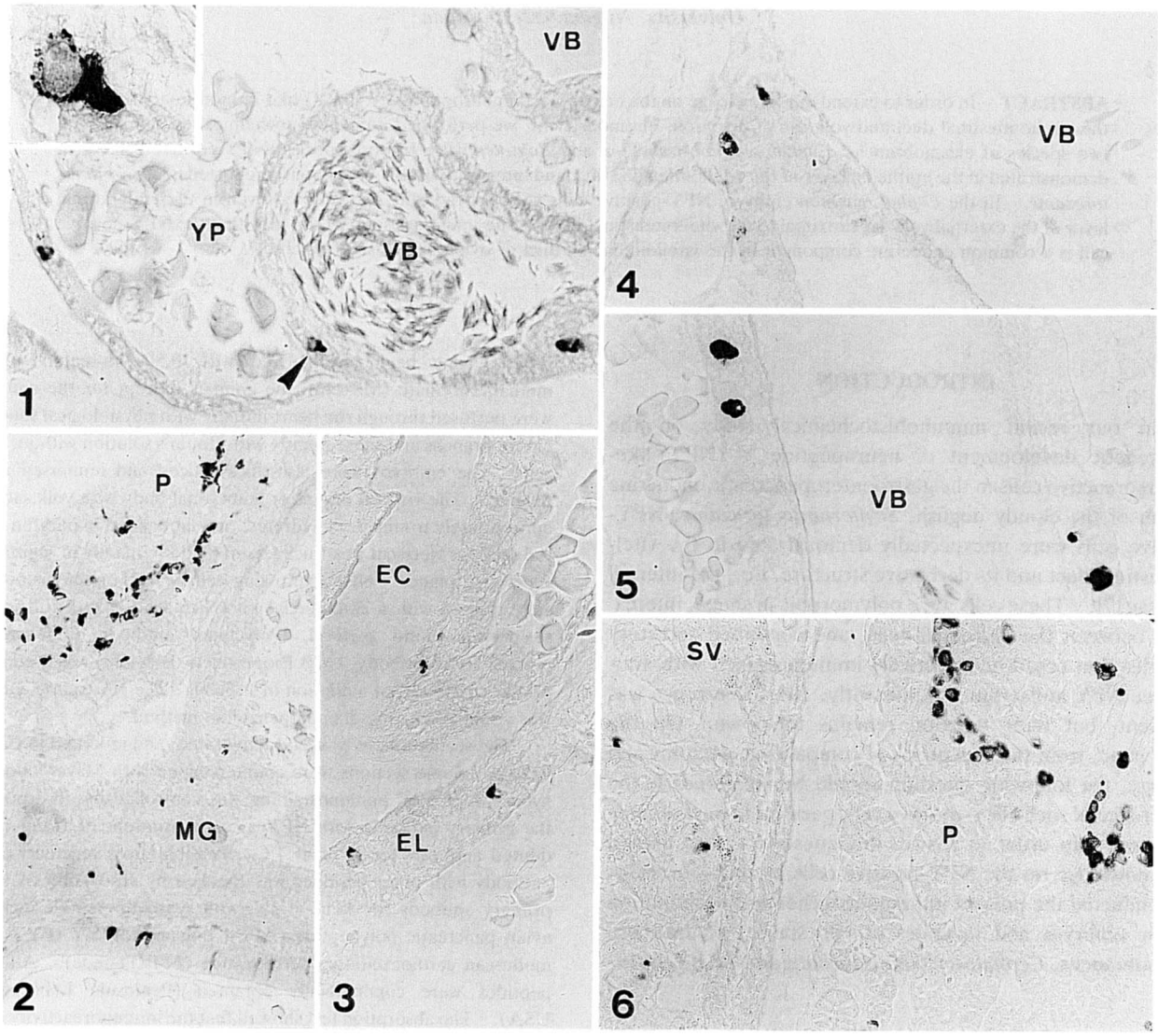

FIG. 1. Cross section of the vitellointestinal duct of an embryo of *Cephaloscyllium isabellum* showing NPY-immunoreactive cells in the epithelial lining. Inset shows an enlarged view of the immunostained cell indicated by the arrowhead. YP, yolk platelets; VB, vitellin blood vessel. × 700. (inset, × 2000).

FIG. 2. Sagittal section of the midgut (MG) and pancreas (P) of the *Cephaloscyllium* embryo showing NPY-positive cells in the gut epithelium and pancreas. × 400.

FIG. 3. Sagittal section of the wall of the external yolk sac proximal to the vitellointestinal duct showing NPY-positive cells in the endodermal layer (EL). EC, extraembryonic coelum. × 400.

FIG. 4. Sagittal section of the vitellointestinal duct of an embryo of *Raja kenojei* at the 42-mm stage showing NPY-positive cells in the epithelial lining. VB, vitelline blood vessel. × 700.

FIG. 5. NPY-positive cells in the epithelial lining of the vitellointestinal duct of an embryo of *Raja* at the 58-mm stage. VB, vitelline blood vessel. × 700.

FIG. 6. A part of the pancreas (P) and spiral valve (SV) of a juvenile fish of *Raja* showing a number of NPY-positive cells in the pancreas. × 400.

cells were detected in the endodermal layer of the external yolk sac. Immunopositive cells were also absent in the shrunken external yolk sac of a prehatching embryo at the 88-mm stage. In this stage, the labeled cells were infrequent in the vitellointestinal duct and internal yolk sac. In a juvenile at the 95-mm stage, around 13 days after hatching, the vitellointestinal duct and internal yolk sac were markedly shrunken due to rapid reabsorption of the yolk platelets, which shrinkage resulted in their appearance as a minute swelling attached to the external wall of the anterior part of the spiral intestine. Immunopositive cells were no longer demonstrated in this swelling. By this stage, NPY-positive cells had increased in number in the pancreas and spiral valve (Fig. 6), and their location extended to the pyloric portion of the stomach. Thus, in the posthatching juvenile, the distribution of the NPY-like-immunoreactive cells in the gastroenteropancreatic endocrine system attained to that of adult fish.

The present study on the embryos and/or juveniles of *Cephaloscyllium* and *Raja* confirmed our previous results on the occurrence of NPY-like-immunoreactive cells in the vitellointestinal duct and internal yolk sac of *Scyliorhinus torazame* [7], but minor differences were also noticed: (1) the NPY-positive cells were less frequent in *Cepahloscyllium* and *Raja* than in *Scyliorhinus*; (2) immunopositive cells with slender processes were occasionally seen in *Scyliorhinus* [7], whereas such cells were hardly demonstrated in *Cephaloscyllium* and *Raja*. Currently, we have no special idea to explain these interspecific differences. In this connection, information from the viewpoint of comparative anatomy is additionally required. In *Scyliorhinus*, the NPY-positive cells in the vitellointestinal duct and internal yolk sac seemed to be transitory [7]. This should be true in *Raja* but the present study failed to demonstrate the degenerative change of the immunostained cells as reported in *Scyliorhinus*. Therefore, in *Raja* it is not clear whether the posthatching disappearance of the immunoreactivity in the embryonic nutritive organs is caused by disappearance of the tissue antigen itself or by disappearance of the immunostained cells themselves. Apart from this problem, discussion should be extended to the possible function of the NPY-positive cells in the embryonic nutritive organs of elasmobranchs. Apparently, the transient occurrence of these cells in the dogfish [7] and skate (the present data) leads us to consider that their function may be essentially involved in embryonic life. In the dogfish, the NPY-positive cells in the embryonic nutritive organs were categorized as a member of the gastroenteropancreatic endocrine system [7]. So, potentially, these cells may have regulatory function(s) in embryonic life, e.g., control of digestion, control of yolk movement from the yolk sac to the embryonal gut, or control of blood streaming or pressure in the yolk stalk. According to TeWinkel [18], movement of yolk platelets from the external yolk sac to the internal yolk sac and from there to the gut lumen is accomplished by ciliary movement along the luminal surface of the vitellointestinal duct, internal yolk sac, and gut epithelium. So far, we have no direct or indirect evidence to correlate the NPY-positive cells with regulation of the ciliary movement.

NPY-like peptide was isolated from the pancreas of the dogfish [10] and skate [11], and its primary structure was reported to be more similar to mammalian NPY than to mammalian PYY or PP. Bioactivity of the dogfish NPY was tested by Conlon *et al.* [10], who showed that this peptide has vasoconstrictory action similar to human NPY. In addition, electrophysiological effects on the gut epithelium of NPY or NPY-like peptide were also reported in teleosts [16]. These data should be taken in consideration for future analysis of the function of the NPY-positive cells in the embryonic nutritive organ of elasmobranchs. Immunohistochemical data hitherto obtained on the gastroenteropancreatic endocrine system of elasmobranchs [7–9], teleosts [1, 3–5, 15], and cyclostome [6] are also helpful for discussion of the NPY-positive cells from the viewpoint of comparative anatomy. The present findings together with our previous data [7] extend our knowledge about the ontogenetic aspect of the endocrine cells containing NPY/PYY/PP-like peptide(s) in the gastroenteropancreatic system of various vertebrate groups [2, 12–14, 17].

REFERENCES

1 Agulleiro B, Lozano MT, Abad ME, Garcia Hernández, MP (1993) Electron microscopic immunocytochemical study of the endocrine pancreas of sea bass (*Dicentrarchus labrax*). Cell Tissue Res 274: 303–314

2 Alumets PC, Håkanson R, Sundler F (1978) Distribution, ontogeny and ultrastructure of pancreatic polypeptide (PP) cells in the pancreas and gut of the chicken. Cell Tissue Res 194: 377–386

3 Barton CL, Shaw C, Halton DW, Johnston CF, Buchanan KD (1992) Distribution and characterisation of neuropeptide Y immunoreactivity in the brain and gastrointestinal tract of the rainbow trout, *Oncorhynchus mykiss*. Comp Biochem Physiol 103C: 175–180

4 Beorlegui C, Martinez A, Sesma P (1992) Some peptide-like colocalizations in endocrine cells of the pyloric caeca and the intestine of *Oncorhynchus mykiss* (Teleostei). Cell Tissue Res 269: 353–357

5 Burkhardt-Holm P, Holmgren S (1989) A comparative study of neuropeptides in the intestine of two stomachless teleosts (*Poecilia reticulata, Leuciscus idus melanotus*) under conditions of feeding and starvation. Cell Tissue Res 255: 245–254

6 Cheung R, Andrews PC, Plisetskaya EM, Youson JH (1991) Immunoreactivity to peptides belonging to the pancreatic polypeptide family (NPY, aPY, PP, PYY) and to glucagon-like peptide in the endocrine pancreas and anterior intestine of adult lampreys, *Petromyzon marinus*: an immunohistochemical study. Gen Comp Endocrinol 81: 51–63

7 Chiba A, Honma Y, Oka S (1995) Ontogenetic development of neuropeptide Y-like immunoreactive cells in the gastroenteropancreatic endocrine system of the dogfish. Cell Tissue Res (in press)

8 Cimini V, Van Noorden S, Nardini V (1989) Peptides of the gastrointestinal tract of the dogfish (*Scyliorhinus stellaris*). J Exp Zool 2: 146–157

9 Cimini V, Van Noorden S, Sansone M (1992) Neuropeptide Y-like immunoreactivity in the dogfish gastroenteropancreatic

tract: light and electron microscopical study. Gen Comp Endocrinol 86: 413–423

10 Conlon JM, Balasubramaniam A, Hazon N (1991a) Structural characterization and biological activity of a neuropeptide Y-related peptide from the dogfish, *Scyliorhinus canicula*. Endocrinology 128: 2273–2279

11 Conlon JM, Bjenning C, Moon TW, Youson JH, Thim L (1991b) Neuropeptide Y related peptides from the pancreas of a teleostean (eel), holostean (bowfin) and elasmobranch (skate) fish. Peptides 12: 221–226

12 El-Salhy M (1984) Immunocytochemical investigation of the gastro-entero-pancreatic (GEP) neurohormonal peptides in the pancreas and gastrointestinal tract of the dogfish *Squalus acanthias*. Histochemistry 80: 193–205

13 El-Salhy M, Grimelius L, Emson PC, Falkmer S (1987) Polypeptide YY- and neuropeptide Y-immunoreactive cells and nerves in the endocrine and exocrine pancreas of some vertebrates: an onto- and phylogenetic study. Histochem J 19: 111–117

14 Garcia Hernández MP, Agulleiro B (1992) Ontogeny of the endocrine pancreas in sea bass (*Dicentrarchus labrax*): an immunocytochemical study. Cell Tissue Res 270: 339–352

15 Jönsson A-C (1993) Co-localization of peptides in the Brockmann bodies of the cod (*Gadus morhua*) and the rainbow trout (*Oncorhynchus mykiss*). Cell Tissue Res 273: 547–555

16 Kiliaan AJ, Holmgren S, Jönsson A-C, Dekker K, Groot JA (1993) Neuropeptides in the intestine of two teleost species (*Oreochromis mossambicus, Carassius auratus*): localization and electrophysiological effects on the epithelium. Cell Tissue Res 271: 123–134

17 Sundler F, Håkanson R, Larsson L-I (1977) Ontogeny of rat pancreatic polypeptide (PP) cells. Cell Tissue Res 178: 303–306

18 TeWinkel LE (1943) Observations on later phases of embryonic nutrition in *Squalus acanthias*. J Morphol 73: 177–205