Gastro-Entero-Pancreatic (GEP) Endocrine System of the Flatfish, *Paralichthys olivaceus*: An Immunocytochemical Study

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Summary. The localization of the gastro-entero-pancreatic (GEP) endocrine cells in the flatfish, *Paralichthys olivaceus* was examined using immunocytochemical methods.

The Brockmann body of the flatfish included a large principal islet and a smaller islet. Three types of endocrine cells, i.e., insulin-, glucagon- and somatostatin-immunoreactive cells were found in both islets. Pancreatic polypeptide (PP)-immunoreactive cells were restricted to the periphery of the smaller islet.

In the digestive tract, somatostatin cells occurred only in the stomach. The pyloric appendages contained cells reactive simultaneously to cholecystokinin (CCK) antiserum and to gastrin antiserum, whereas the middle portion of the intestine contained cells reactive only to the CCK antiserum. The intestinal endocrine cells showing N-terminal glucagon-immunoreactivity were also reactive to a glicentin antiserum.

The endocrine pancreas of teleost fish is mainly composed of two types of islets: 1) one, two or even multiple large islets, called principal islets and 2) numerous, widely scattered small islets (Falkmer and Östberg, 1977). The largest of the principal islets, sometimes including a few associated smaller ones, may form a body visible by the naked eye, called the Brockmann body. The Brockmann body is well-developed in a group of the teleosts, i.e., the Ctenosquamata among Euteleostei (Falkmer and Östberg, 1977), including the flatfish, which is the subject of this study.

The endocrine pancreas of teleosts have been studied by several research groups using immunocytochemical techniques (Johnson et al., 1976, 1982; Klein and Lange, 1977; Klein and Van Noorden, 1978, 1980; Stefan et al., 1978; Van Noorden and Patent, 1978; Langer et al., 1979; Rombout et al., 1979; Stefan and Falkmer, 1980; Wagner and McKeown, 1981; Rombout and Taverne-Thiele, 1982). There have been four types of endocrine cells found that are immunoreactive to the antisera against mammalian insulin, glucagon, somatostatin and pancreatic polypeptide (PP), respectively, but the localization of the cells within the islets and the cell population seem considerably variable among species. It is especially the case in the occurrence of PP-immunoreactive cells.

As to the immunocytochemical studies of the endocrine cells occurring in the teleostean digestive tract, several reports are available (Langer et al., 1979; Noailiac-Depeyre and Hollande, 1981; Holmgren et al., 1982; Rombout and Taverne-Thiele,
1982), but our knowledge on their types and distribution is meager as yet. The glucagon-, somatostatin-, PP-, gastrin/cholecystokinin(CCK)-, met-enkephalin-, substance P- and bombesin-like immunoreactivities have been demonstrated in the gut endocrine cells of teleosts.

This paper deals immunocytochemically with the endocrine cells in the pancreas of the flatfish, *Paralichthys olivaceus*, which comprise a typical Brockmann body, as well as the endocrine cells dispersed in the gastrointestinal tract.

**MATERIALS AND METHODS**

Ten flatfish, *Paralichthys olivaceus* (average body length, 38 cm) captured near Niigata in the Japan Sea were used in this study. After decapitation, the Brockmann body and various regions of the digestive tract, i.e., the esophagus, gastric corpus, pyloric antrum, pyloric appendages, middle portion of the intestine and rectum were dissected out and fixed in Bouin's fluid for 2-6 hrs. All specimens were dehydrated through an ethanol-xylene series, embedded in paraffin and cut at about 4 μm in thickness, sometimes serially cut at about 2 μm.

Dewaxed paraffin sections were submitted to the indirect immunoperoxidase method (NAKANE and PIERCE, 1966) for the staining of insulin and the peroxidase-antiperoxidase (PAP) method (STERNBERGER, 1974) for the other hormones or bioactive peptides. The following antisera against various hormones or peptides were used in this study: a guinea pig antisemum against bovine/porcine insulin (at a dilution of 1:160) and rabbit antisera against synthetic somatostatin 14 (1:600), bovine pancreatic polypeptide (BPP) (1:500), human gastrin I (specific for the middle region of gastrin, R 1301, 1:500), cholecystokinin (CCK) 1-27 (R 5905, 1:600), porcine secretin (R 801, 1:600), porcine vasoactive intestinal polypeptide (VIP) (R 501, 1:500) and glicentin C-terminal fragment corresponding to the 49-69 sequence (R 4804, 1:500). Glucagon antisera used in the present study were N-terminal specific (1:500) and C-terminal specific antisera (OAL 123, 1:500).

The specificity of the immunostaining was checked by using, instead of the first

| Table 1. Distribution of immunoreactivities of peptide hormones in the GEP endocrine system of the flatfish, *Paralichthys olivaceus* |
|---------------------------------------------------------------|
| Pancreas | Esophagus | Stomach | Pyloric appendages | Middle portion of intestine | Rectum |
|----------|-----------|---------|-------------------|-------------------------|--------|
| Insulin  | +         | -       | -                 | -                       | -      |
| Glucagon | +         | -       | -                 | -                       | -      |
| (C-terminus) |         |         |                   |                         |        |
| Glucagon | +         | -       | -                 | +                       | +      |
| (N-terminus) |         |         |                   |                         |        |
| Glicentin| +         | -       | +                 | +                       | +      |
| Somatostatin | +       | -       | +                 | -                       | -      |
| PP (BPP) | +         | -       | -                 | -                       | -      |
| Gastrin  | -         | -       | -                 | +                       | -      |
| CCK      | -         | -       | -                 | +                       | +      |
| Secretin | -         | -       | -                 | -                       | -      |
| VIP      | -         | -       | -                 | -                       | -      |

+: Occurrence of immunoreactive cells, -: immunoreactive cells not detectable.
antiserum, a normal guinea pig serum or rabbit serum, and by using the antiserum preincubated with the corresponding antigen (10–20 μg peptide/ml diluted antiserum).

Some of sections from the Brockmann body were stained by aldehyde-fuchsin, combined with Masson-Goldner’s trichrome method.

RESULTS

The results of the immunocytochemical examination are shown in Table 1. All immunostaining controls were negative in reaction, supporting the specificity of the immunoreactions concerned.

Endocrine cells of the pancreas

The Brockmann body of the flatfish was an ellipsoid, measuring 2–3 mm in the major axis and 1–2 mm in the minor axis, and was located between the gall bladder and the cranial portion of the stomach. The major part of the Brockmann body was occupied by a large islet, i.e., the principal islet. Exocrine pancreatic parenchyma, in some places only a thin layer of the parenchyma, surrounded the islet. In addition, a smaller islet was found near the principal islet within the exocrine zone of the Brockmann body (Fig. 1).

The principal islet was often clearly distinguishable into the central and peripheral regions by their cellular composition. Round to oval somatostatin-immunoreactive cells were dispersed through the peripheral region (Fig. 3). The remaining spaces in the peripheral region were occupied by stellate glucagon-immunoreactive cells which extended their cytoplasmic processes among the somatostatin cells (Fig. 4). The glucagon cells were immunoreactive both to the N-terminus- and C-terminus-specific anti-glucagon sera. In the central region of the principal islet, insulin-immunoreactive cells were predominant. They were oval or spindle-like in shape and loosely gathered, tending to form cell cords (Fig. 2). The remaining spaces in the central region were occupied by the somatostatin cells of irregular shapes. No PP-immunoreactive cells was observed in the principal islet.

The smaller islet had the similar cellular composition to the principal islet, although it was not so easy to distinguish the central and peripheral regions. Insulin and glucagon cells were present in the central and peripheral region, respectively. Somatostatin cells were distributed through the islet. This accessory islet

Fig. 1. The principal islet and accessory islet (arrow) in the flatfish pancreas. Aldehyde-fuchsin and Masson-Goldner’s staining. Insulin cells, which occupy the central region of both islets, are dyed with aldehyde-fuchsin, appearing dark in the picture. ×50
Fig. 2-5. Legends on the opposite page.
was characterized by a rim of PP-immunoreactive cells located in the most peripheral part of the islet (Fig. 5).

**Gut endocrine cells**

Somatostatin-immunoreactive cells were found only in the gastric corpus and pyloric antrum (Fig. 6). In the gastric corpus, they were gathered in the neck portion of the fundic glands, and were dispersed in the surface epithelium. Most of them showed a thickened pyramidal shape and extended their apical process to the lumen: they were open in type. The cells were also open-type in the pyloric antrum.

Gastrin-immunoreactive cells were restricted to the mucosa of the pyloric appendages, which corresponded histologically to the duodenum of the higher vertebrates (Fig. 8a). CCK-immunoreactive cells occurred in the pyloric appendages and middle portion of the intestine of this fish (Fig. 8b). Observation of serially cut, adjacent sections revealed that some of gastrin cells in the pyloric appendages simultaneously reacted to the CCK antiserum (Fig. 8a, b).

Dispersed epithelial cells in the pyloric appendages and the middle portion of the intestine were reactive to the N-terminus-specific anti-glucagon serum (Fig. 7). The cells reacted similarly to the glicentin antiserum, but not to the C-terminus-specific anti-glucagon serum.

The gastrin, CCK and glucagon (glicentin)-like immunoreactive cells in the pyloric appendages and intestine were slender in shape and were all open in type.

No epithelial cells in the esophagus and rectum were reactive to any of the antisera used.

**DISCUSSION**

This study revealed that the endocrine pancreas of the flatfish contains insulin-, glucagon-, somatostatin- and PP-immunoreactive cells. It seems a general rule in the endocrine pancreas of teleosts, in both principal and dispersed smaller islets, that the insulin and glucagon cells occur in the central and peripheral regions, respectively (KLEIN and LANGE, 1977; LANGER et al., 1979; KLEIN and VAN NOORDEN, 1980; STEFAN and FALKMER, 1980; WAGNER and MCKEOWN, 1981; ROMBOUT and TAVERNE-THIELE, 1982). This distribution pattern of the cells was confirmed to occur in the principal and accessory islets of the flatfish in the present study.

Somatostatin cells of the teleostean islets have been reported to be dispersed mainly

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**Fig. 2-5.** Islet cells stained with the antisera against insulin (Fig. 2), somatostatin (Fig. 3), glucagon (Fig. 4) and PP (Fig. 5). Fig. 2a, 3a and 4a (×230) show the same field from successive serial sections of the principal islet, and their closer views are shown in Fig. 2b, 3b and 4b (×570). Fig. 5a (×200) and its closer view Fig. 5b (×700) are from the accessory islet.

**Fig. 2.** a and b. Numerous insulin cells are present in the central region of the islet, and not seen in the peripheral region (*).

**Fig. 3.** a and b. Somatostatin cells of rounded shapes are seen in the peripheral region of the principal islet, whereas those of irregular shape are dispersed in the central region. In Fig. 3b, the arrows show a boundary line between the peripheral and central regions.

**Fig. 4.** a and b. Glucagon cells extend their slender cytoplasm through the peripheral region, leaving the spaces for somatostatin cells.

**Fig. 5.** a. PP cells are restricted to the periphery of the accessory islet. Fig. 5b is the closer view of the rim of the islet composed of PP cells.
in the central region, intermingled with insulin cells (KLEIN and VAN NOORDEN, 1978; LANGER et al., 1979; STEFAN and FALKMER, 1980; ROMBOUT and TAVERNE-THIELE, 1982). In the flatfish, however, numerous somatostatin-immunoreactive cells occur in the peripheral region of the islet, besides the common central region. It was a remarkable finding in the present study that the somatostatin-immunoreactive cells were different in shape due to their location as demonstrated above.

PP cells as the fourth cell type were demonstrated first by STEFAN et al. (1978) and VAN NOORDEN and PATENT (1978) in the pancreas of some teleosts. Later, it has been revealed that PP cells were conspicuously variable in distribution among species, although the cells, if they occur, were always located at the periphery of the islets. In Barbus conchonius, PP cells were scarce in the principal islet, but were more numerous in the periphery of the smaller islets (ROMBOUT and TAVERNE-THIELE, 1982). Another species of teleost, Cottus scorpius possessed two principal islets, one juxtasplenic islet which was lacking in PP cell and the other, juxtapyloric islet which contained numerous PP cells at the periphery (STEFAN and FALKMER, 1980). In a teleostean fish, Xiphophorus helleri examined by KLEIN and VAN NOORDEN (1980), many PP cells were located

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**Fig. 6.** Somatostatin cells in the gastric corpus. $\times 270$

**Fig. 7.** Slender endocrine cells in the middle portion of the intestine are immunoreactive to the N-terminal glucagon antiserum. $\times 520$

**Fig. 8.** Adjacent serial sections from the pyloric appendage are stained with the antisera against gastrin (a) and CCK (b). As the arrow shows, one of the gastrin cells apparently corresponds to the CCK cell. $\times 340$
in the principal islet. Johnson et al. (1982) reported that in Lophius americanus (anglerfish), PP cells were localized in all of the principal (splenic), secondary (pyloric), and tertiary (mesenteric) islets. On the other hand, no PP cells could be found in the endocrine pancreas of some species of teleosts previously examined (Van Noorden and Patent, 1978; Langer et al., 1979). The pancreas of the flatfish, in which the PP cells were present only in the periphery of the accessory islet, may correspond to the condition of the Barbus conchonius. Moreover, the principal and accessory islets of the flatfish may be similar in the localization of pancreatic endocrine cells including PP cells to the splenic and pyloric principal islets of Cottus scorpius, respectively.

Only somatostatin- and glucagon-like immunoreactivities among four peptides found in the endocrine pancreas of the flatfish were recognized in the digestive tract. It has been reported that somatostatin cells in the teleostean gut were restricted to the stomach (Langer et al., 1979; Noailiac-Depeyre and Hollande, 1981; Holmgren et al., 1982). The present study confirms this finding in the flatfish. This is contrasted by the mammalian gut, in which somatostatin cells are widely distributed from the stomach down to the large intestine. Judging from the immunoreactivity to the three kinds of antisera against glucagon-related peptides, the glucagon-like immunoreactivity in the gut of this fish seems due to the occurrence of glicentin-like substance, as in mammals.

The presence of gastrin-like or CCK-like immunoreactivity in the gut of many teleosts using the immunocytochemical methods (Larsson and Rehfeld, 1977; Langer et al., 1979; Noailiac-Depeyre and Hollande, 1981; Rombout and Taverne-Thiele, 1982). Gastrin or CCK-related peptides in the teleostean gut seem to show as complicated a mode of occurrence as that in mammalian gut. Our findings on the gastrin-immunoreactive cells in the flatfish are in agreement with those reported by Langer et al. (1979) in five species of teleosts. These authors revealed that the endocrine cells reactive to the N-terminal gastrin antiserum, but non-reactive to the C-terminal antiserum occurred only in the upper part of the intestine. In our flatfish, some of gastrin cells in the pyloric appendages corresponded to the CCK cells, and in the middle portion of the intestine only CCK cells were present. We have no definite explanation accounting for the coexistence of gastrin and CCK-like immunoreactivities in the pyloric appendages of the flatfish. Interestingly, Vigna (1979) have suggested using a biological assay method that the gastrins may have evolved from a CCK-like factor at the level of the divergence of chondrichthyeans and osteichthyeans.

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