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Comparative study of endocrine cells in the principal pancreatic islets of two teleosts, *Silurus asotus* (Siluridae) and *Siniperca scherzeri* (Centropomidae)

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The regional distribution and relative frequency of some endocrine cells in the principal pancreatic islets of two teleosts, *Silurus asotus* Linne (Siluridae) and *Siniperca scherzeri* Steindachner (Centropomidae), which have similar feeding habits, were observed using specific antisera against insulin, glucagon, somatostatin and bovine pancreatic polypeptide (bovine PP) using the peroxidase antiperoxidase (PAP) method. Spherical to spindle shaped cells were demonstrated in the principal pancreatic islets in both species of teleost fishes. However, they were not detected in the exocrine portions nor the pancreatic ducts. Insulin-immunoreactive cells were located in the central regions of the principal pancreatic islets at high frequency in both species. Glucagon-immunoreactive cells were restricted to the peripheral regions of the principal pancreatic islets in both species. They formed a mantle zone in the peripheral regions of *Silurus asotus* with moderate frequency, and occupied a narrower mantle zone in *Siniperca scherzeri* with moderate frequency. In addition, glucagon-immunoreactive cell cores were also found in the peripheral zone of some principal pancreatic islets of *Siniperca scherzeri*. Somatostatin-immunoreactive cells were dispersed in the central zone of the principal pancreatic islets of *Silurus asotus* with moderate frequency, but were located in the peripheral regions with low frequency in *Siniperca scherzeri*. Bovine PP-immunoreactive cells were found in the peripheral region and the mantle zone of the principal pancreatic islets with low and rare frequency, respectively in both species. In conclusion, the regional distribution and relative frequency of endocrine cells in the principal pancreatic islets of *Silurus asotus* showed general patterns similar to those of other teleostean fishes. But, some species-dependent distributional patterns and/or relative frequencies, particularly in glucagon-, somatostatin- and bovine PP-immunoreactive cells, were detected in the principal pancreatic islets of *Siniperca scherzeri*.

Key words: Pancreas, teleosts, *Silurus asotus*, *Siniperca scherzeri*, endocrine cell, immunohistochemistry, principal pancreatic islets

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Introduction

Catfish, *Silurus asotus* Linne, belonging to the Siluridae in the order Siluriformes, are well recognized as stomach teleost freshwater fish, which are dispersed worldwide. *Siniperca scherzeri* Steindachner belonging to family Centropomidae in the order Perciformes are also stomach teleost freshwater fish but their habitations are limited to Korea and a part of China.

It is generally known that the pancreas of vertebrates is subdivided into two portions. One is exocrine where the digestive enzymes are released and the other is endocrine, where regulatory hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide (PP) are released into the blood vessels. The appearance, regional distribution and relative frequency of these regulatory hormones secreted by endocrine cells in the pancreas are well identified by histochemistry (Kobayashi and Ali, 1981), immunofluorescence (Orci, 1982) and immunohistochemistry (Sternberger *et al*., 1970). In addition to the above regulatory hormones, peptide YY-, neuropeptide YY- (Ali-Rachedi *et al*., 1984), motilin- (Yamada *et al*., 1986) and the chromogranin family- (Rindi *et al*., 1986; Ito *et al*., 1987) immunoreactive cells have also been demonstrated in the vertebrate pancreas. The pancreas has been treated as a valuable organ for endocrine studies and the endocrine pancreas has been extensively

studied in association with diabetes (Jansson and Sundler, 1988). Until now, investigations of the gastroenteropancreatic (GEP) endocrine cells have been considered to be an important part of a phylogenetic study (D'Este *et al*., 1994).

The endocrine pancreas of teleost fish is mainly composed of two types of pancreatic islets: 1) one, two or even more multiple large islets, called principal pancreatic islets and 2) numerous, widely scattered small islets (Falkmer and Östberg, 1977). Although studies have elucidated the regional distribution and relative frequency of endocrine cells, immunoreactivity to antisera against mammalian insulin, glucagon, somatostatin and PP, in the pancreas of teleosts, localization of endocrine cells in the principal pancreas of *Silurus asotus* and *Siniperca scherzeri* have not yet been reported. In addition, the localization of these endocrine cells within the pancreatic islets and cell population seemed considerably variable among species, especially in the case of PP-immunoreactive cells (Yoshida *et al*., 1983). Among teleosts, PP-immunoreactive cells, which were generally demonstrated, were not detected in the pancreas of the channel catfish and the lungfish (McNeill *et al*., 1984; Hansen *et al*., 1987).

In the present study, the regional distribution and relative frequency of endocrine cells in the principal pancreatic islets of two species teleosts, *Silurus asotus* Linne (Siluridae) and *Siniperca scherzeri* Steindachner (Centropomidae) which have similar feeding habits, were examined, using specific antisera against insulin, glucagon, somatostatin and bovine PP by the peroxidase antiperoxidase (PAP) method.

Materials and Methods

Each of five adult *Silurus asotus* Linne (Siluridae) and *Siniperca scherzeri* Steindachner (Centropomidae) was purchased from a merchant in Taegu, Korea and used in this study without sexual distinction. After decapitation, samples from the pancreas were fixed in Bouin's solution. After paraffin embedding, 3-4 sections were prepared, and representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal alimentary architecture.

Deparaffinized sections were rehydrated and submitted for PAP (Sternberger, 1979). Background blocking was performed with normal goat serum prior to incubation with specific antisera (Table 1). After rinsing in phosphate buffered saline (PBS, 0.01 M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution of 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05 M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive cells were observed under a light microscope.

Results

The principal islets were often clearly distinguishable in the central, mantle and the peripheral regions by their cellular composition. The regional distribution and relative frequency of endocrine cells in the principal islets of two species of teleostean fishes are summarized in Table 2.

Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
Insulin	PUO290395	BioGenex, San Ramon	1 : 24
Glucagon	PUO390699	BioGenex, San Ramon	1 : 20
Somatostatin	PUO420198	BioGenex, San Ramon	1 : 20
Bovine pancreatic polypeptide (bovine PP)	i607	UCB bioproducts, Drogenbos	1 : 5,000

*All antisera were raised in rabbits except for insulin which was raised in guinea pigs

Table 2. Regional distribution and relative frequency of the endocrine cells in the principal pancreatic islets of two stomach teleostean fishes, *Silurus asotus* and *Siniperca scherzeri*

Hormones	*Silurus asotus*			*Siniperca scherzeri*		
	Central region	Mantle zone	Peripheral region	Central region	Mantle zone	Peripheral region
Insulin	+++	–	–	+++	–	–
Glucagon	–	++	±	–	±	++
Somatostatin	++	±	±	–	±	+
Bovine pancreatic polypeptide	–	±	+	±	±	+

+++: numerous, ++: moderate, +: a few, ±: rare, –: not detected.

Spherical to spindle shaped immunoreactive cells having cytoplasmic process were demonstrated in the pancreatic islets, but no cells were detected in the exocrine portions nor the pancreatic ducts. Different distribution patterns of these immunoreactive cells, especially glucagon- and somatostatin-immunoreactive cells, were shown by the principal pancreases of the two species.

Endocrine cells in the principal pancreatic islets of *Silurus asotus*

Insulin-immunoreactive cells were located in the central regions of the principal pancreatic islets with high frequency, forming central cores. However, no insulin-immunoreactive cells were detetcted in the mantle and peripheral regions (Figs. 1A and B). Glucagon-immunoreactive cells were situated in the peripheral regions and formed a two to five cell thickness mantle zone, at relatively lower frequency than that of insulin-immunoreactive cells (Figs. 1C and D). Somatostatin-immunoreactive cells were randomly dispersed in the central regions with moderate frequency and their cytoplasmic processes were extended among the insulin-immunoreactive cells in that region. In addition, rarely somatostatin-immunoreactive cells were also found in the mantle zone and peripheral regions. In the mantle zone where most of glucagon-immunoreactive cells were situated, their cytoplasmic processes extended among the glucagon- and bovine PP-immunoreactive cells, and similar distributional patterns were seen in the peripheral zone, which was mainly occupied by bovine PP-immunoreactive cells (Figs. 1E and F). Bovine PP-immunoreactive cells were detected in the peripheral regions of the principal pancreas, and a small number of cells were also observed in the mantle zone where their cytoplasmic processes extended between the large mass of glucagon-immunoreactive cells (Figs. 1G and H).

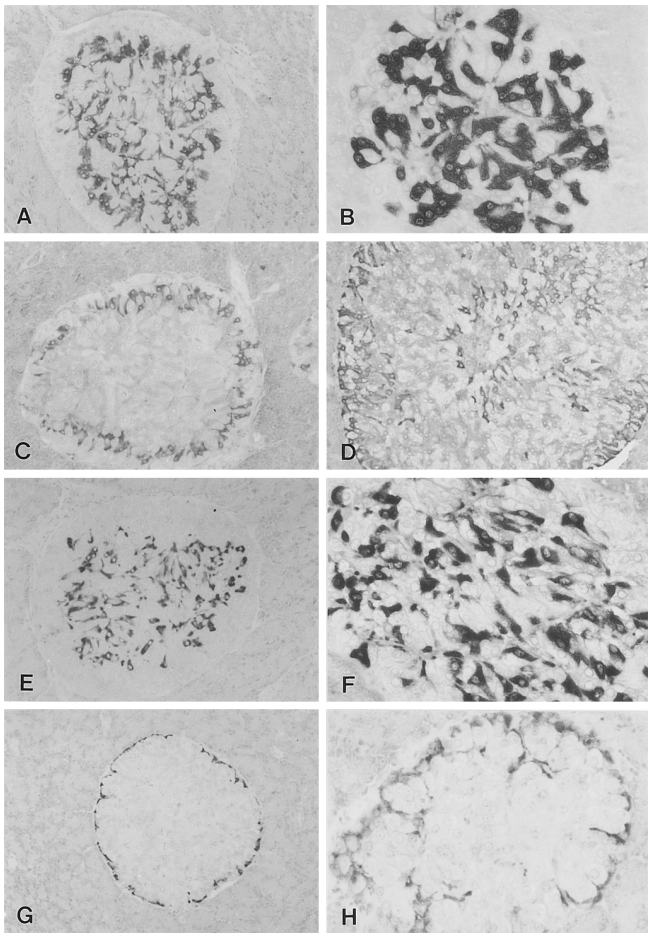

Fig. 1. Immunoreactive cells in the principal pancreatic islets of the *Silurus asotus*. Insulin-immunoreactive cells were located in the central region (A and B). Glucagon-immunoreactive cells surrounded the central regions, where insulin- and somatostatin-immunoreactive cells were located, and formed a mantle zone (C and D). Somatostatin-immunoreactive cells were observed in the central regions, intermingled with insulin-immunoreactive cells (E and F). Bovine pancreatic polypeptide-immunoreactive cells were situated in the peripheral regions and occasionally in the mantle zone, intermingled with glucagon-immunoreactive cells (G and H). A, C-E, G: ×175; B, F,H: ×350. PAP method.

Endocrine cells in the principal pancreatic islets of *Siniperca scherzeri*

Insulin-immunoreactive cells were situated in the central regions of the principal pancreatic islets with sufficient frequency to form a central core. However, no insulin-immunoreactive cells were detetcted in the mantle and peripheral regions (Figs. 2A and B). Glucagon-immunoreactive cells were located in the peripheral regions and formed a one to two cell thickness mantle zone in these region with relatively lower frequency than that of the insulin-immunoreactive cells. In addition, glucagon-immunoreactive cell cores, which consisted of numerous cells, were also found in the peripheral zone of some principal pancreatic islets (Figs. 1C and D). Somatostatin-immunoreactive cells were restricted to the mantle and peripheral regions at rare and low frequencies, respectively, and their cytoplasmic processes extended among the glucagon- and bovine PP-immunoreactive cells in these regions (Figs. 1E and F). Bovine PP-immunoreactive cells were detected in the regions similar to those of glucagon-immunoreactive cells, but their relative frequencies in these regions was somewhat lower than that of the glucagon-immunoreactive cells. In that regions, their cytoplasmic processes were extended between glucagon-immunoreactive cells. In addition, endocrine cells were also rarely distributed in the central regions, which were occupied by numerous insulin-immunoreactive cells (Figs. 1G and H).

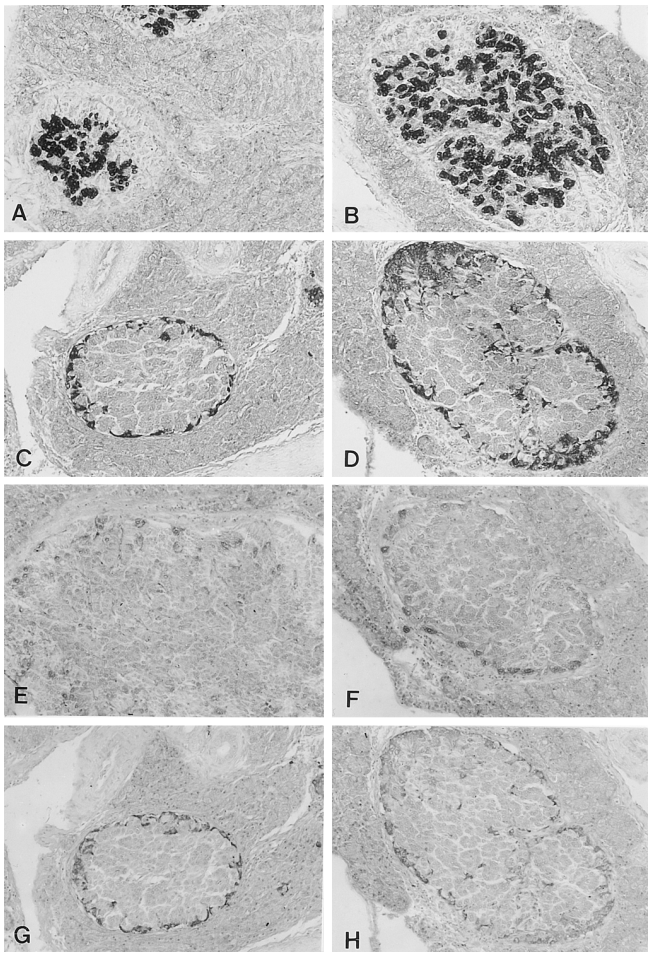

Fig. 2. Immunoreactive cells in the principal pancreatic islets of *Siniperca scherzeri*. Insulin-immunoreactive cells were located in the central region (A and B). Glucagon-immunoreactive cells surrounded the central regions, where insulin-immunoreactive cells were located, and formed a mantle zone. In addition, glucagon cells masses were also detected in some islets (C and D). Somatostatin-immunoreactive cells were demonstrated in the peripheral regions, intermingled with bovine pancreatic polypeptide- and glucagon-immunoreactive cells (E and F). Bovine pancreatic polypeptide-immunoreactive cells were situated in the peripheral regions and occasionally in the mantle zone, intermingled with glucagon- and somatostatin-immunoreactive cells (G and H). A-H: ×175. PAP method.

Discussion

This study revealed that the principal pancreatic islets of the two species of teleostean fishes, having similar feeding habits, contained insulin-, glucagon, somatostatin- and bovine PP-immunoreactive cells. In the present study, somewhat different distributional patterns of these four types of immunoreactive cells were observed in the two species. In addition, species-dependent distributional patterns were also observed, especially for the somatostatin- and bovine PP-immunoreactive cells.

Insulin is synthesized in the B cells of the pancreatic islets and regulates the serum glucose levels (Hsu and Crump, 1989). The regional distribution and relative frequency of the insulin-immunoreactive cells in the pancreas of numerous teleosts have been reported in the lungfish (Hansen *et al*., 1987), flatfish (Yoshida, 1983), gilt-head sea bream (Guyot *et al*., 1998), five species of osteoglossomorpha, an ancient teleostean group (Al-Mahrouki and Youson, 1998), *Protopterus annectens* (Tagliafierro *et al*., 1996), dipnoan fish (Scheuermann *et al*., 1996), anglerfish and channel catfish (Johnson *et al*., 1976). From these previous reports, it seems to be a general rule that in the pancreatic islets of teleosts, insulin-immunoreactive cells occur in the central regions and our results correspond well in these respect to previous reports (Johnson *et al*., 1976; Yoshida, 1983; Hansen *et al*., 1987; Scheuermann *et al*., 1996; Tagliafierro *et al*., 1996; Al-Mahrouki and Youson, 1998; Guyot *et al*., 1998), insulin-immunoreactive cells were found in the central regions of the principal pancreatic islets of both species in the present study.

Glucagon is synthesized in the A cells of the pancreas and regulates glucose levels in the blood (Hsu and Crump, 1989). Morphologically similar cells are also observed in the digestive tract of the dog. The regional distribution and relative frequency of glucagon-immunoreactive cells in the teleostean pancreas have been reported in the flatfish (Yoshida *et al*., 1983), Carp (Rombout *et al*., 1986), five species of osteoglossomorpha, and an ancient teleostean group (Al-Mahrouki and Youson, 1998), gar (Groff and Youson, 1997), *Protopterus annectens* (Tagliafierro *et al*., 1996), dipnoan fish (Scheuermann *et al*., 1996), anglerfish and channel catfish (Johnson *et al*., 1976), and *Xiphophorus helleri* (Klein and Van Noorden, 1980). It also seems to be a general rule in the pancreatic islets of teleosts that glucagon-immunoreactive cells occur in the peripheral regions and that they form a small mantle zone or rim surrounding centrally located insulin-immunoreactive cells except in the case of osteoglossomorpha, which shows a scattered immunoreactivity throughout the central portion of the islets in addition to the common peripheral regions. In the present study, glucagon-immunoreactive cells were found to be located in the peripheral regions of the principal islets of both species and formed a two to five (in *Silurus asotus*) or one to two (in *Siniperca scherzeri*) cell thickness mantle zone. These results were similar to those of previous studies (Johnson *et al*., 1976; Klein and Van Noorden, 1980; Yoshida *et al*., 1983; Rombout *et al*., 1986; Scheuermann *et al*., 1996; Tagliafierro *et al*., 1996; Groff and Youson, 1997). However, cell masses consisting of numerous glucagon-immunoreactive cells were also found in the principal pancreatic islets of *Siniperca scherzeri*, which were restricted in some islets. These findings are considered to represent a species-dependent unique

distributional pattern. In addition, the cell layer of the mantle zone also differed in the two species.

Somatostatin, which consists of 14 amino acids, was isolated from the hypothalamus of sheep, and exists in straight and cyclic forms (Brazeau *et al*., 1973). This substance inhibits the secretion of the gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid (Kitamura *et al*., 1984) and the absorption of amino acid, glucose and fatty acid in the gastrointestinal tract (Brazeau *et al*., 1973). Somatostatin-immunoreactive cells of the teleostean islets have been reported to be dispersed mainly in the central region, intermingled with insulin-immunoreactive cells (Stefan and Falkmer, 1980; Rombout and Taverne-Thiele, 1982). However, Yoshida *et al.* (1983) suggested that somatostatin-immunoreactive cells occur in the peripheral regions of islets intermingled with insulin cells, in addition to the central regions, and similar distributional patterns have also been seen in *Protopterus annectens* (Tagliaflerro *et al*., 1996). In addition, Scheuermann *et al*. (1991) reported that somatostatin-immunoreactive cells were scattered throughout the islets of dipnoan fish. Although in the present study somatostatin-immunoreactive cells in the principal pancreatic islets of *Silurus asotus* were found to be located in the central regions, which in similar to previous reports (Stefan and Falkmer, 1980; Rombout and Taverne-Thiele, 1982), in *Siniperca scherzeri*, they were restricted to the peripheral regions with glucagon- and bovine PP-immunoreactive cells. This is considered to be an unique distributional pattern of *Siniperca scherzeri*.

PP-immunoreactive cells, 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 was revealed that PP-immunoreactive cells were conspicuously variable in distribution among species, although the cells, if they occured, were always located in the peripheral regions of the pancreatic islets. PP-immunoreactive cells were detected in the principal pancreas of *Cottus scorpius* (Stefan and Falkmer, 1980), *Barbus conchonius* (Rombout and Taverne-Thiele, 1982), *Xiphophorus helleri* (Klein and Van Noorden, 1980), anglerfish (Johnson *et al*., 1982), flatfish (Yoshida *et al*., 1983), five species of osteoglossomorpha, an ancient teleostean group (Al-Mahrouki and Youson, 1998) and gar (Groff and Youson, 1997). However, no PP-immunoreactive cells were found in the pancreas of the channel catfish (McNeill *et al*., 1984) and lungfish (Hansen *et al*., 1987). In the present study, and as has been found in other teleostean fishes (Stefan *et al*., 1978; Van Noorden and Patent, 1978; Klein and Van Noorden, 1980; Stefan and Falkmer, 1980; Johnson *et al*., 1982; Yoshida *et al*., 1983; Groff and Youson, 1997; Al-Mahrouki and Youson, 1998), bovine PP-immunoreactive cells were mainly distributed in the peripheral regions of the principal pancreatic islets of both species with glucagon-immunoreactive cells and occasionally with somatostatin-immunoreactive cells. However, rarely cells were dispersed in the central regions of pancreatic islets of *Siniperca scherzeri*, *which* was considered to be a species-dependent distributional patterns.

In conclusion, the regional distribution and relative frequency of insulin-, glucagon-, somatostatin- and bovine PP-immunoreactive cells in the principal pancreatic islets of *Silurus asotus* showed the general patterns observed in teleostean fishes, but some species-dependent distributional patterns and/or relative frequencies, particularly in glucagon-, somatostatin- and bovine PP-immunoreactive cells, were detected in the principal pancreatic islets of *Siniperca scherzeri*.

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