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Immunohistochemical Study of the Endocrine Cells in the Pancreas of the Carp, *Cyprinus carpio* (*Cyprinidae*)

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Abstract

The regional distribution and relative frequency of some endocrine cells in the pancreas of the carp, *Cyprinus carpio* Linnaeus, belonging to the family Cyprinidae in the order Cypriniformes, were observed using specific mammalian antisera against insulin, glucagon, somatostatin and human pancreatic polypeptide (hPP) by peroxidase antiperoxidase (PAP) method. The pancreas was divided into four regions (principal and secondary islets, exocrine and pancreatic duct regions). In addition, the pancreatic islet regions were further subdivided into three regions (central, mantle and peripheral regions) and the pancreatic duct regions were subdivided into two regions (epithelial and subepithelial regions). Spherical to spindle or occasionally round to oval shaped immunoreactive (IR) cells were demonstrated in the pancreatic islets, exocrine and pancreatic duct. In the principal islet regions, some cells were also detected in the other regions, most of insulin- and somatostatin-IR cells were located in the central regions, and glucagon- and hPP-IR cells were situated in the peripheral regions. In this regions, insulin-IR cells were most predominant cell types and then, glucagon, somatostatin and hPP in that order. In the secondary islet regions, the regional distribution and relative frequency of these four types of endocrine cells were quite similar to those of the principal islets except for cell clusters consisted of hPP-IR cells that were situated in the peripheral to mantle regions. In the pancreatic duct regions, all four major pancreatic endocrine cells were demonstrated in the inter-epithelial cells and/or basal regions of the epithelial linning. In addition, cell clusters composed of numerous insulin-, moderate glucagon- and somatostatin-IR cells of low frequency were also observed in the subepithelial regions of the pancreatic duct. In the exocrine regions, insulin-, glucagon-, somatostatin- and hPP-IR cells were located in the inter-acinus regions with rare, a few, moderate and moderate frequencies, respectively. In conclusion, the regional distribution and relative frequency of four major pancreatic endocrine cells, insulin-, glucagon-, somatostatin- and hPP-IR cells, in the pancreas of the carp showed general patterns which were observed in other stomachless teleost. However, some species-dependent different distributional patterns and/or relative frequencies were also demonstrated.

Key words : Carps, pancreas, hepatopancreas, immunohistochemistry, immunoreactive cells, endocrine cells

Introduction

The carp, *Cyprinus carpio* Linnaeus, belonging to the family Cyprinidae in the order Cypriniformes is a freshwater stomachless teleost. Although this species was originated from Asia, now their habitation is distributed throughout the world except for some regions of South America and Australia. Among teleost, the pancreas of this stomachless teleost has been widely studied because their unique anatomical and histological profiles, so called hepatopancreas in there, pancreas was dispersed throughout the whole liver parenchyma, of course some pancreatic parenchyma having large islets was also demonstrated between liver and mesenteric membrane [21].

It is generally known that pancreas of vertebrates is subdivided into two regions. One is an exocrine region where digestive enzymes are released and the other is an endocrine portion where regulatory hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide (PP) are released into blood vessels. The appearance, regional distribution and relative frequency of these regulatory hormones secreted by endocrine cells in the pancreas are well recognized by

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histochemistry [28], immunofluorescence method [39] and immunohistochemistry [52]. In addition to four regulatory hormones mentioned above, peptide YY-, neuropeptide Y- [2], and chromogranin family- [22, 43] immunoreactive (IR) cells were also demonstrated in the vertebrate pancreas. The pancreas has been treated as a valuable organ for endocrine studies and endocrine pancreas was extensively studied associated with diabetes [23]. Until now, investigations of gastroenteropancraetic (GEP) endocrine cells have been considered to be an important part of a phylogenetic study [11] and the distribution and relative frequency of these endocrine cells in the pancreas were varied with animal species and feeding habits. Recently most intensive studies have been done on the Pisces because some endocrine cells were demonstrated in the skin, gills and airways [58], and the alteration of regional distribution and relative frequency of these cells by heavy metal intoxication such as lead was also demonstrated [41]. In addition, the possibility of using the teleost fish endocrine tissues for treatment hormonal disorder such as diabetes was suggested [37]. 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, called secondary pancreatic islets [14]. Until now, the appearance, regional distribution and relative frequency of numerous types of regulatory peptides have been demonstrated in the pancreas of the Pisces. Insulin-, glucagon-, somatostatin- and pancreatic polypeptide (PP)-IR cells, which were major four endocrine cell types detected in mammalian pancreas, were also observed in the pancreas of the five species of Osteoglossomorpha [3, 4], the gar [16], the southern-hemisphere lampreys [56], the sea bream [1], the lamprey [7, 8], the sea bass [35], the dipnoan fish [47], the cartilaginous fish [15], the rainbow trout [38], the coho salmon [38], the arctic lamprey [57], the anglerfish [24], the channel catfish [24], the ray [48] and the telecostei [27] by immunohistochemical and/or electron microscopical methods. In addition, similar to that of mammals, the appearance of neuropeptide Y and peptide YY-IR cells and/or nerve fibers was also demonstrated in the pancreas of the eel [12], the dogfish [9, 10] and the spiny dogfish [40]. The ontogenic changes and changes of distribution and relative frequency of some endocrine cells with developmental stages were also well documented in the Japanese flounder [29], the dogfish [9], the lamprey [13], the sea bream [46] and the sea bass [5].

Well corresponding to those of mammals, the regional distribution and relative frequency of endocrine cells within the pancreas, and the cell population seemed to be considerably variable among species and feeding habits, especially in the case of occurrence in PP cells [55]. Namely, these IR cells that were generally demonstrated in teleost, were not detected in the pancreas of channel catfish and lungfish [18, 36]. In addition, it is also reported that somewhat different distributional patterns of pancreatic endocrine cells were found in two species of stomach fresh water teleost having similar feeding habits [34]. Although many studies have elucidated regional distribution and relative frequency of endocrine cells, IR to the antisera against mammalian insulin, glucagon, somatostatin and PP, in the pancreas of teleost, localization of endocrine cells on the pancreas of the carp has not yet been reported except for insulin. Regulation of insulin biosynthesis was reported in the carp [20] and the carp insulin was isolated and crystallized [42].

In the present study, the regional distribution and relative frequency of some endocrine cells in the pancreas of stomachless fresh-water teleost, the carp, *Cyprinus carpio* Linnaeus (Cyprinidae) having unique hepatopancreas, were observed using specific antisera against mammalian insulin, glucagon, somatostatin and PP by peroxidase antiperoxidase (PAP) method.

Materials and Methods

Experimental animals

Five adult carp, *Cyprinus carpio* Linnaeus (Cyprinidae), about 40cm in length, were purchased from a merchant in Taegu, Korea and used in this study without sexual distinction.

Histological procedures

After decapitation, samples from the pancreas were fixed in Bouin's solution. After paraffin embedding, 3 4㎛ sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal pancreatic architecture.

Immunohistochemical procedures

The each representative section was deparaffinized, rehydrated and immunostained with the peroxidase antiperoxidase (PAP) method [51]. Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsed in phosphate buffered saline (PBS; 0.01M, 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 3,3′-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05M, pH 7.6). After immunostained, the sections were lightly counterstained with Mayer's hematoxylin and the IR cells were observed under light microscope

Specificity of antiserum reaction

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger [51], including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen.

Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
Insulin	842613	Diasorin, Stillwater, USA	1:1,000
Glucagon	927604	Diasorin, Stillwater, USA	1:2,000
Somatostatin	917600	Diasorin, Stillwater, USA	1:600
HPP1)	A61P	DAKO Corp., Carpenteria, USA	1:100

*All antisera were raised in rabbits except for insulin, which were raised in a guinea pig.
1) hPP: human pancreatic polypeptide

Category of relative frequency

The relative frequency of occurrence of each type of IR cell was placed into one of five categories, not detected (—), rare (±), a few (+), moderate (++) and numerous (+++), according to their observed numbers as seen using light microscopy.

Classification of pancreatic regions

The distribution of IR cells was divided into four regions, 1) the principal and 2) secondary islets regions, 3) the exocrine regions and 4) pancreatic duct regions according to modified classifications of Lee *et al*. [34] and Ku *et al*. [32] which were classified by their histological profiles. In addition, the regional distribution and relative frequency of endocrine cells in the pancreatic islets were further subdivided into three regions from centrally to marginally, central, mantle and peripheral regions according to types of cell composition. The pancreatic duct regions were also subdivided into two distinct regions according to their histological profiles, epithelial lining and sub-epithelial regions.

Results

In the present study, all four kinds of the IR endocrine cells were detected using antisera against mammalian insulin, glucagon, somatostatin and hPP in the pancreatic islets, pancreatic duct and exocrine regions. Different regional distributions and relative frequencies of these IR cells were observed in the different pancreatic regions, and these differences are shown in Table 24. Spherical to spindle or occasionally oval to round-shaped immunoreactive cells were observed in this study.

Insulin-IR cells

In the principal pancreatic islets, spherical to spindle shaped cells having cytoplasmic process were demonstrated in the central regions with numerous frequencies but they were situated in the mantle regions with a few frequencies and no cells were found in the peripheral regions. In there, their cytoplasmic processes were intermingled with other IR cells especially with somatostatin-IR cells (Figs. 2ac). In the pancreatic duct, spindle shaped insulin-IR cells were detected in the inter-epithelial cells of duct epithelium with a few frequencies (Figs. 2df) and some cells were located in the basal regions of the pancreatic duct epithelial lining. In addition, insulin-IR cells were also located in the cell clusters situated in the sub-epithelial regions in the case of large pancreatic ducts (Figs. 2d and e) with moderate frequency. In the secondary islet regions, they were mainly located in the central regions with similar shape compared to that of principal islets and showing numerous frequencies. In addition, some cells were also demonstrated in the mantle regions in there their cytoplasmic processes were intermingled with other endocrine cells especially glucagon-IR cells and showing rare frequency. However, no insulin-IR cells were found in the peripheral regions (Fig. 2g). In the exocrine regions, round to oval shaped insulin-IR cells were detected between acinar cells with rare frequencies (Fig. 2h).

Table 2. Regional distribution and relative frequency of the endocrine cells in the principal pancreatic islets of the carp

Hormones	Regions of principal pancreatic islets		
	Central	Mantle	Peripheral
Insulin	+++	+	+
Glucagon	±	+	+
Somatostatin	++	+	±
hPP*	±	±	+

— : not detected, ± : rare, + : a few, ++ : moderate and +++ : numerous.
*hPP : human pancreatic polypeptide.

Glucagon-IR cells

In the principal pancreatic islets, spherical to spindle shaped cells having cytoplasmic process were demonstrated in the peripheral regions with moderate frequency and some of these IR cells were situated in the mantle and central regions with a few and rare frequencies respectively. In there, their cytoplasmic processes were intermingled with other IR cells especially with insulin-IR cells (Figs. 3ad). In the pancreatic duct, spindle shaped glucagon-IR cells were detected in the inter-epithelial cells of duct epithelium with moderate frequency (Figs. 3eg), and some cells were located

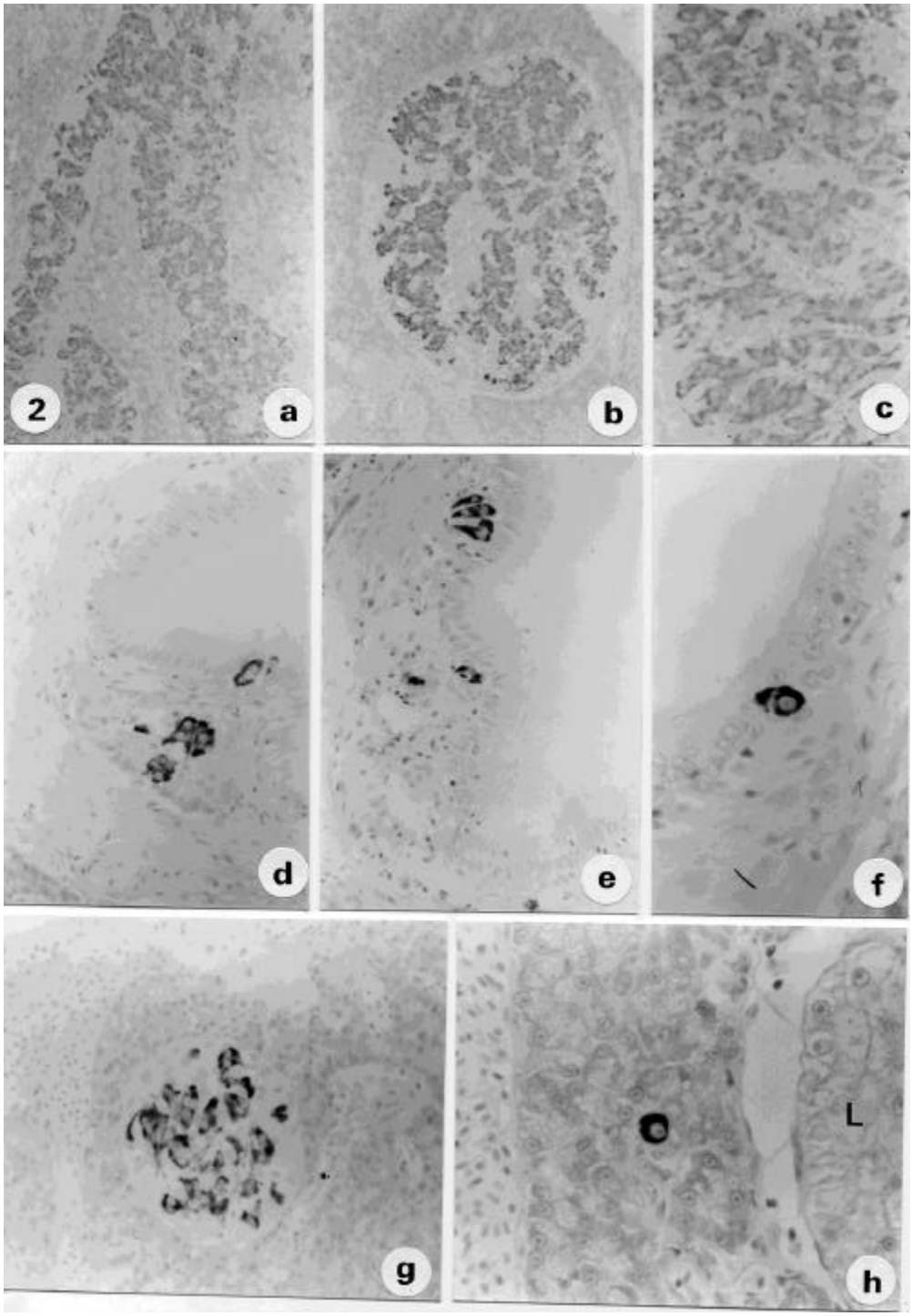

Fig. 2. Insulin-IR cells in the pancreas of the carp. Note that these IR cells were dispersed throughout whole central regions of the principal islets and the cells of lower frequncy were also demonstrated in the mantle regions (a, b). In the pancreatic duct, they were located in the inter-epithelial cells or basal regions of epithelial lining (df). In addition, insulin-IR cells were also detected in the cell clusters located in the sub-epithelial regions of the large types of pancreatic duct (d, e). The regional distribution of insulin-IR cells in the secondary islets was quite similar to those of the principal islets (g). Some cells were also demonstrated in the inter-acinus regions of the exocrine regions (h) that were dispersed between liver parenchyma (L). a, b: ×120; ce, g: ×240; f, h: ×480. PAP method.

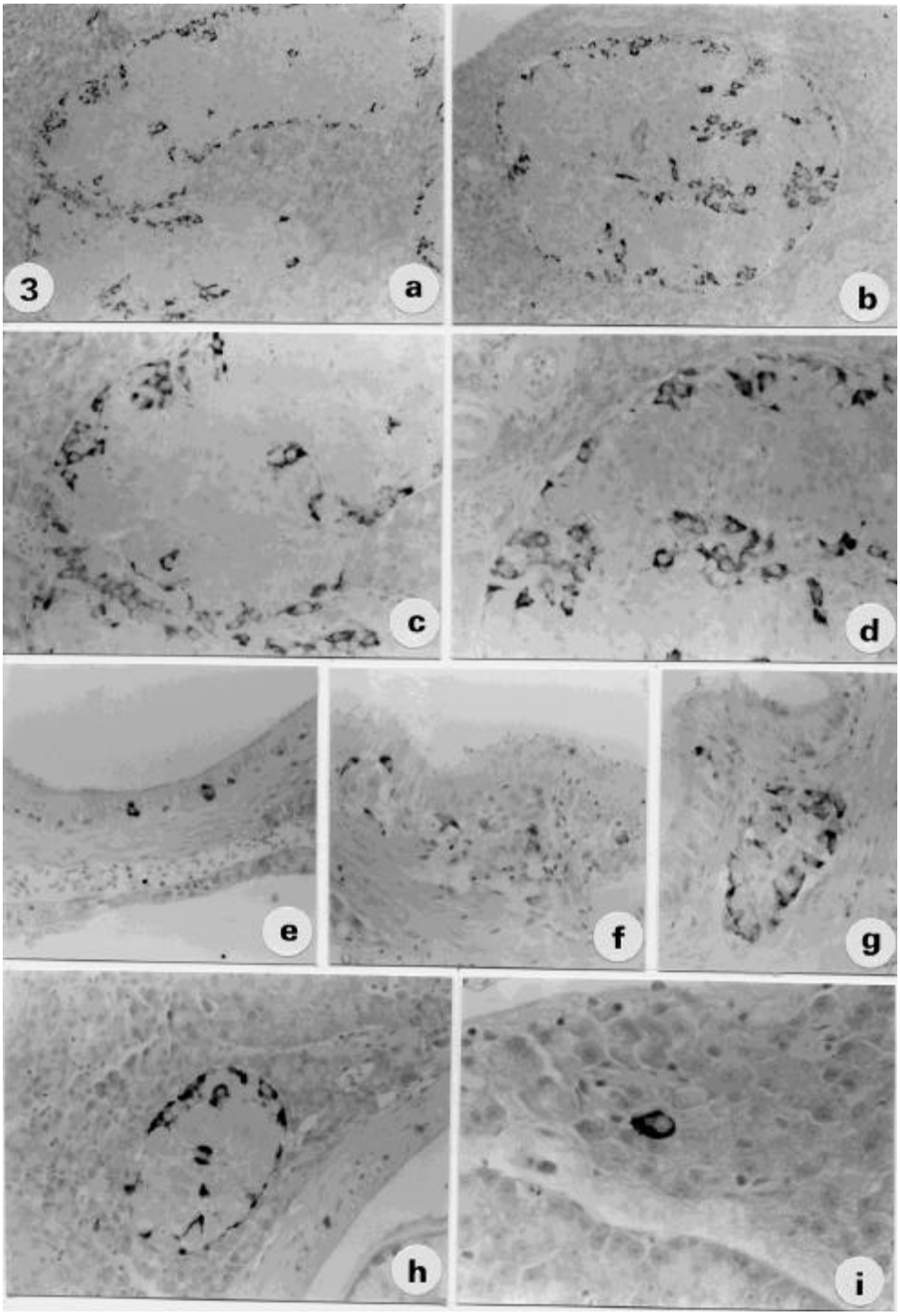

Fig. 3. Glucagon-IR cells in the pancreas of the carp. Note that most of glucagon-IR cells were restricted to the peripheral and mantle regions of the principal islets and the cells of low frequency were also demonstrated in the central regions (ad). In the pancreatic duct, they were located in the inter-epithelial cells or basal regions of epithelial lining (e, f). In addition, some cells were also detected in the cell clusters located in the sub-epithelial regions of the large types of pancreatic duct (g). The regional distribution of glucagon-IR cells in the secondary islets was quite similar to those of the principal islets (h). Glucagon-IR cells were also observed in the inter-acinus regions of the exocrine regions (i). a, b: ×120; ch: ×240; i: ×480. PAP method.

in the basal regions of the pancreatic duct epithelial lining. In addition, glucagon-IR cells were also located in the cell clusters situated in the lamina propria regions in the case of large pancreatic ducts (Fig. 3g) with moderate frequency and in there, the cytoplasmic process of glucagon-IR cells was intermingled with that of insulin-IR cells. In the secondary islet regions, glucagon-IR cells were mainly located in the peripheral regions with similar shape compared to that of principal islets and showing moderate frequency. In addition, some cells were also demonstrated in the mantle regions in there their cytoplasmic processes were intermingled with other endocrine cells especially insulin-IR cells and showing a few frequency. However, no glucagon-IR cells were found in the central regions (Fig. 3h). In the exocrine regions, spherical to spindle shaped glucagon-IR cells were detected between acinar cells with a few frequencies (Fig. 3i).

Table 3. Regional distribution and relative frequency of the endocrine cells in the secondary pancreatic islets of the carp

Hormones	Regions of principal pancreatic islets		
	Central	Mantle	Peripheral
Insulin	+++	+	−
Glucagon	−	+	++
Somatostatin	++	+	±
hPP*	±	±	+

− : not detected, ± : rare, + : a few, ++ : moderate and +++ : numerous.
*hPP : human pancreatic polypeptide.

Somatostatin-IR cells

In the principal pancreatic islets, spherical to spindle shaped cells having cytoplasmic process were dispersed throughout the whole central regions with moderate frequency and some of these IR cells were situated in the mantle and peripheral regions with a few and rare frequencies respectively. In there, their cytoplasmic processes were intermingled with other IR cells especially with insulin- (in the case of central regions) and glucagon- (in the case of mantle and peripheral regions) IR cells (Figs. 4ac). In the pancreatic duct, spindle shaped somatostatin-IR cells were detected in the inter-epithelial cells of duct epithelium with moderate frequency (Figs. 4de) and some cells were located in the basal regions of the pancreatic duct epithelial lining. In addition, somatostatin-IR cells were also located in the cell clusters situated in the sub-epithelial regions in the case of large pancreatic ducts with rare frequency and in there, the cytoplasmic process of somatostatin-IR cells was intermingled with that of glucagon- and insulin-IR cells (Fig. 4e). In the secondary islet regions, somatostatin-IR cells were dispersed in the central regions with similar shape compared to that of principal islets and showing moderate frequency. In addition, some cells were also demonstrated in the mantle and peripheral regions in there their cytoplasmic processes were intermingled with other endocrine cells similar to those of principal islets and showing a few and rare frequencies, respectively (Fig. 4f). In the exocrine regions, spherical to spindle shaped or occasionally round to oval shaped somatostatin-IR cells were detected between acinar cells with moderate frequency (Fig. 4g).

HPP-IR cells

In the principal pancreatic islets, spherical to spindle shaped hPP-IR cells having cytoplasmic process were demonstrated in the peripheral to mantle regions with a few and rare frequencies and some of these IR cells were also situated in the central regions with rare frequency. In there, their cytoplasmic processes were intermingled with other IR cells especially with glucagon-IR cells (Figs. 5ac). In the pancreatic duct, spindle shaped glucagon-IR cells were detected in the inter-epithelial cells of duct epithelium with moderate frequency (Figs. 5d, e) and some cells were located in the basal regions of the pancreatic duct epithelial lining. However, no hPP-IR cells were demonstrated in the cell clusters situated in the sub-epithelial regions in the case of large pancreatic ducts where numerous insulin- and glucagon-IR cells were detected and rare somatostatin-IR cells were also demonstrated (Figs. 2d, e; Fig. 3g; Fig. 4e; Fig. 5d) in there, the cytoplasmic process of insulin-, glucagon- and somatostatin-IR cells were intermingled with each other. In the secondary islet regions, hPP-IR cells were mainly located in the peripheral regions with similar shape compared to that of principal islets and showing a few frequencies. In

Table 4. Regional distribution and relative frequency of the endocrine cells in the pancreatic duct and exocrine regions of the carp

Hormones	Regions of Pancreatic ducts		Exocrine regions
	Epithelial lining	Subepithelial regions	
Insulin	+	++	±
Glucagon	++	++	+
Somatostatin	++	±	++
hPP*	++	−	++

− : not detected, ± : rare, + : a few, ++ : moderate and +++ : numerous.
*hPP : human pancreatic polypeptide.

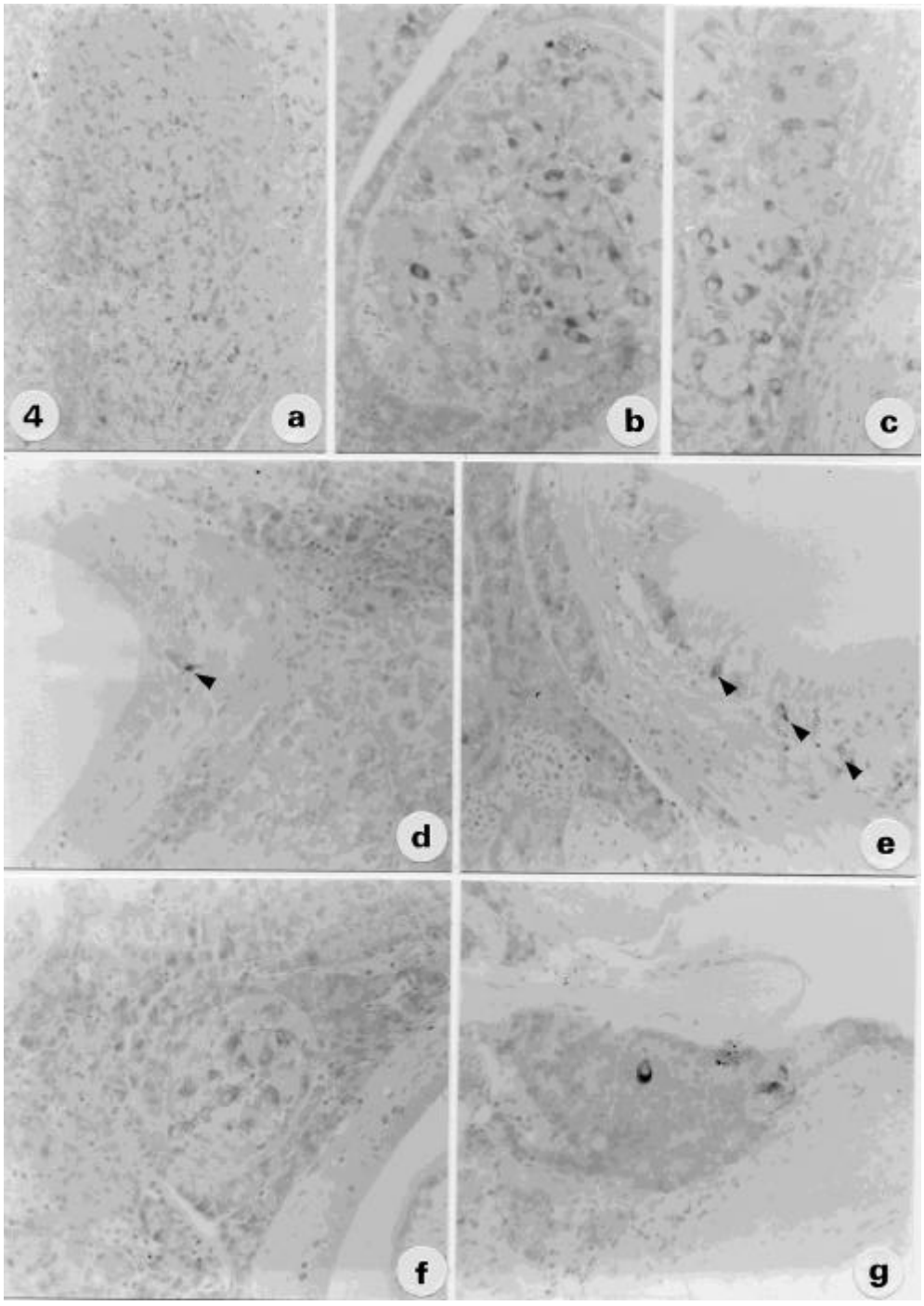

Fig. 4. Somatostatin-IR cells in the pancreas of the carp. Note that these cells were dispersed throughout whole central regions of the principal islets and the cells of low frequency were also demonstrated in the mantle and peripheral regions (ac). In the pancreatic duct, they were located in the inter-epithelial cells or basal regions of epithelial lining (d, e; arrow heads). In addition, rare somatostatin-IR cells were also detected in the cell clusters located in the sub-epithelial regions of the large types of pancreatic duct (e). The regional distribution of somatostatin-IR cells in the secondary islets was quite similar to those of the principal islets (f). Some cells were also demonstrated in the inter-acinus regions of the exocrine regions (g). a: ×120; bg: ×240. PAP method.

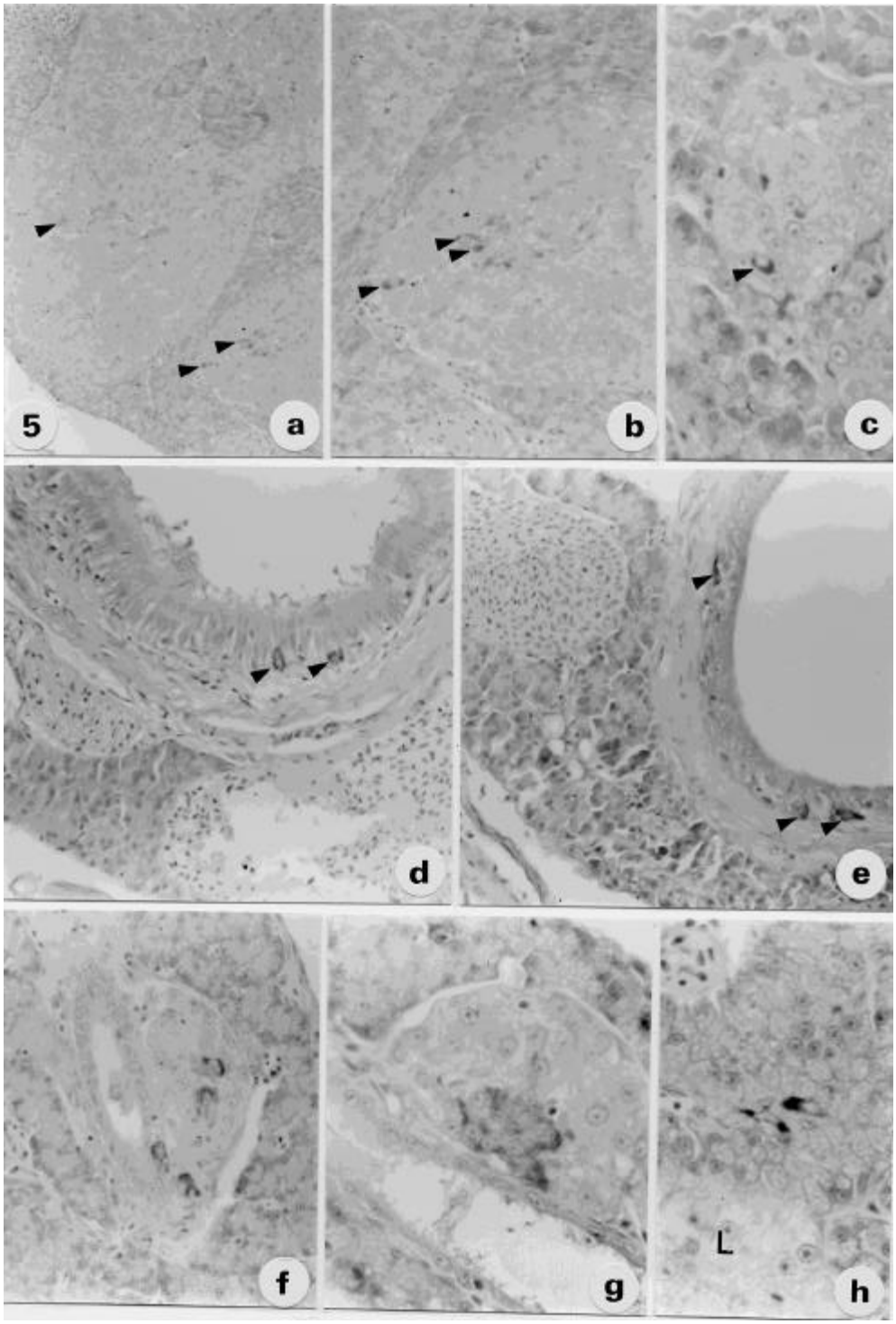

Fig. 5. hPP-IR cells in the pancreas of the carp. Note that these cells were detected in the peripheral regions of the principal islets and the cells of low frequency were also demonstrated in the mantle and central regions (ac; arrow heads). In the pancreatic duct, they were located in the inter-epithelial cells or basal regions of epithelial lining (d, e; arrow heads). The regional distribution of hPP-IR cells in the secondary islets were quite similar to those of the principal islets (f) but in some case of the secondary islets, cell clusters consisted of hPP-IR cells located in the mantle to peripheral regions were also detected (g). Some cells were also demonstrated in the inter-acinus regions of the exocrine regions dispersed between liver parenchyma (h; L). a: ×120; b, df: ×240; c, g, h: ×480. PAP method.

addition, some cells were also demonstrated in the mantle and central regions in there their cytoplasmic processes were intermingled with other endocrine cells especially insulin-IR cells and showing a few and rare frequencies, respectively (Fig. 5f). Although these findings were restricted to some islets, cell clusters composed of hPP-IR cells were detected in the mantle to peripheral regions of secondary islets (Fig. 5g). In the exocrine regions, spherical to spindle shaped hPP-IR cells were detected between acinar cells with moderate frequency (Fig. 5h).

Discussion

This study revealed that the pancreatic endocrine cells of stomachless fresh-water teleostean fish, the carp *(Cyprinus carpio)* having unique histological profiles of pancreas hepatopancreas contained insulin-, glucagon, somatostatin- and hPP-IR cells. In the present study, somewhat different distributional patterns of these four types of IR cells were also demonstrated according to region of pancreas and types of IR cells. In addition, species-dependent unique distributional patterns were also observed especially in hPP-IR cells.

Insulin is synthesized in the B cells of the pancreatic islets and regulates the serum glucose levels [19]. The regional distribution and relative frequency of the insulin-IR cells in the pancreas of numerous teleost have been reported in the lungfish [18], flatfish [55], gilt-head sea bream [17], five species of osteoglossomorpha, an ancient teleostean group [3], *Protopterus annectens* [53], dipnoan fish [47], anglerfish and channel catfish [25]. From these previous reports, it seems to be a general rule in the pancreatic islets of teleost that insulin-IR cells occur in central regions regardless their types of pancreatic islets. Although somewhat lower relative frequencies were demonstrated, compared to those of pancreatic islets, some insulin-IR cells were also located in the exocrine and pancreatic duct. In the present study, well corresponded to those of previous reports [3, 17, 18, 25, 47, 53, 55], insulin-IR cells were found in the central regions of the pancreatic islets of the carp regardless of their types and some cells were also demonstrated in the exocrine and pancreatic duct. Some insulin-IR cells detected in this study in the cell clusters which were located in the sub-epithelial regions of pancreatic duct. Although these findings were ordinarily demonstrated in higher vertebrates [31], it is seldom in the case of teleost pancreas and this appearance was regarded as species-dependent characteristic of this species of stomachless fresh-water teleost, the carp.

Glucagon is synthesized in the A cells of the pancreas and regulates glucose levels in blood [19]. Morphologically similar cells are also observed in the digestive tract of the dog. The regional distribution and relative frequency of glucagon-IR cells in the teleostean pancreas have been reported in the flatfish [55], *Barbus conchonius* [45], five species of osteoglossomorpha, an ancient teleostean group [3], gar [16], *Protopterus annectens* [53], dipnoan fish [47], anglerfish and channel catfish [25] and *Xiphophorus helleri* [27]. It seems to be a general rule in the pancreatic islets of teleost that glucagon-IR cells occur in the peripheral regions and they formed a small mantle zone or rim surrounding centrally located insulin-IR cells except for osteoglossomorpha [3] which shows a scattered immunoreactivity throughout the central region of the islets besides the common peripheral regions regardless of principal and secondary types. Similar to those of insulin-IR cells, some cells were also demonstrated in the exocrine and pancreatic duct with lower relative frequencies compared to those of pancreatic islets. In the present study, although some IR cells of low frequency were demonstrated in the mantle and central regions of islets, most of glucagon-IR cells were located in the peripheral regions of the principal and secondary islets. In addition, some cells were also demonstrated in the exocrine and pancreatic duct. These results were similar to those of previous studies [16, 25, 27, 31, 47, 53, 55]. However, glucagon-IR cells intermingled with insulin-IR cells were also detected in the cell clusters, which were located in the sub-epithelial regions of pancreatic duct in this study. Although these findings were ordinarily demonstrated in higher vertebrates [30], it is seldom in the case of teleost pancreas and this appearance was regarded as species-dependent characteristic of this species of stomachless fresh-water teleost, the carp.

Somatostatin, which consisted of 14 amino acids, was isolated from hypothalamus of sheep for the first time and it could be divided into straight form and cyclic form [6]. This substance inhibits the secretion of the gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid [26] and absorption of amino acid, glucose and fatty acid in the gastrointestinal tract [6]. Somatostatin-IR cells of the teleostean pancreas have been reported to be dispersed mainly in the central region, intermingled with insulin-IR cells [44, 50]. However, Yoshida et al. [55] suggested that somatostatin-IR cells occur in the peripheral regions of islets intermingled with insulin cells, besides the common central regions, and similar distributional patterns were also seen in *Protopterus annectens* [53]. In addition, Scheuermann *et al*. [47] reported that somatostatin-IR cells were scattered throughout the islets of dipnoan fish. Although somatostatin-IR cells were demonstrated in the exocrine and pancreatic duct, more numerous IR cells were dispersed in the central regions of principal and secondary pancreatic islets of carp used in this study similar to those of previous reports [44, 50]. In addition, somatostatin-IR cells intermingled with insulin- and glucagon-IR cells were also detected in the cell clusters, which were located in the sub-epithelial regions of pancreatic duct in this study. Although these findings were ordinarily demonstrated in higher vertebrates [33], it is seldom in the case of teleost pancreas and this appearance was regarded as species-dependent characteristic of this species of stomachless fresh-water teleost, the carp.

PP-IR cells as the fourth cell type were demonstrated first by Stefan *et al*. [49] and Van Noorden and Patent [54] in the pancreas of some teleost. Later, it has been revealed that PP-IR cells were conspicuously variable in distribution among species, although the cells, if they occur, were always located at the peripheral regions of the pancreatic islets. PP-IR cells were detected in the exocrine and endocrine pancreas of the *Cottus scorpius* [50], *Barbus conchonius* [44], *Xiphophorus helleri* [27], anglerfish [24], flatfish [55], five species of osteoglossomorpha, an ancient teleostean group [3] and gar [16]. However, no PP-IR cells were found in the pancreas of the channel catfish [36] and lungfish [18]. In the present study, similar to those of other teleostean fishes [3, 16, 24, 27, 44, 49, 50, 54, 55], hPP-IR cells were mainly distributed in the peripheral regions of principal and secondary pancreatic islets of carp in this study except for some cell clusters consist of hPP-IR cells demonstrated in the some case of secondary islets which were considered as species-dependent characteristic of this stomachless freshwater teleost, the carp.

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