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Identification, localization and morphology of APUD cells in gastroenteropan creatic system of stomach-containing teleosts

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Abstract

AIM **To identify the type localization and morphology of APUD endocrine cells in the gastroenteropancreatic (GEP) system of stomach-containing teleosts, and study APUD endocrine system in the stomach, intestine and pancreas of fish species.**

METHODS **Two kinds of immunocytochemical (ICC) techniques of the streptavidin biotin-peroxidase complex (SABC) and streptavidin-peroxidase (S-P) method were used. The identification, localization and morphology of APUD endocrine cells scattered in the mucosa of digestive tract, intermuscular nerve plexus and glandular body of northern snakehead (Channa argus), ricefield eel (*Monopterus albus*), yellow catfish (*Pelteobagrus ful vidraco*), mandarinfish (*Siniperca chuatsi*), largemouth bass(*Micropterus salmoides*), oriental sheatfish (*Silurus asotus*), freshwater pomfret (*Colossoma brachypomum*) and nile tilapia (*Tilapia nilotica*) were investi gated with 8 kinds of antisera.**

RESULTS **The positive reaction of 5-hydroxytryptamine (5-HT) immunoreactive endocrine (IRE) cells was found in the digestive tract and glandu lar body of 8 fish species in different degree. Only a few gastrin (GAS)-IRE cells were seen in C. argus, M. albus and *P. fulvidraco*. Glucagon (GLU)-IRE cells were not found in the digestive tract and glandular body but existed in pancreatic island of most fish species. The positive rea ction of growth hormone (GH)-IRE cells was found only in pancreatic island of *S. Chuatsi* and *S. Asotus*, no positive reaction in the other 6 fish species. *Somatostatin (SOM)*, calcitonin (CAL), neurofilament (NF) and insulin (INS)-IRE cells in the stomach, intestine and pancreas of 8 kinds of fish were different in distribution and types. The distribution of all 8 APUD cells was the most in gastrointestinal epithelium mucosa and then in digestive glands. The positive reaction of SOM-and 5-HT-IRE cells was found in intermuscular nerve plexus of intestine of *P. fulvidraco* and *S. chuatsi* . Only GH-IRE cells were densely scattered in the pancreatic islands of *S. chuatsi* and *S. asotus*, and odd distribution in the pancreas of *S. asotus*. SOM-IRE cells were distributed in the pancreatic islands of *S. asotus*, *C. Brachypomum* and *T. nilotica*. There were INS-IRE cells in the pancreatic islands of *S. chuatsi* and *S. asolus*. Eight kinds of APUD cel ls had longer cell body and cytoplasmic process when they were located in the gastrointestinal epithelium, and had shorter cell body and cytoplasmic process in the gastric gland, and irregular shape in the esophagus and pancreatic island.**

CONCLUSION **Eight kinds of IRE cells were identified in the GEP system of stomach-containing teleosts. These endocrine cells were scattered in gastrointestinal mucosa, intermuscular nerve plexus, gland body, pancreatic gland and islands under APUD system. CAL-and GH-IRE cells in the pancreatic islands of fishes showed functional diversity for these two hormones. Their morphological feature provides evidence of endocrine-paracrine and endocrine-exocrine acting mode. This research can morphologically prove that the GEP endocrine system of fish (the lowest vertebrate) is almost the same as of mammal and human.**

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INTRODUCTION

In recent years, APUD (amine precursor uptake and decarboxylation) cells in the digestive tract attracted worldwide attention. It was a research domain developed quickly in recent years, especially gastrointestinal hormones[1-7] . Gastrointestinal tract was not only the digestive organ but also the biggest and most complex endocrine organ in animal[8]. It has been proved that gastrointestinal hormones were not only related with feeding behavior but also with nutrition of gastrointestinal tract. At present it has been applied to clinical practice[9-24]. The endocrine tumor of digestive tract could occur in gastrointestinal tract and liver or in pancreatic gland[25,26], even over ninety per cent of carcinoid occurred in gastrointestin[27-40]. What is the hormone's effect on it So it is of special significance to study endocrine cells in digestive system. Up till now, researches about digestive tract in animal have been reported[41], but the research data about fish having close relations with human food are scarce. The distribution of APUD cells in the gut of 8 stomachless teleosts was reported[42]. In the present paper, ICC studies on APUD cells in the GEP system of stomach-containing teleosts were reported further, delving into difference of gastrointestinal hormone types, secretory way, distribution and cell's shapes among fish, mammal and human, in order to provide basic materials for studying gastrointestinal endocrinology, gastroenterology, origin and prevention of disease in digestive system and its treatment.

MATERIALS AND METHODS

Specimens and section

Ricefield eel (*M. albus*), northern snakehead (*C. argus*), oriental sheatfish (*S. asotus*), mandarinfish (*S. chuatsi*), and yellow catfish (*P. ful vidraco*) were bought separately from the aquatic products market in Wuhan; freshwater pomfret (*C. brachypomum*) and nile tilapia (*T. nilotica*) were bought from The Second Fish and Stock Farm in Hongshan District of Wuhan; largem outh bass (*M. Salmoides*) was bought from Nanhu Fish Farm. We reared above 8 kinds of fish temporarily in fresh water for 24 hours, then according to the methods in references[43-46], and collected the samples and made the sections. Monoclonal antibody of GH was used only in SABC ICC stain method[46], S-P ICC stain method was used in the other 7 kinds of antibody[43].

Reagents and antisera

The details of the different antisera, the working dilutions and main regents used in this study are listed in Table 1.

ICC staining steps and control

The steps of S-P ICC stain and ontrol references and the SABC ICC staining steps and control followed references[43,46].

Observation, photomicrograph and count

Five fishes used for each species in all 8 kinds of teleost studied, 7 specimens of each fish were observed and photomicrographed under the Olympus (BH-2) photomicroscope. The dark brown positive cells on section were counted under 10×20 times field. The average number of positive cells from 10 fields selected randomly in each specimen part was the IRE cell number of this part in each fish. The average number of 5 fishes of each species was quantified IRE cell's distribution density in every part. The distribution density was showed with five grades.

RESULTS

Types and distribution of APUD cells

APUD cell types, distribution and density in different parts of GEP system of 8 kinds of stomach-containing teleosts are listed in Table 2. It can be seen from Table 2 that IRE cell types and distribution quantity were the least in the esophageal epithelium, only GAS, SOM and CAL-IRE cells were found in the esophageal epithelium mucosae of M.albus (Figure 1). IRE cells were the most common types and the highest distributiond ensity in the gastric epithelium and glands of 8 kinds of fish (Figures 3, 5, 7, 9, 10, 12); then in the intestine (Figures 2, 4, 8, 11, 13, 14). GLU- and INS -IRE cells were mostly located in every specimen of pancreatic island. SOM- and 5-HT-IRE cells were found in the gastrointestinal intermuscular nerve plexus of *P.fulvidraco* and *S. chuatsi* separately (Figure 8, Table 2). No GAS, 5-HT, CAL- and NF-IRE cells were seen in the pancreatic island of all 8 kinds of teleosts. There were 5-HT-IRE cells in the digestive tract of all fish species; only a few GAS-IRE cells were distributed in the esophagus and stomach of *C. argus*, *M.albus* and *P.fulvidraco*; GH-IRE cells were distributed in a small amount in the pancreas of *S.asolus*, but were scattered in the pancreatic islands of *S.chuatsi* and *S.asotus*; and were not seen in the gastrointestinal tract of 8 kinds of fish species. In gastrointestinal tract, APUD cells were distributed bet ween epithelium mucosa and glandular epithelium (Figures 1-14). Negative reaction was found in all controls.

Morphological feature of APUD cells

According to the distribution of APUD cells, their morphology was of great diversity. APUD cells located in stratified squamous epithelium of esophagus were scattered or piled in distribution, and irregular in shape, and had a shorter cytoplasmic process (Figure 1). While the APUD cells distributed betw een gastrointestinal columnar epithelium had a longer cell body, an apical

cytoplasmic process extended to the gastrointestinal lumen or a basal process extended to the basement membrane (Figures 2, 4, 9, 11-14). The APUD cells located in gastric glands were mainly pyramid-shaped (Figures 5-7,10), their cytoplasmic process extended to the gastrointestinal lumen. In the pancreatic islands of *S.chuatsi* and *S.asotus*, GLU-IRE cells were located in the edge of pancreatic islands (Figures 15, 18) and SOM, INS- and GH-IRE cells scattered in the whole pancreatic islands (Figures 16, 17, 19). Their shapes were irregular, some had a longer cytoplasmic process (Figure 18), the secretory granules were clear (Figure 19) in some of IRE cells. There were a few scattered GH-IRE cells in the pancreatic exocrine area of *S.asotus* (Figure 19).

Table 1 Details of antisera and main regents used

Antisera & regents	Working dilution	Specificity	Source
Human CAL	1:400		ZYMED Lab. Inc.,USA
Grass carp GH	1:600		Yangtze River Fisheries Institute, Chinese Academy of Fishery Sciences
Human NF	1:400		ZYMED Lab. Inc., USA
Human INS	1:1000		ZYMED Lab. Inc., USA
Synthetic human GAS	1:5000	No cross reaction with Cholecystokinin-8	Dr. N Yanaihara & Shizuoka
Procine GLU	1:1000	Wholly cross react with Pancreatic & intestinal glucagon	Amersham International pl.
Synthetic human SOM	1:3000		Dr. S Ito Niigata
5-HT	1:10000		Immunonuclear Corp., Stillwater
S-P Kit	1:100		ZYMED Lab. Inc., USA
SABC Kit(mouse IgG)	1:100		Boster Biotechnology Co.
SABC Kit(rabbit IgG)	1:100		LTD., Wuhan
DAB	1:2000		Dr.Kinji INOUE

Table 2 APUD cells types and distribution feature and density i n GEP system of 8 kinds of stomach-containing teleosts

Fish species	GAS	SOM	5-HT	GLU	CAL	GH	NF	INS
Northern snakehead (*C .argus*)	3 +++/+ 4 +/		5 +++/	ND	2 +/ 3 ++++/ 4 +++/		1-6 ND	1-6ND
Ricefield eel (*M. albus*)	1 +++/+	1 +++/+ 3 +++/++	4 +++/	ND	1 ++++/ 3 +/ 4 +/		4 /++	4/+
Yellow catfish (*P. fulvidraco*)	3 /++	3 /++++ 4 /++++ 5 /++	3 ++/ 5 ++/	7 +++/ 2 +/ 3 ++/ 4 +++/ 5 +/	1 +/		3 ++++/	2 /++ 4 / +
Mandarinfish (*S. chuatsi*)		2 +++/++ 4 ++/++	3 +++/++ 5 ++/ 6 ++	7 +++/		7 +++/	1-6 ND	1-6 ND 7 +++/
Largemouth Bass (M.salmoides)			3 +/ 4 +/ 5 +++/	7 ++++/	7 +++/		1-6 ND	1-6 ND
Oriental Sheatfish (*S. asotus*)		7 +++/	3 ++/ 4 ++/ 5 ++++/	7 ++++/	2 +/ 3 /++ 4 /++ 5 +++/ 7 +++/	7 ++++/ +	1-6ND	1-6ND 7 ++/
Freshwater Pomfret (*C.brachypomum*)		4 +/ 7 ++/	2 +/+ 3 ++/ + 4 +++/+ 5 +/	7 ++++/	1 +/		1-7ND	1-7ND
Nile tilapia (*T.nilotica*)		3 +++/ 4 ++/ 5 +/ 7 ++/	2 +++/ + 3 ++/+ 4 ++/+ 5 +/	7 ++++/			1-7ND	1-7ND

Notes: 1=esophageal epithelium / esophageal gland; 2=cardiace pithelium / cardiac gland; 3=gastric epithelium / fundus gland; 4=pyloric epithe lium / pyloric gland; 5=intestinal epithelium / intestinal gland; 6=intermus cular nerve plexus of digestive tract; 7=pancreatic island / pancreas; ++++=above 30 IRE cells in one field 10×20; +++=20-29 cells; ++=10-19 cells; +=below 10 cells; No IRE cell was found if the parts of sample were not listed in table; ND=not detected.

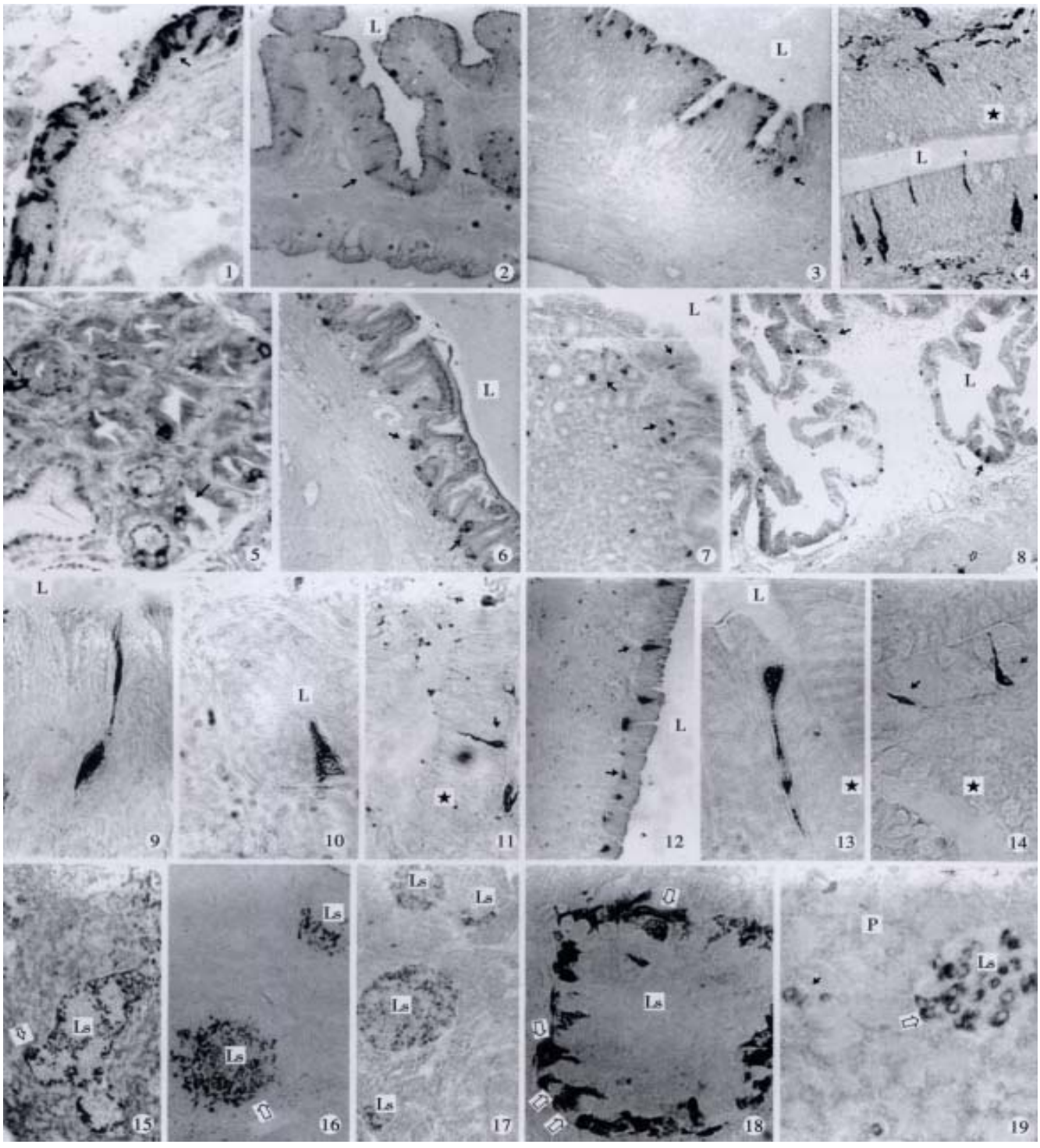

Figure 1 Distribution of CAL-IRE cells (⬆) in esophageal epithelium of *M. albus*. ×66
Figure 2 SOM-IRE cells (⬆) in intestinal epithelium of *T. nilotica*. ×33
Figure 3 GAS-IRE cells (⬆) in gastric epithelium of *C. argus*. ×33
Figure 4 5-HT-IRE cells (⬆) in intestinal epithelium of *C.argus*. ×100
Figure 5 SOM-IRE cells (⬆) in gastric gland of *P. fulvidraco*. ×152
Figure 6 SOM-IRE cells (⬆) in pyloric gland of *P. fulvidraco*. ×33
Figure 7 SOM-IRE cells (⬆) in cardiac epithelium and gland of *S. chuatsi*. ×66
Figure 8 5-HT-IRE cells in intestinal epithelium (⬆) and intermuscular nerve plexus (⇧) of *S. chuatsi*. ×33
Figures 9-10 Shape of 5-HT-IRE cells (⬆) in gastric epithelium and glands of *M. salmoides*. ×200
Figure 11 5-HT-IRE cells (⬆) in intestinal epithelium of *M. salmoides*. ×100
Figure 12 5-HT-IRE cells (⬆) in gastric epithelium of *S. asotus*. ×33
Figure 13 Shape of 5-HT-IRE cells (⬆)in intestinal epithelium of *S. asotus*. ×200
Figure 14 5-HT-IRE cells (⬆) in intestinal epithelium of *C. brachypomum*. ×200
Figure 15 GLU-IRE cells (⇧) in pancreatic islands of *S. chuatsi*. ×33
Figure 16 SOM-IRE cells (⇧) in pancreatic islands of *S. asotus*. ×33
Figure 17 INS-IRE cells (⇧) in pancreatic islands of *S. chuatsi*. ×33
Figure 18 Shape of GLU-IRE cells (⇧) in pancreatic islands *S. asotus*. ×268
Figure 19 GH-IRE cells in pancreatic islands (⇧) and pancreas (⬆) of *S. asotus*. ×200

Note: ★:goblet cell; L: gastrointestinal lumen; Is: pancreatic island; P: pancreas

DISCUSSION

The distribution of APUD cells in the GEP system of stomach-containing teleosts and in the digestive system of human and stomachless teleosts is quite different. GAS-IRE cells existed in human GEP system[8,14-16] and in the intestine of 7 kinds of stomachless teleosts[42], but at present study, GAS-IRE cells were not seen in the digestive system of 7 fish species except in the esophagus of *M. albus* and in the stomach of *C. argus* and *P. fulvidraco*. After ICC identification of APUD cells in the gut of 7 stomachless teleosts, the 5-HT-IRE cells were not found in the gut[42]; but in the intestine of 7 stomach-containing teleosts except *M. albus*. Rombout and Reinecke[47] found SOM-IRE cells only existing in the stomach of stomach-containing teleosts; but at present studies, SOM-IRE cells were seen in the esophagus of *M. albus,* the gastric epithelium and intermuscular nerve plexus of *P. fulvidraco,* the gastric epithelium of *S. chuatsi* and *T. nilotica* . GLU-IRE cells existed universally in the gut of stomachless teleosts[42]; but in this study they were not found in the gastrointestinal tract of 8 stomach-containing teleosts.

It was reported that there were 4 APUD cell types: B cell (secreting INS), PP cell (secreting pancreatic polypeptide), A cell (secreting GLU) and D cell (secreting SOM) in the pancreatic island of fish species[48]. In our studies,CAL-IRE cells were found in the pancreatic island of *S. asotus* and *M. salmoides*. Calcitonin was a hormone secreted by ultimobranchial gland of fish species[49], and also was found in the secretory cells of prolactin hormone of rat hypophysis[50]. But up to now, no report showed that CAL-IRE cells exist in the pancreatic island of fish species. CAL-IRE cells in the pancreatic island of *S. asotus* and *M. salmoides* were morphologically long shuttle-shaped which was different from other endocrine cells in the pancreatic island which had the shorter cytoplasmic process and irregular shape. This specific morphological feature provides evidences that CAL-IRE cells in the pancreatic island of *S. asotus* and *M. salmoides* may adapt to some specific physiological functions and conduct endocrine paracrine action[8].

It is a common knowledge that there are GH-IRE cells in the meso-adenohypophysis of fish species and can promote the growth of them. Our studies demonstrated that there was no GH-IRE cell in the gastrointestinal tract of fish species[46], and GH was not gastrointestinal hormone. However our studies found for the first time that GH-IRE cells in the pancreatic islands of *S. chuatsi* and *S. asotus*, and in the exocrine pancreas of *S. asotus* had scattering distribution. It is evidenced that GH-IRE cells were not only located in the endocrine organ-pancreatic island but in the digestive gland in a small amount. It shows morphological evidence of brain-gutpeptide with an endocrine-exocrine mode of action[51]. It suggests that the GH-IRE cells existed simultaneously in the hypophysis, pancreatic island and pancreas, and the other brain-gut peptide or brain-pancreas peptide varied in pattern of distribution, and the diversity of physiological function of GH-IRE cells based on their morphology.

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