

An immunohistochemical study of the gastro-entero endocrine cells in the snakehead, *Channa(Ophicephalus) argus*

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가물치 위장관에 있어서 내분비세포의 면역조직화학적 연구

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초록 : 가물치의 위장관 내분비세포의 부위별 분포 및 출현빈도를 면역조직화학적으로 관찰하였던 바, secretin 면역반응세포들은 위의 분문부와 위저부의 상피 또는 장선부에서 소수로 출현하였다. Motilin과 GRP 면역반응세포는 장의 원위부와 위저부의 상피에서 극소수로, PYY 면역반응세포들은 유문맹낭부위와 장의 근위부에서 소수 또는 극소수로 각각 관찰되었다. Substance P 면역반응세포들은 위저부에서 장의 원위부에 걸쳐 소수 내지는 비교적 다수의 출현빈도를 보였으며 또한 장의 원위부 고유층에서는 신경세포가 양성 반응을 보였다. 그러나 neurotensin, met-Enk, GIP 면역반응세포들은 전장관에서 관찰할 수 없었다.

Key words : gastrointestinal tract(GIT), snakehead, endocrine cell, immunoreactive cell.

Introduction

The carnivorous snakeheads are distributed in northeastern Asia including the Korea, China and Japan. In recent years various peptides have been described in endocrine cells of the gastrointestinal tract(GIT) of most

vertebrates. However, less is known about information concerning the occurrence of endocrine cells in the GIT of fish. It has been reported the distribution and frequency of endocrine cells in the GIT of fish showed with regard to different species. Although four endocrine cells were observed in the GIT of the snake-

head¹, the occurrence of the other endocrine cells has not been investigated.

The present study was undertaken to demonstrate the regional distribution and relative frequency of each endocrine cell type in the GIT of the snakehead, *Channa (Ophicephalus) argus*, by specific immunohistochemistry.

Materials and Methods

Five adult specimens of both sexes of the snakehead, *Channa (Ophicephalus) argus*, were used in this study. The snakehead were decapitated and the esophagus, stomach(cardia, fundus, pylorus), pyloric caeca and intestine (proximal, distal) were dissected out. The tissue samples were fixed in Bouin's fluid, dehydrated and embedded in paraffin. After deparaffinizing, immunohistochemical staining was performed using the avidin-biotin complexes (ABC) technique². Background blocking was performed with normal serum prior to incubation with the primary antisera (Table 1).

After rinsing in PBS buffer, the sections were incubated with biotinylated goat anti-rabbit IgG(1 : 200) and biotinylated rabbit anti-rat IgG(1 : 200). They were then washed in PBS buffer and finally followed by a third incubation with avidin-biotin complexes(Vector). The peroxidase reactions were developed in a solution of 3,3' diaminobenzidine tetrahydrochloride containing 0.01% H₂O₂ in Tris-HCl buffer. After immunostaining,

the sections were lightly counterstained with Mayer's hematoxylin.

Results

By means of immunohistochemical method five endocrine cell types were found, namely secretin, motilin, GRP, PYY and substance P-immunoreactive cells. Also, nerve cells positive to substance P were observed. However, neurotensin, met Enk and GIP immunoreactive cells were not found in the GIT of the snakehead (Table 2). The distribution and frequency of the immunoreactive cells in the various parts of the GIT are given in Table in the stomach, the glandular region was less development in the snakehead than in that of the other vertebrates. The immunoreactive cells occurred in the GIT of the snakehead were mainly located in between the columnar cells of mucosa, having long cytoplasmic processes.

A few number of secretin-immunoreactive cells were distributed in the cardia and the fundus of the stomach (Fig 1a, d). In the cardiac region, they were detected not only in the columnar cells of mucosa but the tubular glands (Fig 1d).

Rarely motilin and GRP-immunoreactive cells were restricted to the distal intestine (Fig 2) and the fundus (Fig 3), respectively.

PYY-immunoreactive cells were a few in the pyloric caeca (Fig 4a) and rarely in the proximal intestine (Fig 4b).

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Dilution
Secretin	R-801	Dr. N.Yanaihara	1:1,000
Neurotensin	R-3501	"	1:1,000
Motilin	R-1104	"	1:1,000
Gastrin releasing peptide (GRP)	R-6902	"	1:6,000
met-Enkephalin-8 (met-Enk)	R-0171	"	1:8,000
Gastric inhibitory peptide (GIP)	G/R/34-111 D	immunonuclear Corps., Stillwater	1:10,000
Polypeptide YY (PYY)	R841303-2	Milab, Malmö, Sweden	1:40,000
Substance P	B9C 35	Sera-Lab., Sussex,	1:1,000

* All antisera were raised in rabbits except that against substance p which monoclonal raised in rat.

Table 2. Regional distribution and relative frequency of endocrine cells in the GIT of the snakehead, *channa (Ophiccephalus)argus*

	Esophagus	Stomach			Pyloric caeca	Intestine	
		Cardia	Fundus	Pylorus		Proximal	Distal
Secretin	--	+	+	--	--	--	--
Neurotensin	--	--	--	--	--	--	--
Motilin	--	--	--	--	--	--	±
GRP	--	--	±	--	--	--	--
m-Enk	--	--	--	--	--	--	--
GIP	--	--	--	--	--	--	--
PYY	--	--	--	--	+	±	--
Substance P	--	--	++	++	++	+	++

--Not detected, ± Rare, + A few, ++ Relatively numerous.

They were a slender spindle shape.

Relatively numerous substance p-immunoreactive cells were found from the fundus(Fig 5a) to the distal intestine (Fig 5b). They were oval shape in the fundic regions(Fig 5a) and a slender spindle shape in the other regions(Fig 5b-d). In addition substance P-immunoreactive nerve cells were detected in the lamina propria in the distal intestine (Fig 5b).

Discussion

Using antisera against mammalian peptide hormones, we have demonstrated the presence of five kinds of immunoreactive cells and nerve cells to substance P in the GIT of the snakehead. Kim and Lee¹ have been previously described for six endocrine cells in the GIT of the same species. In the present study, however, neurotensin-, met-Enk- and GIP-immunoreactive cells were not detected.

Although the antisera raised against mammalian neurotensin do not detect immunoreactive cells in teleosts³⁻⁷, they have been found scattered in the entire intestine of the goldfish⁸. In cartilaginous fishes, El-Salhy⁹ reported that neurotensin molecule is not identical to that of mammals due to detect only by N-terminal specific antiserum. Our result is in accordance with a previous reports on the teleosts.

Enkephalin-immunoreactive cells have been reported to occur in the GIT of teleosts^{4,10-12} and cartilaginous fish¹³. In particular, they occurred in the GIT of all ver-

tebrates except amphibians¹⁴ and reptiles⁹. Nevertheless, these cells were not detected in this study.

The presence of GIP-immunoreactive cells has been demonstrated previously in the GIT of cartilaginous fishes^{13,15}. However, they were not detected in the GIT of the snakehead in our study, coinciding with a previous report on the teleosts⁷.

Secretin- and motilin-immunoreactive cells in the GIT of teleosts have not been detected previously⁷. The present study showed that secretin-immunoreactive cells occurred in the cardia and fundus, and motilin-immunoreactive cells were found only in the distal intestine. The distribution pattern of these cells in the snakehead differs from those of the other teleosts⁷.

Bombesin/GRP-immunoreactive cells have been identified previously in the GIT of amphibians^{14,16}, reptiles⁹ and birds¹⁷. Also, in fishes these cells have been described previously in the GIT in teleosts^{4,5,18} and cartilaginous fishes^{12,13,22}. In the present study, GRP-immunoreactive cells were found mainly in the stomach, agreeing with a previous report on teleosts^{4,5,18}.

PYY-immunoreactive cells have been identified in the GIT of some teleosts^{4,10,19,20} and cartilaginous fishes^{13,21}. As reported in those studies, the distribution pattern of these cells were mainly appeared in the stomach and the small intestine of fishes. Our result showed that such cells detected in the pyloric caeca and the proximal intestine in the snakehead.

This finding is similar to those reported previous studies.

Substance P-immunoreactive cells were mainly found

in the epithelium of the intestines^{7,13,22}, where as they were distributed throughout the GIT of a few teleosts^{4, 18}. On the other hands, the occurrence of substance P-immunoreactive nerve cells were also investigated in entire GIT^{4,18} and the intestines¹³. El-Salhy¹³ reported that substance P peptide has already been established in the gut at the evolutionary level of the cartilaginous fishes. Our study demonstrated the occurrence of these endocrin cells from the fundus to the distal intestine and these nerve cells in the distal intestine of the snakehead. This results differ from a previous reports on the other species.

In conclusion, we have demonstrated the characteristics patterns of distribution of five kinds of endocrine cells including substance P-immunoreactive nerve cell and their relative frequency of the snakehead.

Summary

The regional distribution and relative frequency of endocrine cells in the GIT of the snakehead, *Channa(Ophicephalus) argus* were studied immunohistochemically. Five kinds of endocrine cells and one kind of nerve cell were identified in this study.

A few numbers of secretin-immunoreactive cells were restricted to the cardia and fundus of the stomach. Motilin- and GRP-immunoreactive cells were rare in the epithelium of the distal and proximal intestines. PYY-immunoreactive cells were found in a few number in the pyloric caeca and were rare in the distal intestine. Substance P-immunoreactive cells were distributed relatively numerous from the fundus to the distal intestine. Also, their nerve cells were detected in occurrence in the lamina propria in the distal intestine. No neurotensin-, met-Enk- and GIP-immunoreactive cells were found in the GIT of the snakehead.

Legends for figures

Fig 1. Secretin-immunoreactive cells(arrowheads) in the cardia(a) and fundus(b). a, b; × 480

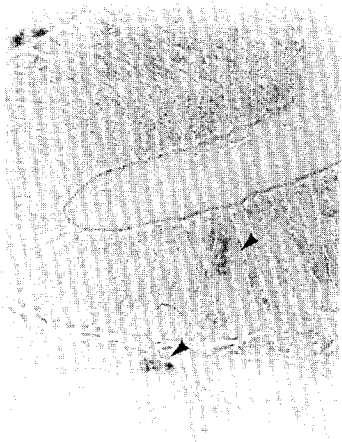
Fig 2. Motilin-immunoreactive cells(arrowheads) in the distal intestine. × 480

Fig 3. GRP-immunoreactive cells(arrowhead) in the fundus. × 480

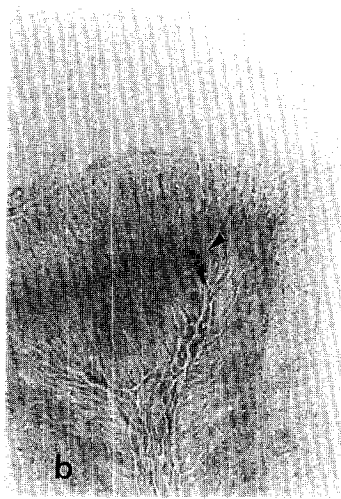
Fig 4. PYY-immunoreactive cells(arrowheads) in the pyloric caeca(a) and proximal intestine(b). × 480

Fig 5. Substance P-immunoreactive cells(arrowheads) in the fundus(a), pylorus(b), pyloric caeca(c) and distal intestine(d). a, b, d; × 480, c; × 240

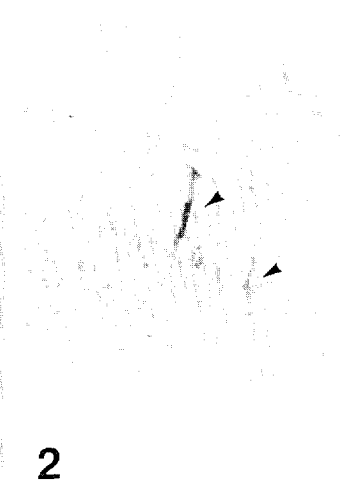
Note numerous substance P-containing nerve cells are located beneath the epithelium(arrows)



1a



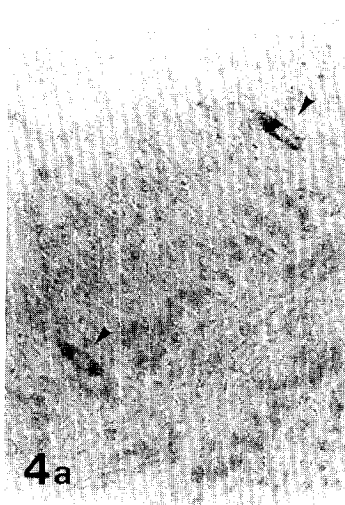
b



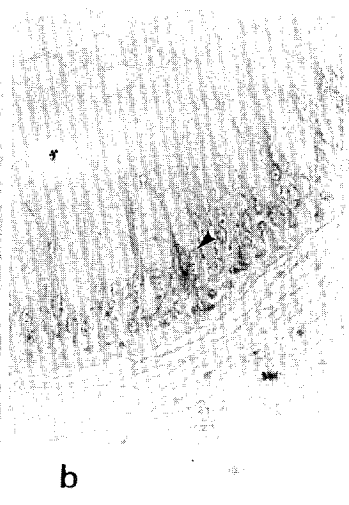
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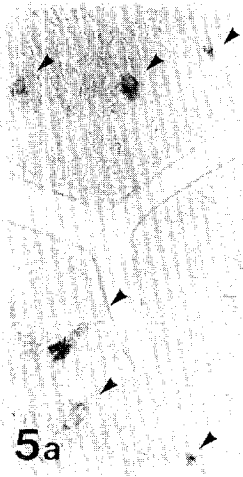
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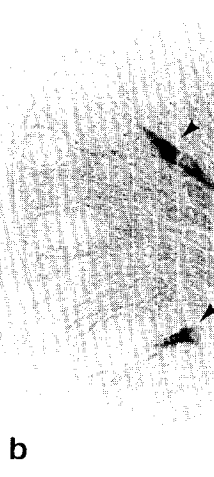
4a



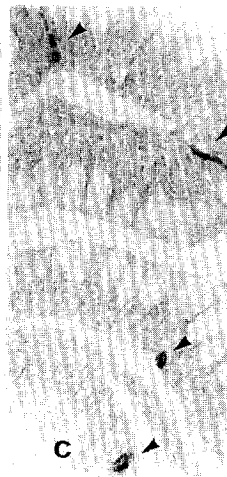
b



5a



b



c



d

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