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An immunohistochemical study of endocrine cells in the alimentary tract of the snake, *Rhabdophis tigrinus tigrinus*

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Abstract : The regional distribution and relative frequency of endocrine cells in the alimentary tract of the snake, *Rhabdophis tigrinus tigrinus*, were investigated by immunohistochemical method using 7 antisera. Chromogranin (Cg)-, glucagon-, somatostatin-, gastrin/cholecystokinin (Gas/CCK)-, serotonin-, bovine pancreatic polypeptide (BPP)-immunoreactive cells were identified in this study.

Cg-immunoreactive cells were detected throughout the alimentary tract including the esophagus, with predominant frequency in the pylorus. Numerous immunoreactive cells were observed from the esophagus to the pylorus but a few cells were detected in the large intestine. Glucagon-immunoreactive cells were observed from the proximal portions to the distal portions of the small intestine. They were increased to the middle portions but thereafter decreased, and no cells were found in the terminal portions. Somatostatin-immunoreactive cells were restricted to the small intestine and these cells were decreased toward to distal portions of the small intestine. Gas/CCK-immunoreactive cells were detected in the pylorus and small intestine. They were most predominant in the pylorus and the proximal portions of the small intestine but thereafter decreased toward to the distal regions. Serotonin-immunoreactive cells were observed throughout the alimentary tract. They were most predominant in the pylorus and proximal portions of the small intestine but a few cells were observed in the large intestine. BPP-immunoreactive cells were restricted to the distal portions of the small intestine with rare frequency. No bombesin-immunoreactive cells were found in this study.

Key words : endocrine cell, alimentary tract, snake, immunohistochemistry.

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Introduction

Rhabdophis tigrinus tigrinus belonging to the Colubridae in order Squamata is widespread throughout in the Korea. Hormones secreting endocrine cells in the alimentary tract are important in regulating digestive function[1]. Although many studies have been elucidated the regional distribution and relative frequency of different endocrine cells in the alimentary tract of the various vertebrates, immunohistochemical studies on the Reptilia have received little attention. Most recently intensive studies have been done on the reptilian species because their phylogenetical tree is situated at middle in the evolution of vertebrates[2].

Many reports have dealt with the identification of regulatory peptides of the alimentary tract in the reptilian species[2-16] by using silver techniques and either radioimmunochemical or immunohistochemical methods. But little data is available on the regional distribution and relative frequency of endocrine cells along the entire length of the gastrointestinal tract (GIT) of the Colubridae. Gastrin/cholecystokinin (Gas/CCK)- and somatostatin-immunoreactive cells were identified in the small intestines of three species of the Viperidae. However pancreatic glucagon-, secretin- or motilin-immunoreactive cells were not observed in those species reported by Masini[17]. In addition, somatostatin-containing cells have been detected in the stomach and small intestine of the European adder *Vipera berus*[18].

The purpose of the present study was to clarify the regional distribution and relative frequency of the alimentary tract of the Colubridae, *Rhabdophis tigrinus tigrinus* by specific immunohistochemistry.

Materials and Methods

Five adult snakes of the Colubridae, *Rhabdophis tigrinus tigrinus*, without sexual distinction were used in this study. As there was no clear demarcation between the regions of alimentary tract, this was divided into 9 portions according to Arena *et al*[8]. Samples from the esophagus, fundus, pylorus, 5 portions of small intestine (S I - V) and large intestine were fixed in Bouin's solution. After paraffin embedding, 3~4μm serial sections were prepared. Representative sections of each tissues were stained with hematoxylin and eosin for light microscopic examination of the normal alimentary architecture.

The each representative sections were deparaffinized, rehydrated and immunostained with the peroxidase antiperoxidase (PAP) method[19]. Background blocking was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing 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 buff-

Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
Chromogranin	8541011	Immunonuclear Corp., Stillwater	1 : 500
Glucagon	8635013	Immunonuclear Corp., Stillwater	1 : 800
Somatostatin	CA325	Cambridge Research Biochemicals, Billerica	1 : 1,000
Gastrin/cholecystokinin	i600/004	Union Chimique Belge, bioproducts	1 : 100
Serotonin	8535028	Immunonuclear Corp., Stillwater	1 : 10,000
Bovine pancreatic polypeptide	i607	Union Chimique Belge, bioproducts	1 : 5,000
Bombesin	08230	Cambridge Research Biochemicals, Billerica	1 : 8,000

* All antisera were raised in rabbits.

er (0.05M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive cells were observed under light microscope.

Results

In this study, 6 kinds of the immunoreactive cells were detected with the antisera against Cg, glucagon, somatostatin, Gas/CCK, serotonin and BPP. The regional distribution and relative frequency of these cells in the alimentary tract of the Colubridae, *Rhabdophis tigrinus tigrinus*, are shown in Table 2.

In addition, most of immunoreactive cells were generally spherical or spindle in shape and Gas/CCK-immunoreactive cells were occasionally round in shape.

Cg-immunoreactive cells, reaching a peak in the pylorus were demonstrated in the epithelia and mucosal glands of the entire alimentary tract. These cells were observed to increased from the esophagus to the pylorus but thereafter decreased to the large intestine (Fig 1a-i).

Glucagon-immunoreactive cells were detected in the epithelia and basal portion of the epithelia in the small intestine except for the most distal portions (SⅤ) (Fig 2a-d). A few cells were observed from the proximal portions (SⅠ, Ⅱ) and increased toward to middle portion (SⅢ) but thereafter decreased to distal portions and no cell was found in the terminal portions (SⅤ).

Somatostatin-immunoreactive cells were found in the epithelia throughout the small intestine (Fig 3a-d). A few cells were detected in the proximal to distal portions (SⅠ-Ⅳ) but rare in the terminal portions (SⅤ).

Gas/CCK-immunoreactive cells were observed throughout the alimentary tract except for the esophagus, fundus and large intestine (Fig 4a-f). They were reaching a peak in the pylorus and proximal portions of the small intestine (SⅠ) and thereafter decreased toward to the distal portion of the alimentary tract.

Serotonin-immunoreactive cells, reaching a peak from the pylorus to proximal portions of the small intestine (SⅠ, Ⅱ) were found in the epithelia throughout the alimentary tract. These cells were increased from the esophagus to the proximal portions of the small intestine (SⅠ, Ⅱ) and thereafter decreased toward to the large intestine (Fig 5a-h).

However, rare number of BPP-immunoreactive cells were restricted to the distal portions of the small intestine (SⅣ) (Fig 6) and no bombesin-immunoreactive cells were detected in this study.

Discussion

In the present study, Cg-, glucagon-, somatostatin-, Gas/CCK-, serotonin- and BPP-immunoreactive cells were identified in the alimentary tract of the snake, *Rhabdophis tigrinus tigrinus*. The regional distribution and relative fre-

Table 2. The regional distribution and relative frequencies of immunoreactive cells in the alimentary tract of the snake

	Esophagus	Stomach		Small inteastine					Large intestine
		Fundus	Pylorus	SⅠ	SⅡ	SⅢ	SⅣ	SⅤ	
Chromogranin	++	++	+++	++	++	++	++	+	+
Glucagon	-	-	-	+	+	++	+	-	-
Somatostatin	-	-	-	+	+	+	+	±	-
Gastrin/Cholecystokinin	-	-	+++	+++	++	++	+	+	-
Serotonin	++	++	+++	+++	+++	++	++	++	+
Bovine pancreatic polypeptide	-	-	-	-	-	-	±	-	-
Bombesin	-	-	-	-	-	-	-	-	-

Remarks : ++ meoderate, +; a few, ±' rare, -; not detected.

quency of immunoreactive cells were essentially similar to those of the other reptilia[2~18]. However, some characteristic differences were observed in this species. According to the previous reports[7,20~22], it can be concluded that these differences might be due to the differences of the antisera tested, the methods or species differences used in those studies.

It have been reported that Cg-immunoreactive cells were widely distributed in the entire gastrointestinal tract(GIT) of the Amur lizard[23] and *Podarcis sicula*[11], and the small intestine of *Podarcis hispanica*[9]. According to these reports, Cg-immunoreactive cells were most predominant in the pylorus. On the other hands, Cg-immunoreactive cells are widely distributed in endocrine cells of mammals[24] and have been used in marker of other endocrine cells[25]. In this study, these cells were detected throughout the alimentary tract including the esophagus, with predominant frequency in the pylorus. This result shows that the regional distribution and relative frequency of these cells are quite similar to those of the lizard species[9,11,23]. However, their distribution and relative frequency were difficult to compare with other works because there were no reports that were carried out in the other snake species.

Glucagon is synthesized in the A cells of the pancreas and regulated serum glucose levels. Glucagon-immunoreactive cells were distributed throughout the GIT except for the cloaca of the caimen[14] or in the fundus and intestine of the alligator[2]. However, no glucagon-immunoreactive cells were detected in the alimentary tract of the King's skink[8] and small intestine of the snake[17]. In addition, Kim *et al*[26] reported that these cells were restricted to the stomach regions of the pond tortoise. According to the above works, it is suggested that the regional distribution of glucagon-immunoreactive cells were different from the other reptilia. In the present study, glucagon-immunoreactive cells were observed from the proximal to distal portions of the small intestine. They were increased toward to the middle portions but thereafter decreased to the distal portions of the small intestine, and no cells were found in the terminal portions. These findings were somewhat different from the previous reports[2,8,17,26].

Somatostatin that was 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[27]. This substance inhibits the secretion of the other hormones[28]. It is known that somatostatin-immunoreactive cells show the widest distribution in the whole GIT of all vertebrate species investigated, including the primitive agnathans[29]. In the present study, somatostatin-immunoreactive cells were restricted to the small intestine and these cells were decreased toward to the distal portions of the small intestinal tract. These results were mostly agreed with those of the other reptilia[2,17,29], but somewhat different from Lee *et al*[23] who reported that no somatostatin-immunoreactive cells were detected in the GIT of the amur lizard.

Gastrin secreted by intestinal G cell, was promoted the gastric acid secretion and CCK secreted by intestinal I cell was stimulated the pancreatic enzyme secretion. Gas/CCK-immunoreactive cells were detected from the pylorus to the small intestine of this species. These results were well corresponded with the other reptilian reports[2,14,17].

Serotonin were consisted of monoamines and widely distributed in nervous system and GEP endocrine cells[30]. Main functions of serotonin were inhibition of gastric acid secretion and contraction of smooth muscle in the GIT[31]. El-Salhy *et al*[30] reported that serotonin-immunoreactive cells found throughout the GIT of all species and established in the GIT at the early stage of vertebrate evolution. In reptilians, these cells are distributed in the whole alimentary tract[8], except for the pylorus and cloaca[13]. Similar to the King's skink[8], these cells were observed throughout the alimentary tract in the present study.

Since PP-immunoreactive cells have described for the first time in the lizard pancreas[32,33], the occurrence of these cells have been demonstrated in the GIT of the reptiles[7~10]. We observed that BPP-immunoreactive cells were distributed in the distal portions of the small intestine, which were somewhat different from the other reptilian reports[7~10].

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Legends for figures

Fig 1. Cg-immunoreactive cells in the alimentary tract.

a. Esophagus	b. Fundus	c. Pylorus	d. S I
e. S II	f. S III	g. SIV	h. S V

i. Large intestine.
a-i : × 480, PAP methods.

Fig 2. Glucagon-immunoreactive cells in the alimentary tract.

a. S I	b. S II	c. S III	d. SIV

a-d : × 480, PAP methods.

Fig 3. Somatostatin-immunoreactive cells in the alimentary tract.

a. S I	b. S III	c. SIV	d. S V

a-i : × 480, PAP methods.

Fig 4. Gas/CCK-immunoreactive cells in the alimentary tract.

a. Pylorus	b. S I	c. S II	d. S III
e. SIV	f. S V		

a, e, f : × 480, b-d : × 240, PAP methods.

Fig 5. Serotonin-immunoreactive cells in the alimentary tract.

a. Esophagus	b. Fundus	c. Pylorus	d. S II
e. S III	f. SIV	g. SV	h. Large intestine.

a-c, e, h : × 480, d, f, g : × 240, PAP methods.

Fig 6. BPP-immunoreactive cells in the distal small intestine(SIV).
× 480, PAP methods.

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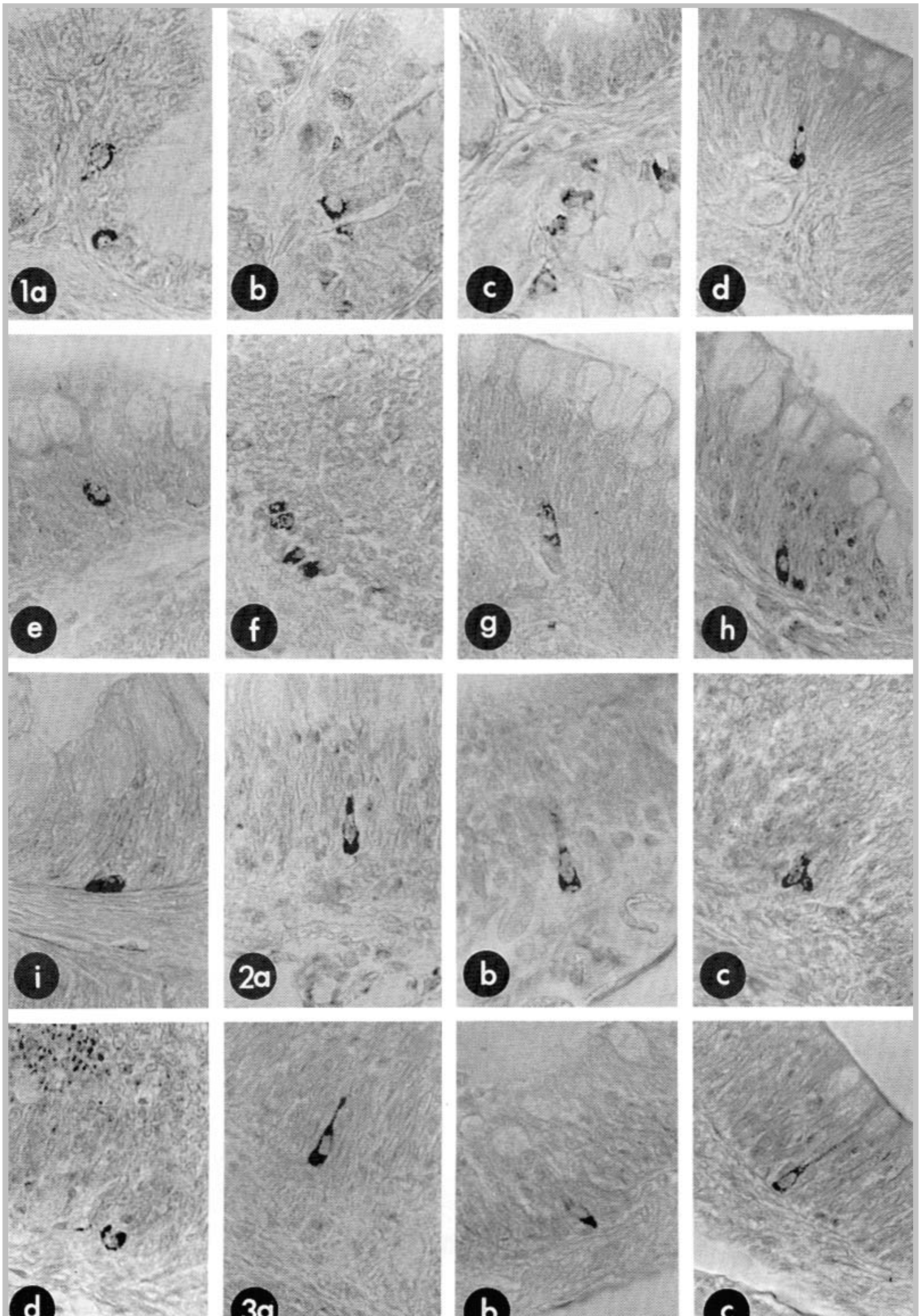
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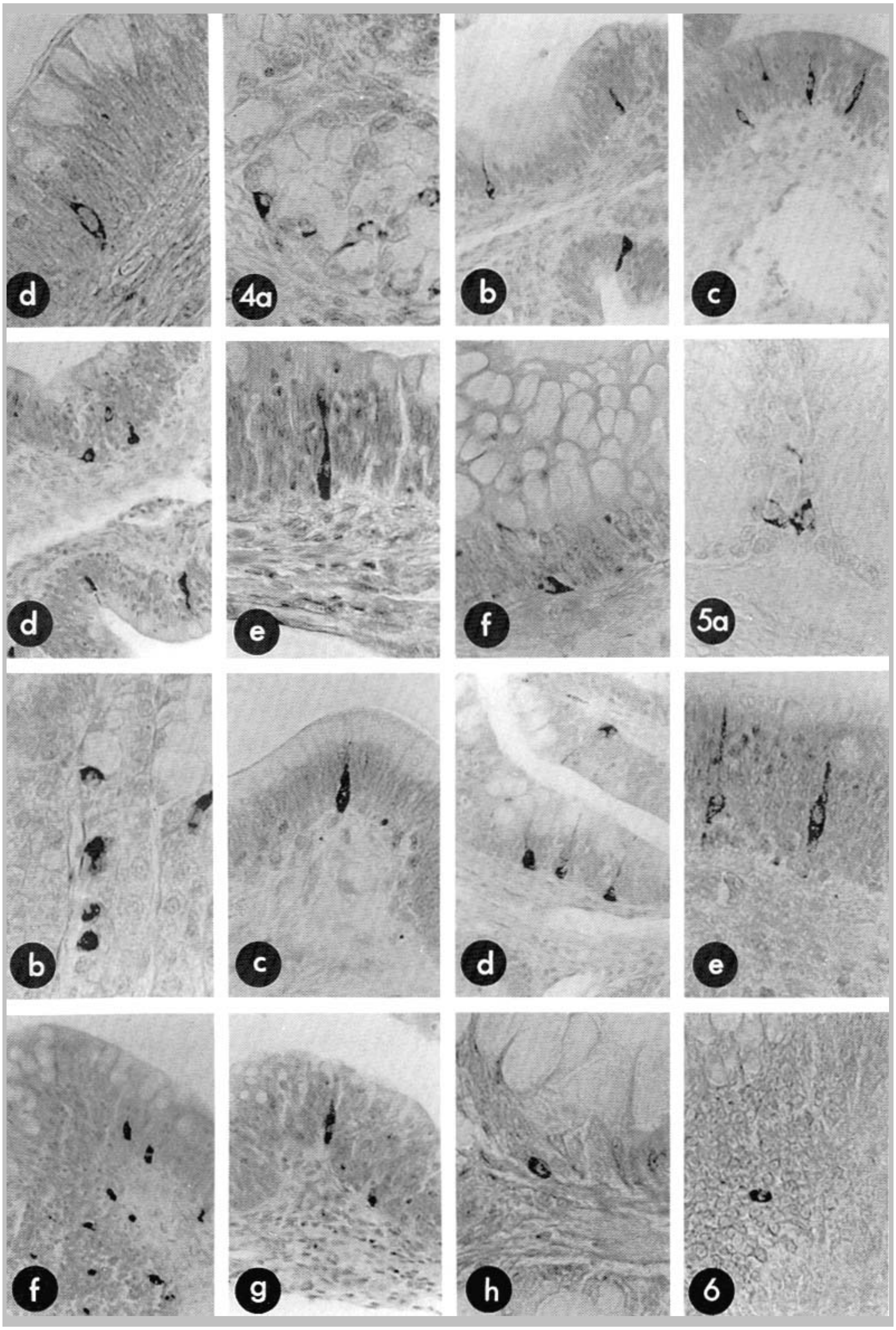
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