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An histological and immunohistochemical study of endocrine cells in the gastrointestinal tract of the Amur lizard (*Takydromus amurensis*)

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아무르장지뱀의 위장관 내분비세포에 관한 조직화학적 및 면역조직화학적 연구

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초록 : 아무르장지뱀(*Takydromus amurensis*)의 위장관 점막에 분포하는 내분비세포의 분포와 출현빈도 및 세포의 종류를 알아보기 위하여 몇가지 도은법 및 면역조직화학적 방법으로 관찰하였다.

Grimelius법에만 염색된 은호성세포(argyrophil cell)는 유문부에 다수로 십이지장에 중등도로 국한되어 출현하였다. 한편 bovine CG면역반응세포는 유문부에서 최고의 빈도로 전장관에 걸쳐 분포하였다. BPP면역반응세포는 소장부위에서만 동정되었다. 따라서 아무르장지뱀의 위장관 내분비세포에서 은호성세포와 bovine CG면역반응세포는 분포와 출현빈도에서 일치하지 않았다.

Key words : endocrine cell, gastrointestinal tract(GIT), Amur lizard, argyrophil cell, immunoreactive cell

Introduction

Although endocrine cells of the gastrointestinal tract(GIT) have been extensively elucidated in mammals, 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.

Many reports have dealted with the identification of regulatory peptides of the GIT in the reptilian species of the order Chelonia[1-4], Squamata including the suborder Lacertilia[5-10] and Serpentes[11,12], and Crocodilia[13-18] by using silver techniques and either radioimmunochemical or immunohistochemical methods.

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In this study, we have reported by immunohistochemical methods the presence of endocrine cells in the GIT of the Amur lizard, *Takydromus amurensis*.

Materials and Methods

Five adult of the Amur lizard(*Takydromus amurensis*) of both sexes were collected from the Kyungsan area. The specimens were lightly anaethetized with ether, dissected out five regions of the GIT and fixed in Bouin's fluid. After paraffin embedding, 4 μm histological sections were prepared. The sctions were stained by haematoxylin-eosin, the Grimelius[19], the Sevier-Munger[20], the Hellerström-Hellman methods[21] for the study of argyrophil cells, and the Masson-Hamperl argentaffin method[22].

For immunohistochemical study, the peroxidase-antiperoxidase(PAP) method[23] was applied. Background blocking was performed with normal goat serum prior to incubation with specific antiserum (Table 1). After rinsing in PBS buffer, the sections were incubated in secondary serum. 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 containg 0.01% H_2O_2 in HCl buffer. After immunostaining, the sections were lightly counterstained with Mayer's haematoxylin.

Results

The results of the silver stains that were present in the GIT of the Amur lizard are summarized in Table 2. Argyrophil cells were only stained by the Grimelius technique but not by the other silver impregnation methods. Also argentaffin cells could not be demonstrated in throughout the GIT of the this species. The argyrophil cells in the pyloric glands were situated almost in the between the gland epithelial cells as oval-shaped(Fig 1a) and in the duodenum, these cells were inserted among the columnar cells of mucosa, being short cytoplasmic processes(Fig 1b).

Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
Bovine chromogranin(Bovine CG)	8541011	Immunonuclear Corp	1:500
Bovine pancreatic polypeptid(BPP)	i607	Union Chimique Belge, bioproducts	1:5,000
Gastrin/cholecystokinin(Gas/CCK)	i600/004	〃	1:100
Somatostatin	CA325	Cambridge Research Biochemical Ltd.	1:1,000
VIP		Dept of Vet Phar Hokkaido Uni	1:2,000

* All antisera were raised in rabbits except for insulin which was raised in a guinea pig.

Table 2. The results obtained with the silver stains in the gastrointestinal tract of the Amur lizard

	Fundus	Pylorus	Duodenum	Ileum	Rectum
Grimelius	-	+++	++	-	-
Sevier-Munger	-	-	-	-	-
Hellerström-Hellman	-	-	-	-	-
Masson-Hamperl	-	-	-	-	-

- not detected, ± rare, + few, ++ moderate, +++ numerous

Table 3. The distribution and frequency of endocrine cells in the gastrointestinal tract of the Amur lizard

	Fundus	Pylorus	Duodenum	Ileum	Rectum
Bovine CG	++	+++	+	±	+
BPP	-	-	++	+	-
Somatostatin	-	-	-	-	-
Gas/CCK	-	-	-	-	-
VIP	-	-	-	-	-

The relative incidence of these cells made using the same five grade scale as in Table 2.

Using immunohistochemical method, two types of immunoreactive cells are shown in the GIT as listed in Table 3.

Bovine CG-immunoreactive cells were distributed in the epithelia throughout the GIT, with predominant frequency in the pyloric glands(Fig 2b) and rarely in the ileum(Fig 2d). In the stomach, they were situated almost always in the glands as pyramidal and oval cells(Fig 2a, b). In the intestine, they were observed in between the columnar cells of mucosa as wedge-shaped cells(Figs 2c-e).

BPP-immunoreactive cells were visualized only in the duodenum and the ileum. Also they were gradually decresed distally along the intestine. They were inserted in the mucosa as thin spindle-shaped cells that contacted the lumen via a long apical cytoplasmic processes(Figs 3a, b).

No immunoreactivity was detected for somatostatin, Gas/CCK and VIP(Table 3).

Discussion

Silver techniques are known to be widely used for the identification of endocrine cells in the GIT of various vertebrates[24,25], despite remains of the problems in chemical nature of these staining reactions[19]. Among silver techniques, only the Grimelius stains was successfully stained endocrine cells and gave a primary result study of the distribution and frequency of the argyrophil cells in the GIT of the Amur lizard. In the present study, our results showed that endocrine cells were distributed numerous in the pyloric glands and moderate in the duodenum. This pattern of regional distribution of these cells that were devoid of the fundic glands, the ileum and the rectum was quite different from that reported on previous studies[5,6,9,10]. The failure to detect gut endocrine cells in the Amur lizard by the other silver methods cannot interpret whether these cells are actually absent from the GIT or whether it is due to the fixative reported by El-Salhy and Grimelius[6].

The identification of endocrine cells in the GIT of the Amur lizard was carried out by immunohistochemical method. In the present study bovine CG-immunoreactive cells were widely demonstrated in the entire GIT of the Amur lizard. CG-immunoreactive cells have been reported throughout the GIT[10] except for the small intestine[7], and on the small intestine of *Podarcis hispanica*[8]. On the other hand, CG A is widely distributed in endocrine cells of mammals[26,27], and some previous studies showed that almost all CG-immunoreactive cells display also argyophil cells with the Grimelius silver method[9,10,28,29]. In *Podarcis hispania*[9] CG-immunoreactive cells were less numerous than argyrophil cells. Our result showed that the distribution of bovine CG-immunoreactive cells actually was not paralleled with those of the Grimelius silver stain in most of endocrine cells. These finding may be due to anatomical differences among the various species and our failure to detect the Grimelius argyrophil cells, it still remains to be worked out.

Since PP-immunoreactive cells have described for the first time in the lizard pancreas[30,31], the occurrence of these cells have been demonstrated in the GIT of

the Lacertidae[6-9]. We also observed that BPP-immunoreactive cells were distributed in the duodenum and the ileum but were absent in the pyloric glands, mostly agreed with results reported the other lizards[6,7]. Generally this pattern of distribution is similar to that other vertebrates[6-8]. Although immunoreactivity to somatostatin, Gas/CCK and VIP was found in the other lizards[6-8], unfortunately these cells were not identified in the Amur lizard. Especially somatostatin-immunoractive cells was resulted in failure to detect in the GIT of the Amur lizard by the Hellerström-Hellman technique and immunohistochemical method.

In the present study, we cannot elucidated whether these cells are actully absent from the GIT of the Amur lizard or whether it is due to our failure to detect the immunohistological reactivity stemming from species differences at the molecular level, still remains to be clarified. In conclusion, we have demonstrated the characteristic patterns of distribution of two kinds of endocrine cells including the argyrophil cells and their relative frequencies of the Amur lizard, *Takydromus amurensis*.

Summary

The distribution and the frequency of endocrine cells in the GIT of the Amur lizard(*Takydromus amurensis*) were investigated using silver techniques and immunohistochemical method.

Only argyrophil cells stained by the Grimelius technique were found numerous in the pyloric glands and moderate in the duodenum.

Two types of immunoreactive endocrine cells were identified by immunohistochemical method. Bovine CG-immunoreactive cells were demonstrated the entire GIT. BPP-immunoreactive cells were restricted in the duodenum and the ileum. The results showed that the number of argyrophil cells was lower than the number of cells stained with bovine CG antiserum. Therefore, bovine CG-immunostaining and the Grimelius silver technique did not correspond with various endocrine cells in the Amur lizard.

Legends for figures

Fig 1. Argyrophil endocrine cells(arrowheads) in the pyloric gland region(a) and the epithelium of the duodenum (b). a, b; ×540.

Fig 2. Bovine CG-immunoreactive cells in the gastrointestinal tract.
a. fundic gland region b. pyloric gland region(arrowheads) c. duodenum d. ileum e. rectum
a-e; ×540.

Fig 3. BPP-immunoreactive cells(arrowheads) in the epithelium of the duodenum(a) and ileum(b).
a, b; ×540.

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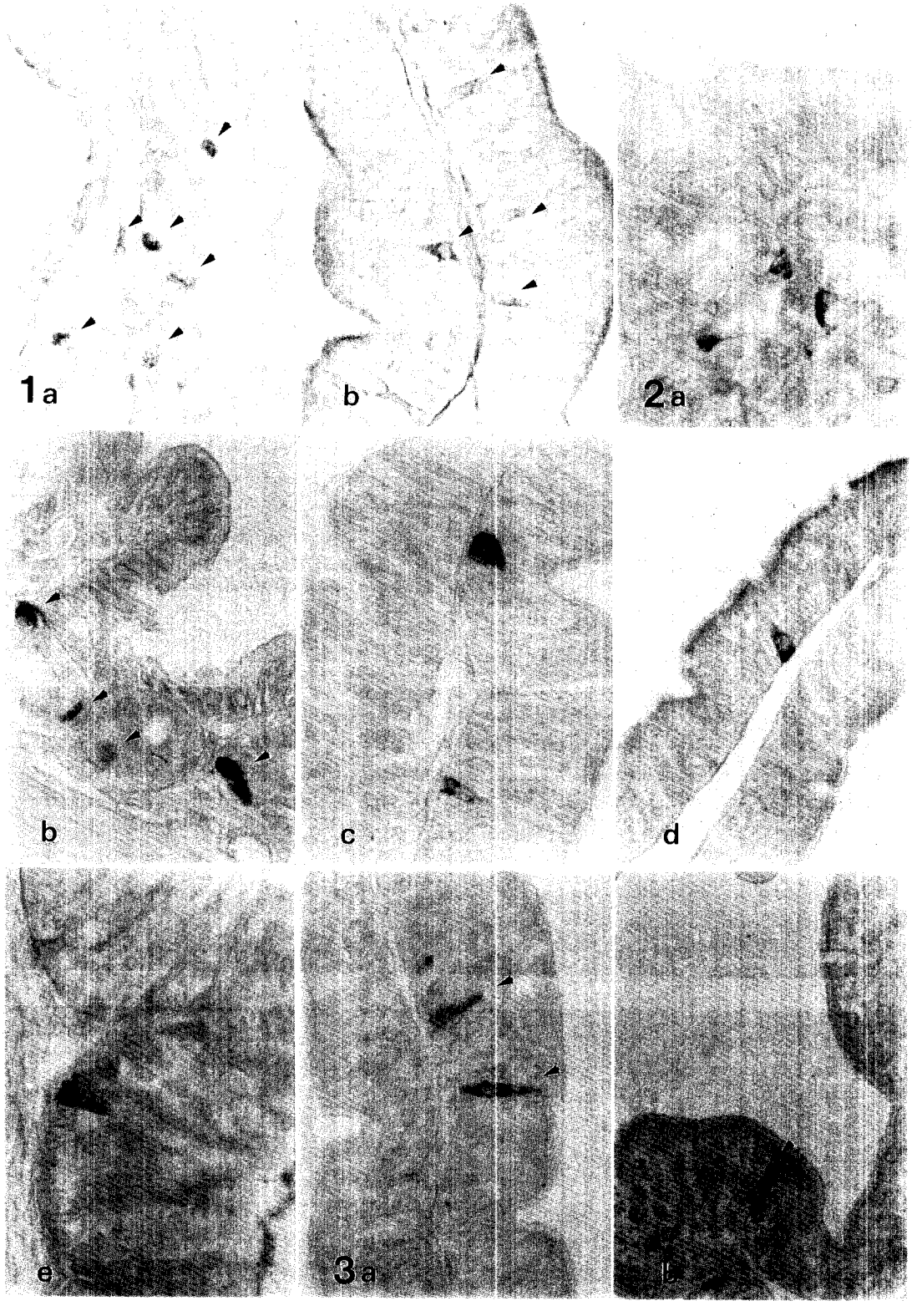
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