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Immunohistochemical study of the endocrine cells of the gastrointestinal mucosa of the African clawed toad, *Xenopus laevis*

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아프리카 발톱두꺼비의 소화관내분비세포에 관한 면역조직화학적 연구

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초 록 : ABC법에 의해 면역조직화학적으로 아프리카발톱두꺼비(*Xenopus laevis*)의 위장관내분비세포를 관찰하였던 바, neurotensin 면역반응세포는 중등도로 소장에서 약하게 반응하였다. 구형의 gastrin releasing peptide(GRP) 면역반응세포는 위에서 위저부의 상피세포 바로 아래와 유문부선에서 다수로, 소장에서는 가는 방추형으로 극소수 또는 중등도로 분포하였다. 한편 substance P 면역반응세포는 방추형으로 장상피에서 관찰되었다. 그러나 secretin, motilin, met-enkephalin-8 (M-Enk) 및 polypeptide YY(PYY) 면역반응세포는 전체 소화관에서 관찰할 수 없었다.

Key words : endocrine cell, *Xenopus laevis,* gastrointestinal tract(GIT), immunoreactive cell.

Introduction

Gastrointestinal hormones have important roles in the regulating of digestive functions. These hormones have been investigated in endocrine cells of the gastroenteropancreatic system of various vertebrates.

In a previous paper[1], although we have been observed five endocrine cell types in the gastroenteropancreatic system of *Xenopus laevis,* less is known about the presence of the other gastrointestinal hormones.

The aim of the present study was to investigate the regional distribution and relative frequency of the occurrence in gastrointestinal endocrine cells such as secretin, neurotensin, motilin, gastrin releasing peptide(GRP), met-enkephalin-8(M-Enk) 및 polypeptide YY(PYY), and substance P by immunohistochemistry.

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Materials and Methods

Five adult the African clawed toads, *Xenopus laevis*, both sexes were used in this study. The African clawed toads were anesthetized with ether and the fundic stomach, pyloric stomach, duodenum, ileum, and rectum were dissected out. The tissue samples were fixed for 24hr in Bouin's fluid and dehydrated in ethanol. After paraffin enbedding, 4μm histological sections were prepared. The immunohistochemical staining was performed using the avidin-biotin complex (ABC) technique[2]. 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). They were then washed in PBS buffer and finally followed by a third incubation with avidin-biotin complex (Vector). The peroxidase reactions were developed in a solution of 3,3'-diaminobezidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer. After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin.

Results

In this study, three cell types of immunoreactive cells were detected with antisera against neurotensin, GRP and substance P. However, no secretin-, motilin-, M-Enk- and PYY-immunoreactive cells were observed in the GIT of the African clawed toad. The frequency and distribution of the immunoreactive endocrine cells in various parts of the GIT are given in Table 2.

In the stomach, GRP-immunoreactive cells were round or oval in shape, and not appeared to reach the lumen. On the other hand, all immunoreactive cells in the intestine seemed to be of the open type.

A moderate number of neurotensin-immunoreactive cells were weakly detected in both the duodenum(Fig 1a) and the

Table 1. Antisera used in this study

	Antisera raised*	Code Source	Dilution
Secretin	R-801	Dr. N.Yanaihara	1 : 1,000
Neurotensin	R-3501	Dr. N.Yanaihara	1 : 1,000
Motilin	R-1104	Dr. N.Yanaihara	1 : 1,000
Gastrin releasing peptide(GRP)	R-6902	Dr. N.Yanaihara	1 : 6,000
Met-Enkephalin-8(M-Enk)	R-0171	Dr. N.Yanaihara	1 : 8,000
Polypeptide YY(PYY)	R841303-2	Milab, Malmö, Sweden	1 : 40,000
Substance P	B9C	Sera-Lab., Sussex, U.K.	1 : 1,000

* All antisera were raised in rabbits.

Table 2. Regional distribution and relative frequency of endocrine cells in the GIT of the African clawed toad, *Xenopus laevis*

	Fundus	Pylorus	Duodenum	Ileum	Rectum
Secretin	-	-	-	-	-
Neurotensin	-	-	+	+	-
Motilin	-	-	-	-	-
GRP	++	++	±	+	-
M-Enk	-	-	-	-	-
PYY	-	-	-	-	-
Substance P	-	-	±	±	++

- : Not detected, ± : Rare, + : Moderate, ++ : Numerous

ileum(Fig 1b), with almost uniform frequency. They were thin, tall, spindle cells.

GRP-immunoreactive cells were distributed throughout the GIT except the rectum. They were numerous in the fundic glands(Fig 2a) and pyloric glands(Fig 2b), but rare in the duodenum(Fig 2c), found in moderate numbers in the ileum (Fig 2d). They were mainly located the upper region of the fundic glands, whereas in the pyloric glands, these cells were found in the basal region. Also, in the intestine, they were inserted among the epithelia.

Substance P-immunoreactive cells were rarely seen in the duodenum and the ileum(Fig 3a) but found in moderate numbers in the rectum(Fig 3b).

Discussion

Comparing the present data with those reported for the other anura, some differences in the frequency and distribution of immunoreactive cells in the GIT were observed.

Although the occurrence of secretin-immunoreactive cells was reported in the GIT of the toad[3], it is well known that these cells are not detected in the lower vertebrates[4,5]. In the present study, no secretin-immunoreactive cells were found in any parts of the GIT, which agrees with above reports[4,5].

Buchan[5] reported that neurotensin-immunoreactive cells were detected in the small intestine of most species. In Ranidae, neurotensin-immunoreactive cells were restricted to the duodenum and the upper small intestine[6], while in Bufonidae these cells confined to the lower small intestine such as the ileum[3,7]. We demonstrated that neurotensin-immunoreactive cells were found in the duodenum and the ileum. This pattern of the distribution is similar to that of Ranidae[6].

In this study, motilin-immunoreactive cells could not be detected in the GIT of the African clawed toad, which agrees with all previous reports on amphibian species[3,8,9].

In many studies, GRP-immunoreactive cells have been reported to be confined to the stomach of anuran species[3,9,10]. However, our results showed that GRP-immunoreactive cells were distributed in the stomach and the small intestine. The presence of immunoreactive cell to GRP was described for the first time in the small intestine of anura[3,9,10].

Enkephalin-immunoreactive cells have been demonstrated in the intestinal EC cells in mammals[11]. Buchan[5] reported that M-Enk-immunoreactive cells were demonstrated in the gastric glands of *Acris crepitans* and the small intestine of *Ambystoma mexicanum* in amphibians. But these cells were not identified in the GIT of the other anura[3,5,11], including this species.

In the toad, PYY-immunoreactive cells of open type were detected in the duodenum and the ileum[7]. However, in this study PYY-immunoreactive cells were not found in the entire GIT.

No occurrence for substance P-immunoreactive cells was observed in *Bufo regularis*[3] and *Bufo bufo*[8], although these cells were seen in the small intestine of several species[12]. In the present study, however, substance P-immunoreactive cells were found in the small and the large intestines, which were quite different from that reported by El-Shally[3].

In conclusion, we have demonstrated the characteristic pattern of distribution of three endocrine cell types in the GIT of the African clawed toad, *Xenopus laevis.*

Summary

The gastrointestinal endocrine cells of the African clawed toad, *Xenopus laevis* have been investigated immunohistochemically using the avidin-biotin method. Seven antisera were tested and three endocrine cell types immunoreacted with antisera to neurotensin, GRP and substance P.

A moderate number of neurotensin-immunoreactive cells were weakly reacted in the small intestine. GRP-immunoreactive cells were mainly situated among the upper portion in the fundic glands, and the basal portion in the pyloric glands. These cells were oval and round in shape. On the other hand, in the intestine they were thin spindly cells with the epithelium. Substance P-immunoreactive cells were observed in among intestinal epithelium. However, no secretin-, motilin-, M-Enk- and PYY-immunoreactive cells were found in the GIT of the African clawed toads.

Legends for figures

Fig 1. Neurotensin-immunoreactive cells(arrowheads) in the duodenum(a) and ileum(b).
a, b ; × 240

Fig 2. GRP-immunoreactive cells throughout the GIT except for the rectum.
a. fundic stomach, b. pyloric stomach, c. duodenum, d. ileum(arrowheads). a-d ; × 240

Fig 3. Substance P-immunoreactive cells in the intestine.
a. duodenum(arrowhead), b. rectum. a-b ; × 480

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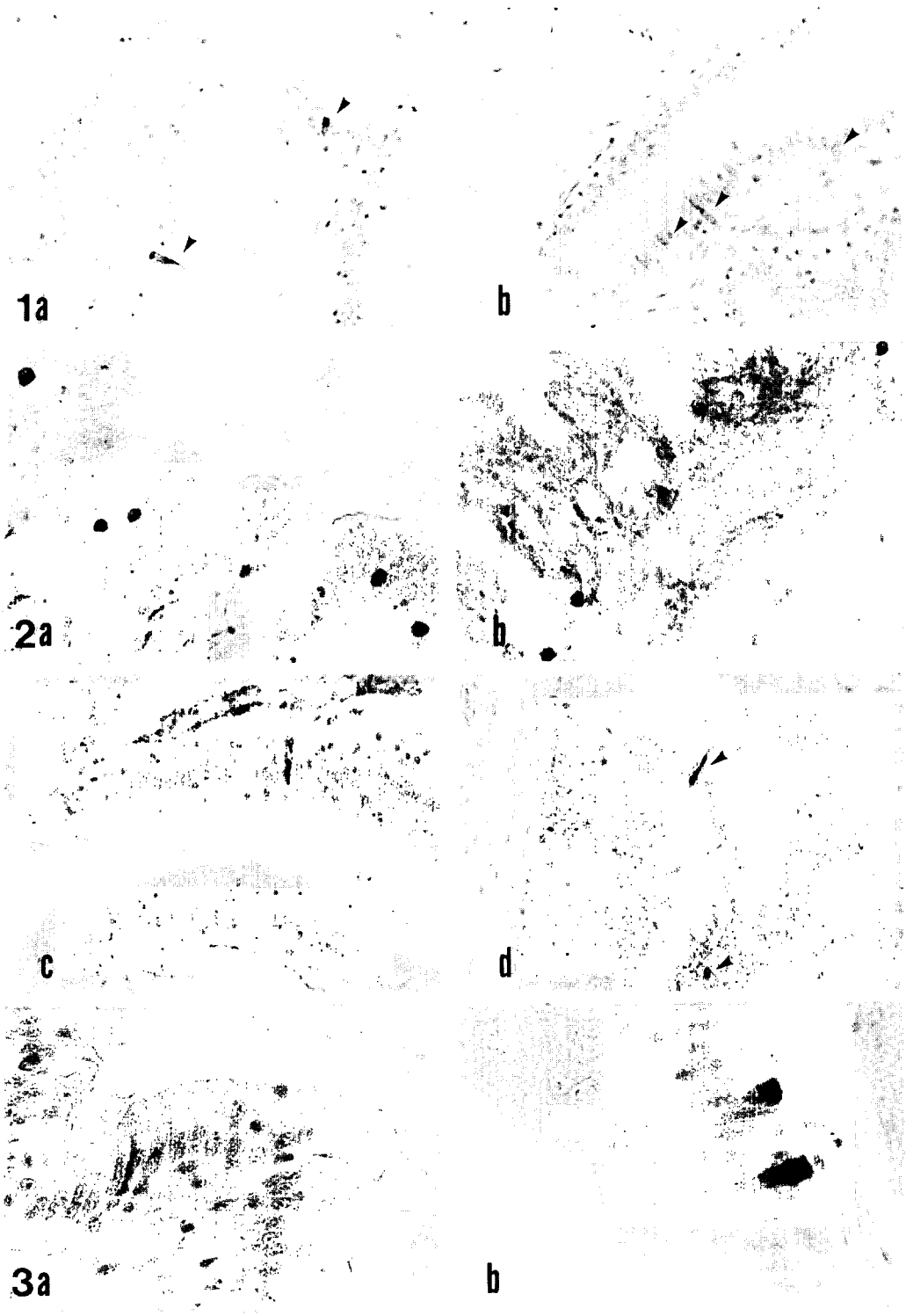
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