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An immunohistochemical study of the endocrine cells on the gastro-entero-pancreatic system of the African clawed toad, *Xenopus laevis*

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아프리카발톱두꺼비(*Xenopus laevis*)의 위장췌내분비세포의 면역조직화학적 연구

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초록 : 아프리카발톱두꺼비(*Xenopus laevis*)의 위장췌내분비세포를 면역조직화학적으로 관찰하였던 바 5-HT면역반응세포들은 중등도로 전장관에 고루 분포하였다. Gas/CCK면역반응세포는 유문부 장선의 기저부와 십이지장 점막상피에서 출현하였다. 소수의 glucagon면역반응세포는 위저부에서 약하게 반응하였고, 췌장에서는 외분비부에서만 관찰되었다. 직장을 제외한 전장관과 췌도 주변부 및 외분비부에서 somatostatin면역반응세포가 관찰되었다. CGs와 insulin면역반응세포는 전장관에서 관찰할 수 없었다. 그러나 insulin세포는 췌도의 중앙부와 외분비부에서 단독으로 출현하였다.

Key words : gastro-entero-pancreatic (GEP) system, African clawed toad, gastrointestinal tract, pancreas.

Introduction

Xenopus laevis, a kind of anura is one species used as the experimental animal in the basic science. Recently, the endocrine cells of the gastro-entero-pnacreatic (GEP) system have been mainly investigated in various vertebrates. Among them, several reports have been covered the distribution and occurrence of different endocrine cell types in the amphibia[1~6], inclucing *Xenopus laevis* that concerned with the bombesin-like producing cells.[7~9]

In the present study, the GEP system of *Xenopus laevis* has been examined by immunohistochemistry for the regional distribution and the relative frequency of presence of endocrine cells using mammalian antisera to the following peptides : 5-HT, glucagon, insulin, bovine CG, porcine CG, Gas/CCK and somatostatin.

Materials and Methods

Five adult the African clawed toads, *Xenopus laevis*, both sexes provied by the Department of Veterinary Anatomy, Hokkaido University, Japan, were used in this study. The African clawed toads were anesthetized with ether and the fundic stomach, pyloric stomach, duodenum, ileum, rectum and pancreas were dissected out and fixed in Bouin's fluid. After paraffin embedding, 4㎛ histological sections were prepared. The representative sections were then deparaffinized, rehydrated and immunostained with the peroxidase antiperoxidase (PAP) method[10]. Background blocking was performed with normal goat serum prior to incubation with the 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 com-

plex was prepared. The peroxidase reaction was carried out in a solution of 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in HCl buffer. After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin.

Results

In the present study, five kinds of immunoreactive cells were observed in the gastrointestinal tract and pancreas of the African clawed toad by specific immunohistochemical methods. However, no CGs-immunoreactive cells were found in the GEP system of the African clawed toad. The regional distribution and relative frequency of immunoreactive cells in the African clawed toad were showed as in Table 2.

In general, the number of immunoreactive cells was lesser frequent in the African clawed toad than in that of other vertebrates. In the fundic stomach, most of the immunoreactive cells were pyramidal or oval in shape. On the other hand, in the pyloric stomach and the intestines, they mainly pyramidal, oval or spindle in shape showed occasionally long or short cytoplasmic processes.

Moderate numbers of 5-HT-immunoreactive cells were detected throughout the gastrointestinal tract, with almost uniform frequency. In the stomach, the immunoreactive cells were situated to the basal portion of the epithelia and between the cells lining to the tubular glands(Figs 1a-c). In the intestines, they were inserted between the columnar cells of mucosa, being long cytoplasmic processes(Figs 1d-f).

Gas/CCK-immunoreactive cells were found in the basal portion of the pyloric gland (Figs 2a, b) and among the epithelia of the duodenum(Fig 2c).

Glucagon-immunoreactive cells were reacted weakly and found rarely on occasions only in the fundic region of the stomach(Fig 3).

Somatostatin-immunoreactive cells were distributed in the epithelia throughout the gastrointestinal tract with except for the rectum. They were a few in the stomach (Figs 4a-d) and rarely in the intestine, duodenum and ileum(Figs 4e, f).

There were no CGs-immunoreactive cells in the GEP

Table 1. Antisera used in this study

Antisera[a)]	Code	Source	Dilution
5-hydroxytryptamine (5-HT)	8535028	Immunonuclear Corp., Stillwater	1 : 10,000
Glucagon	8635013	〃	1 : 800
Insulin	8622014	〃	1 : 2,000
Bovine chromogranin (Bovine CG)	8541011	〃	1 : 500
Porcine chromogranin (Porcine CG)	8541012	〃	1 : 2,000
Gastrin/Cholecystokinin (Gas/CCK)	i600/004	Union Chimique Belge, bioproducts	1 : 100
Somatostatin	CA325	Cambrigde Research Biochemical Billerica	1 : 1,000

a) All antisera were raised in rabbits except for insulin that was raised in a guinea pig.

Table 2. Regional distribution and relative freguency of endocrine cells in the GEP system of the African clawed toad, *Xenopus laevis*

	Fundus	Pylorus	Duodenum	Ileum	Rectum	Pancreas
5-HT	++	+++	++	++	++	−
Gas/CCK	−	+	±	−	−	−
Glucagon	±	−	−	−	−	+
Somatostatin	+	+	±	±	−	+
Bovine CG	-	−	−	−	−	−
Porcine CG	-	−	−	−	−	−
Insulin	-	−	−	−	−	+++

− Not detected, ± Rare, + A few, ++ Moderate, +++ Numerous

system of the African clawed toad.

In the pancreas, glucagon-, insulin- and somatostatin-immunoreactive cells were detected. Singly glucagon-immunoreactive cells were only confined to the exocrine portions (Fig 5). Insulin-immunoreactive cells were distributed as the columnar shaped in the central portion the islets and found only one cell type in the exocrine portions (Fig 6). Somatostatin-immunoreactive cells were weakly stained to the periphery of the islets and were scattered singly in the exocrine portions (Figs 7a, b).

Discussion

The present study was proposed to give precise data about the regional distribution and relative frequency of the GEP endocrine cells in the African clawed toad, *Xenopus laevis*. In this work, such finding is also similar to those of higher vertebrates.[11, 12] But some characteristic differences were observed in this species.

The highest frequency of 5-HT-immunoreactive cells was found in the duodenum of the mammals[13~17], in the colon of the Japanese field vole[18] and the Korean hedgehog.[19] However in the pyloric stomach, these cells contain remarkable more than the other intestinal portions. This diversity of cell distribution in the intestines may be due to a certain reflex of gastro-physiological functions related to the nutritional and anatomical differences among the various species.

Buchan[1] reported that the regional distribution of Gas/CCK-immunoreactive cells in the gastrointestinal tract was differed depending on the species. And then in most species, the pyloric glands and duodenal regions were contained numerous immunoreactive cells in amphibia. We demonstrated that Gas/CCK-immunoreactive cells were found in the pyloric glands and duodenal region in this study. This distributional pattern would be agree with the findings in the other amphibians.[1,3]

Interestingly, glucagon-immunoreactive cells were restricted exclusively within the fundic glands. Usually they are confined to the whole gastrointestinal tract in *Salamandra salamandra*[3] and *Bufo regularis*[4], while the intestinal mucosa was without these cells.

It is known that somatostatin-immunoreactive cells show the widest distribution in the whole gastrointestinal tract of all vertebrate species investigated including the primitive agnathans.[20] Especially in the amphibians, the occurrences of somatostatin-immunoreactive cells have been reported in *Bufo regularis*[4], *Rana catesbeiana*[21], *Salamandra salamandra*[3], and two urodele and eight anuran species[1]. The result for somatostatin cells in the African clawed toad is quite similar to that of *Bufo regularis*[4] and the urodele.[1]

Although CGs-immunoreactive cells have been shown to be present in all identifiable endocrine cell types in the gastrointestinal tract[22~25], these cells could not be found in the present study. Whether CG-immunoreactive cells are actually absent in the African clawed toad intestine or whether it is due to our failure to detect the immunohistological staining from species differences at the molecular form, remains to be clarified.

In the pancreatic islets, the typical peripheral distribution of glucagon- and somatostatin-immunoreactive cells and the central location of insulin-immunoreactive cells were known in the other vertebrates. We observed that the regional distribution of glucagon-immunoreactive cells was without the islets, while these cells only detected in the exocrine portions, insulin cells in the whole islets, and somatostatin cells in the periphery of the islets. Besides insulin- and somatostatin-immunoreactive cells were also scattered singly in the exocrine portions. However, the regional difference of insulin- and somatostatin-immunoreactive cells in the exocrine portions and glucagon cells in the islets were quite different from that of the newt[6] and *Rana pipiens*[26] respectively, but showed a similar pattern to that reported earlier.[27]

In conclusion, we have thus demonstrated the characteristic patterns of distribution of five kinds of the immunoreactive cells in the GEP system of the African clawed toad, *Xenopus laevis*.

Summary

The GEP endocrine cells of the African clawed toad, *Xenopus laevis*, were studied immunohistochemically. Five kinds of the endocrine cells were identified in this study. A moderated number of 5-HT-immunoreactive cells were detected throughout the gastro intestinal tract, being almost uniform frequency. Gas/CCK-immunoreactive cells were restricted to the basal portion of the pyloric gland and among the duodenal mucosa. A rare glucagon-immunoreactive cells were weakly reacted in the fundic region of the stomach and observed in the exocrine portions of

the pancreas. Somatostatine-immunoreactive cells were distributed throughout the gastrointestinal tract with except for the rectum, and not only the periphery of the islets but also the exocrine portions in the pancreas. No CGs- and insulin-immunoreactive cells were found in the gastrointestinal tract, whereas in the pancreas, the later was seen in the central region of the islets and the exocrine portions.

Legends for figures

Fig 1. 5-HT-immunoreactive cells throughout the gastrointestinal tract. a. fundic stomach, b, c. pyloric stomach, d. duodenum, e. ileum, f. rectum. a, c-f ; × 480, b ; × 240

Fig 2. Gas/CCK-immunoreactive cells in the pyloric gland region (a, b) and duodenum(c). a-c ; × 480

Fig 3. Glucagon-immunoreactive cell(arrowhead) in the fundic mucosa. × 480

Fig 4. Somatostatin-immunoreactive cells (arrowheads) throughout the gastrointestinal tract with the exception the rectum. a, b. fundic stomach, c, d. pyloric stomach, e. duodenum, f.ileum. a-f ; × 480

Fig 5. Glucagon-immunoreactive cells in the exocrine portions of the pancreas. Note these cells were not detected in the islets(dots). × 240.

Fig 6. Insulin-immunoreactive cells in both the islet and the exocrine portions. × 240.

Fig 7. Somatostatin-immunoreactive cells (arrowheads) in the periphery of the islets(a), and the exocrine regions (b). a, b : × 480

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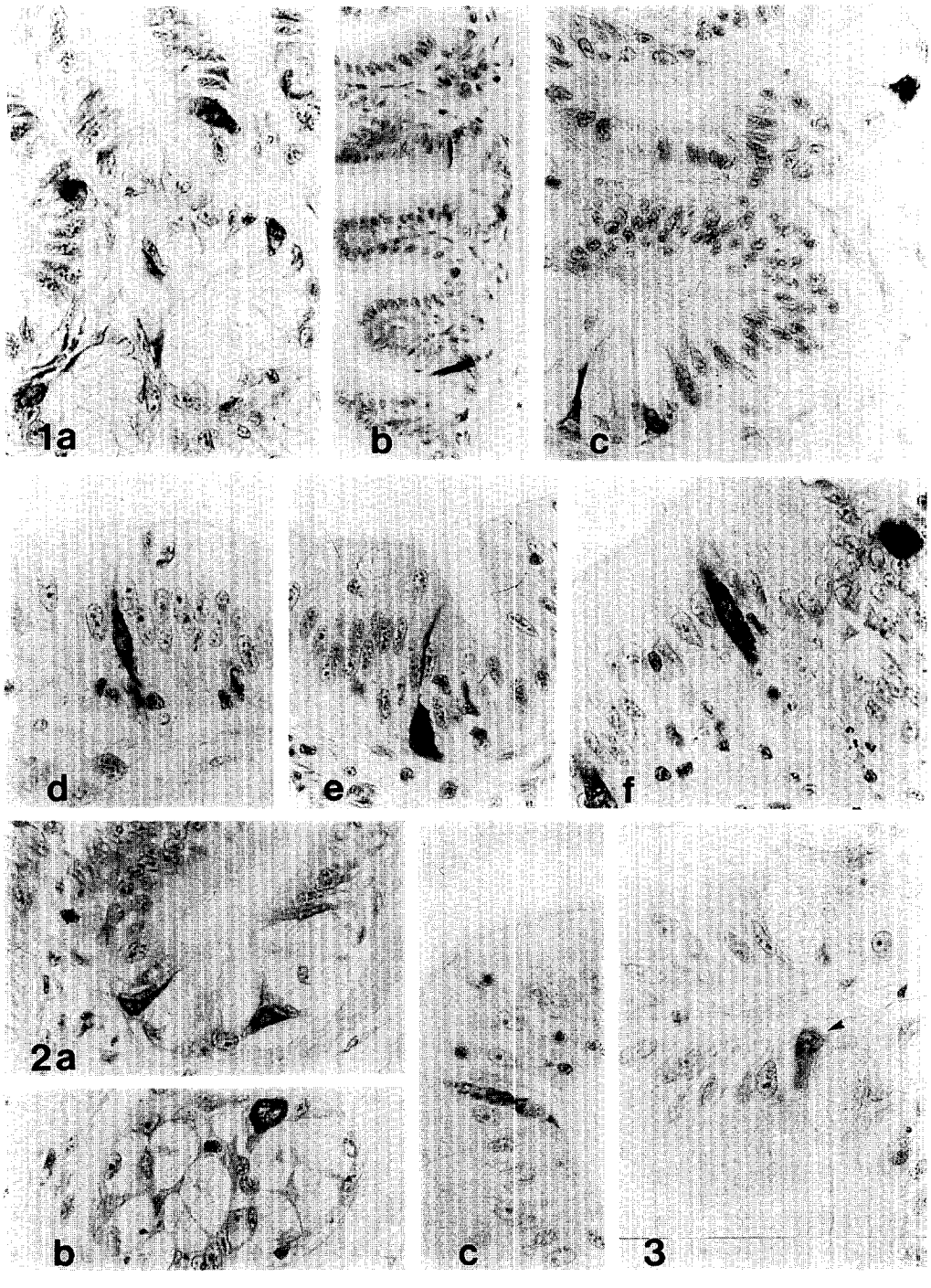
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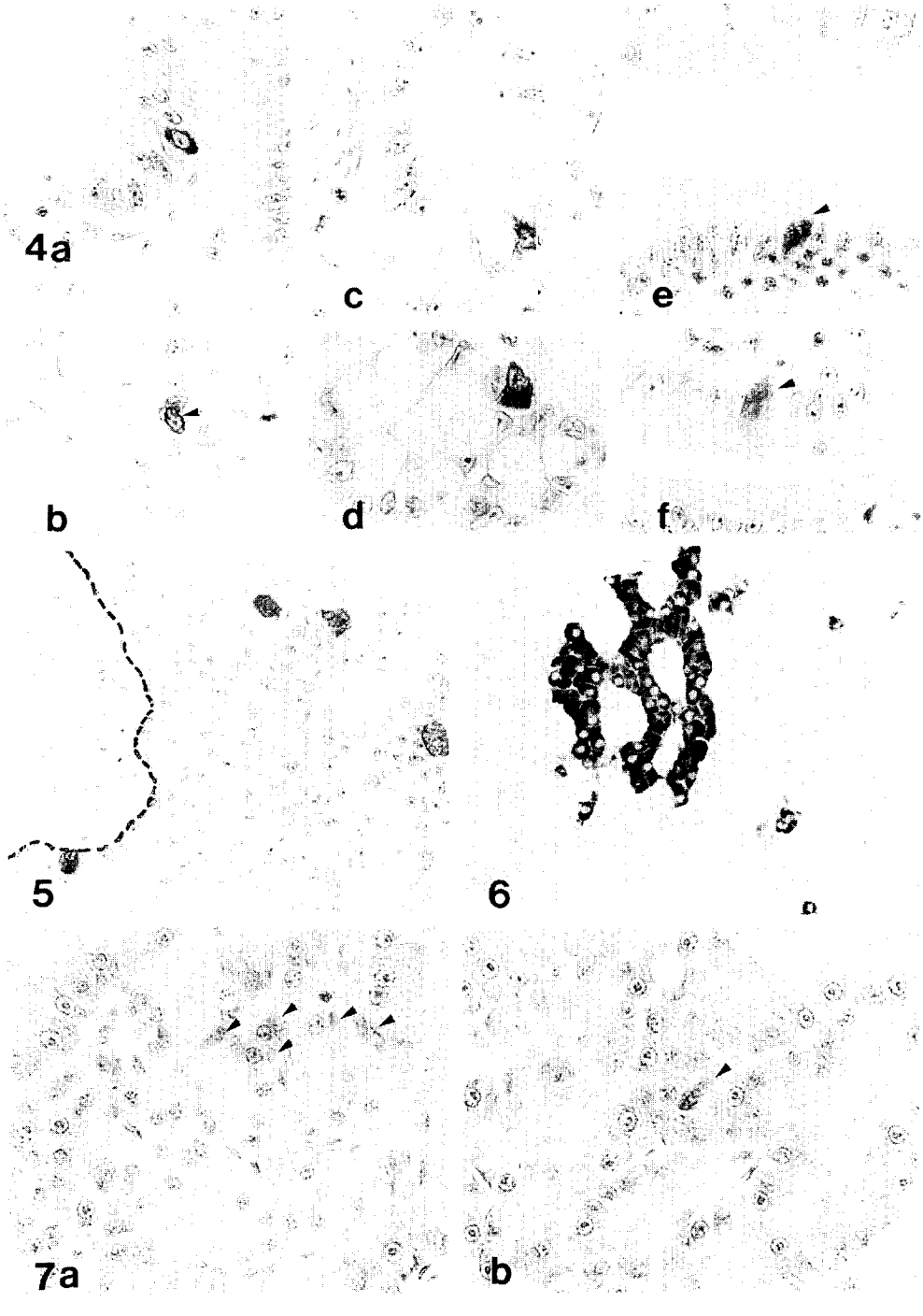
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