

## An immunohistochemical study of the gut hormones in *Hynobius leechii*

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### 도롱뇽의 위장관 내분비세포에 관한 면역조직화학적 연구

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초록 : 도롱뇽(*Hynobius leechii*)의 위장관 내분비세포를 면역조직화학적으로 관찰하였던 바, BPP 면역반응세포는 유문부에서 중등도, 십이지장과 소장에서 소수로 관찰되었다. 중등도의 somatostatin 면역반응세포는 유문부에 국한되어 출현하였다. 5-HT 면역반응세포는 전장관에 걸쳐 출현하였으나 유문부에서 다수 그리고 직장에서 극소수로 분포하였다.

특히 BPP와 5-HT 면역반응세포는 도롱뇽에서 최초로 동정하였다.

**Key words** : gut hormone, gastrointestinal tract(GIT), salamander, endocrine cell, immunoreactive cell

### Introduction

*Hynobius leechii* belonging to the Hynobiidae in order Caudata is widespread throughout the Korea.

Although many studies have been reported the presence of regulatory peptides in the gastrointestinal tract(GIT) of the anura, only a few papers have been carried out on endocrine cells within that of the urodele<sup>1,3</sup>.

In the present study, the GIT of *Hynobius leechii* has been examined by immunohistochemistry for the regional distribution and type of endocrine cells using mammalian antisera.

### Materials and Methods

Five adult of the Korean salamanders(*Hynobius leechii*) of both sexes were sampled in springtime. Five regions of the GIT in the Korea salamanders were dissected out and fixed in Bouin's fluid. After paraffin embedding, 4 $\mu$ m histological sections were prepared. The representative sections were then deparaffinized, rehydrated and immunostained with the peroxidase antiperoxidase(PAP) method<sup>4</sup>. Background blocking was performed with normal goat serum prior to incubation with the specific antiserum(Table 1). After

Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
Bovine pancreatic polypeptide (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
Motilin	AB17	"	1:800
Bombesin	08230	"	1:8,000
Bovine chromogranin(Bovine CG)	8541011	Immunonuclear Corp	1:500
5-hydroxytryptamine(5-HT)	8535028	"	1:2,000
Insulin	8622014	"	1:2,000
Glucagon	8635013	"	1:800
Substance P		Dept of Vet Phar Hokkaido Uni	1:3,000
VIP		"	1:2,000
Neurotensin	54091	Advance	1:1,000

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

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 containing 0.01% H<sub>2</sub>O<sub>2</sub> in HCl buffer. After immunostaining, the sections were lightly counterstained with Mayer's haematoxylin.

## Results

Table 2 summarizes the endocrine cell types and distributions found in the GIT of *Hynobius leechii*. Amongst twelve antisera, only three endocrine cell types immunoreacted with antisera to BPP, somatostatin and 5-HT. Especially, endocrine cell types in *Hynobius leechii* were very scarce compared with the other Urodele. These cells in mucosa were mainly scattered among the columnar cells for exception of the pyloric region that situated to the basal portion of the epithelia(Fig 3b) and between the cells lining to the tubular gland(Fig 2). Most of the immunoreactive cells were principal oval or spindle in shape with the

fine processes.

While the frequency of BPP-immunoreactive cells detected in the pyloric regions was moderate(Fig 1a), there were a few in the duodenum(Fig 1b) and the small intestine(Fig 1c), and could not be seen in the fundic region and the rectum.

Moderate numbers of somatostatin-immunoreactive cells were found to be restricted to the basal tubular glands and among the epithelia of the pyloric regions (Fig 2).

Almost all the GIT were demonstrated in 5-HT-immunoreactive cells, reaching a peak in the pyloric regions(Fig 3b) and a minimum in the rectum(Fig 3e).

## Discussion

This study was designed to investigate the distribution and type of endocrine cells in the GIT of *Hynobius leechii*. In this work, comparing the present results with those reported for other Urodele species, distinct differences in the regional distribution and type of endocrine cells in GIT were observed.

Falkmer and Stefan<sup>5</sup> had described for the first time

**Table 2.** Regional distribution and relative frequency of the gut peptides in *Hynobius leechii*

	Fundus	Pylorus	Duodenum	Small intestine	Rectum
BPP	-	++	+	+	-
Gas/CCK	-	-	-	-	-
Somatostatin	-	++	-	-	-
Motilin	-	-	-	-	-
Bombesin	-	-	-	-	-
Bovine CG	-	-	-	-	-
5-HT	+	+++	++	+	±
Insulin	-	-	-	-	-
Glucagon	-	-	-	-	-
Substance P	-	-	-	-	-
VIP	-	-	-	-	-
Neurotensin	-	-	-	-	-

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

PP-immunoreactive cells in the GIT of the amphibia. Also, Van Noorden and Falkmer<sup>6</sup> reported that these cells in the GIT occurred from early evolution. Our results showed that moderate BPP-immunoreactive cells were demonstrated in the pyloric regions, but a few in the duodenum and the small intestines. Although BPP-immunoreactive cells have been reported in the intestine of *Bufo regularis*<sup>7</sup> and *Rana catesbeiana*<sup>8</sup>, these cells were not detected in the intestine of several amphibian species confirming a previous study<sup>3</sup>. Accordingly this seems to be the first description of BPP-immunoreactive cells in the urodele. This pattern of distribution is similar to that of *Bufo regularis*<sup>7</sup>.

Somatostatin-immunoreactive cells have been demonstrated the entire GIT of all vertebrate species<sup>9</sup>. In the amphibia, the existence of these cells has been reported in *Bufo regularis*<sup>7</sup>, *Rana catesbeiana*<sup>8</sup>, *Salamandra salamandra*<sup>2</sup>, *Xenopus laevis*<sup>10</sup> and two urodele<sup>3</sup> distributed from the stomach to the colon. The present data was restricted to the pyloric regions of the Korean salamander. However, the other intestines except of the pyloric regions were devoid of these cells. Lee et al<sup>11</sup> suggested that the peaked pattern of regional distribution of these cells are consisted of two groups, the fundic regions and the pyloric regions, according to the various species. So, these cell in

*Hynobius leechii* is a unique pattern.

Although 5-HT-immunoreactive cells were distributed throughout the GIT, the highest frequency of these cells were found in the duodenum<sup>11-15</sup> and the colon<sup>16,17</sup> of the mammals. However, they contained remarkable more in the pyloric regions than in the other intestinal portions of *Hynobius leechii*. This finding showed a similar pattern to that reported a previous work<sup>10</sup>.

Gas/CCK, motilin, bombesin, bovine CG, insulin, glucagon, substance P, VIP and neurtansin were not detected in the Korean salamander, although these were found in the other amphibians<sup>2,3,7,18,19</sup>. Therefore, the present study cannot determine whether the antisera tested in the Korean salamander were raised in mammals or whether these cells were actually absent in the GIT.

In conclusion, we have described the characteristic pattern of distribution and type of gut endocrine cells and have identified BPP and 5-HT-immunoreactive cells for the first time in salamander of the urodele.

## Summary

Using immunohistochemical technique, three en-

doocrine cell types were observed in the GIT of the Korean salamander, *Hynobius leechii*. BPP-immunoreactive cells were distributed from the pyloric region to the small intestine. A moderated number of somatostatin-immunoreactive cells were restricted to

the pyloric region. 5-HT-immunoreactive cells were demonstrated throughout the GIT, reaching a peak in the pyloric region. The presence of BPP- and 5-HT-immunoreactive cells are identified for the first time in *Hynobius leechii*.

### Legends for figures

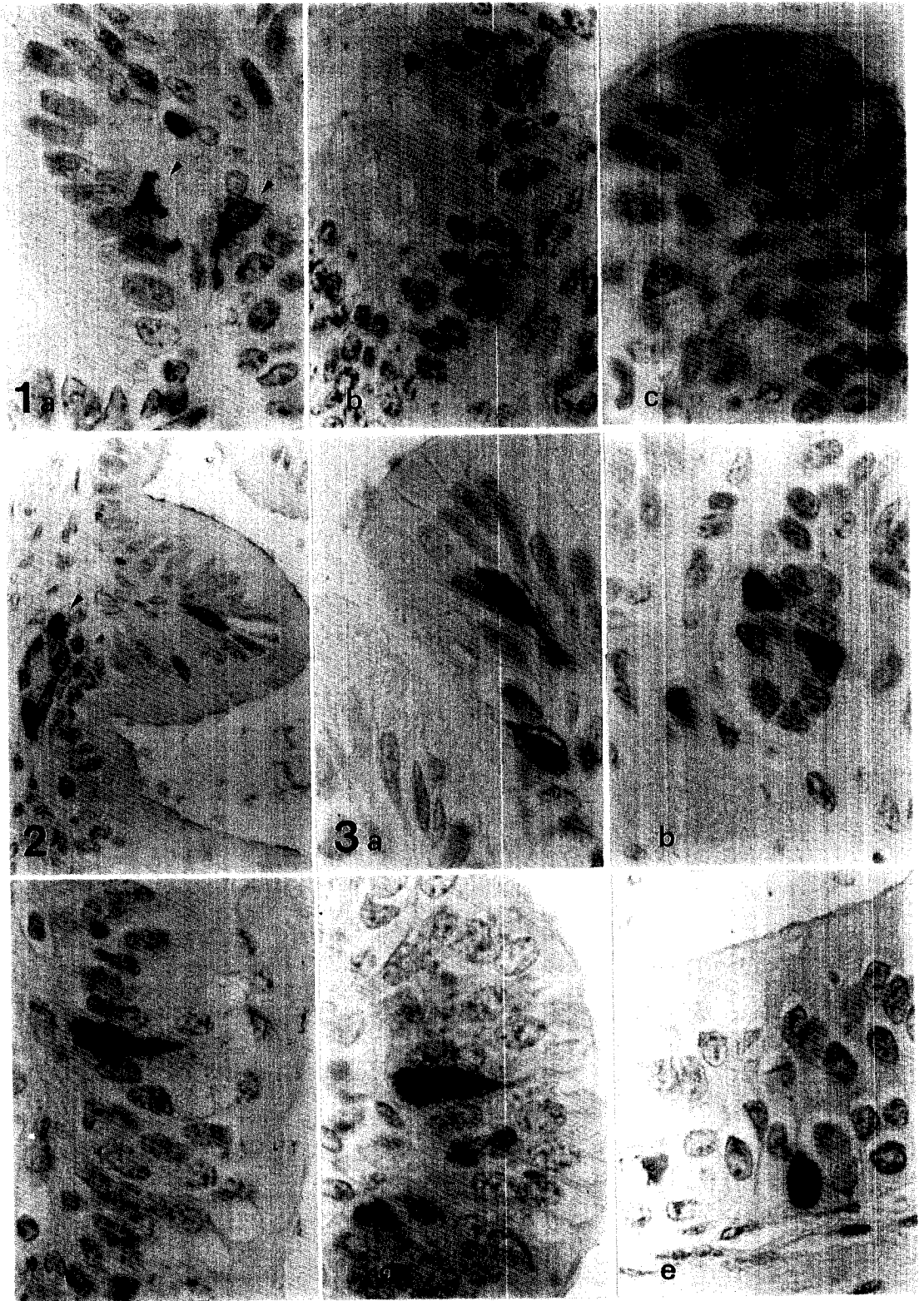
Fig 1. BPP-immunoreactive cells in the pyloric gland region(a; arrowheads), duodenum(b; arrowhead) and small intestine(c). a-c;  $\times 540$ .

Fig 2. Somatostatin-immunoreactive cells in the pyloric gland region(arrowheads).  $\times 200$ .

Fig 3. 5-HT-immunoreactive cells throughout the gastrointestinal tract. a-e;  $\times 540$ .

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