

Changes in the Serotonin-, Somatostatin- and Motilin-Immunoreactive Cells in the Gastrointestinal Tract of the Bullfrog, *Rana catesbeiana*, at Various Developmental Stages

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In order to investigate the regional distribution and relative frequency of the serotonin-, somatostatin- and motilin-immunoreactive cells in the gastrointestinal tract (GIT) of the bullfrog (*Rana catesbeiana*) with developmental stages, group of bullfrogs subdivided into the tadpole with hindlegs, metamorphosed bullfrog with tail, 2 wk after metamorphosed bullfrog and adult bullfrog, were stained by immunohistochemical methods (PAP methods). Multifarious changes of the regional distribution and relative frequency of the gastrointestinal endocrine cells were observed as developmental stages but motilin-immunoreactive cell were not detected in this study. Serotonin-immunoreactive cells were detected from 2 wk after metamorphosed bullfrog in the whole GIT. The positive cells were predominant in the pylorus regions. In the adult bullfrog, these cells increased in fundus. On the other hand, somatostatin-immunoreactive cells were observed from the tadpole with hindlegs in the stomach regions. In the metamorphosed bullfrog with tail, they were found in the whole GIT except for the ileum, and increased with developmental stages except for rectum. In the rectum, these cells were not detected after that period. Our results show that most of the immunoreactive cells appear from completed metamorphosed periods when the histological structure of GIT is completely differentiated.

The bullfrog, *Rana catesbeiana*, belonging to the order Anura have been widely distributed in Korea where the destruction of the ecosystem has occurred. The gastrointestinal endocrine cells dispersed in the epithelium and mucosal glands of the alimentary tract, synthesize various kinds of gastrointestinal hormones (Bell, 1979) and play an important role in the physiological functions of the gastrointestinal tract.

There has been a surge of interest concerning endocrine cells of the GIT in recent years. This is remarkable, considering the fact that so many gastroenteropancreatic (GEP) neuropeptides have been isolated from the amphibian skin (Nakajima et al., 1979; Van Noorden and Polak, 1979). GEP endocrine cells in various adult amphibians have been studied extensively through histochemical (Kim and Chung, 1973), electron microscopic (Geuze, 1971; Chung and Kwun, 1983) and immunohistochemical (Buchan, 1986) methods. Although about 17 types of endocrine cells have been detected in *Rana dybowskii* (Lee and Lee, 1996), *Rana*

pipens (Lechago et al, 1978), *Xenopus laevis* (Lechago et al, 1978; Lee and Lee, 1997; Lee and Lee, 1992), *Rana esculenta* (Trandaburu and Nürnberger, 1995), *Bufo regularis* (El-Salhy et al, 1981, 1982), and 8 species of the Anura amphibian (Buchan, 1986), little work has been published on the endocrine cells of the bullfrog (Lechago et al, 1978; Lee, 1993).

On the other hand, the developmental stages of the frog have been subdivided into 4 (Houillon, 1973) and 25 stages (Taylor and Kollros, 1946), and the histological changes according to this classification are well elucidated. However, no reports carried out the changes of regional distribution and relative frequency of GEP endocrine cells with developmental stages except that of Bodegas et al. (1997), who reported the existence of serotonin-, somatostatin-, bombesin- and cholecystokinin-immunoreactive cells in the larval anterior intestine of *Rana temporaria*. Lee and Lee (1996) reported the characteristic difference of the relative frequency and regional distribution between the hibernating and active phase of GEP endocrine cells in the *Rana dybowskii* using immunohistochemical methods.

In this work, the regional distribution and relative frequency of the serotonin-, somatostatin- and motilin-

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Table 1. Antisera used in this study

Antisera*	Code	Centralize source	Dilution
Serotonin	PUD681196	BioGenex Lab.	1 : 20
Somatostatin	PUD421295	BioGenex Lab.	1 : 20
Motilin	052	DAKO Corp.	1 : 200

*All antisera were raised in rabbits.

immunoreactive cells in the gastrointestinal tract (GIT) of the bullfrog (*Rana catesbeiana*) according to developmental stages (tadpole with hindlegs, metamorphosed bullfrog with tail, the 2 wk after metamorphosed bullfrog and adult) were examined by immunohistochemical methods (PAP methods).

Materials and Methods

According to Houillon (1973), each type of *Rana catesbeiana*, tadpoles with hindlegs, metamorphosed bullfrog with a tail, the 2 wk after metamorphosed bullfrog and adult bullfrog, were captured in Kyungsan and used in this study. Samples of the fundus, pylorus, duodenum, ileum, and rectum obtained from each developmental stage of the bullfrog were fixed in Bouin's solution. After paraffin embedding, 3-4 μm serial sections were prepared using routine histological methods. Each representative sections were deparaffinized, rehydrated, and immunostained using the peroxidase antiperoxidase (PAP) method (Sternberger, 1979). Background blocking was performed with normal goat serum prior to incubation with specific antisera (Table 1). After rinsing in PBS buffer (0.01 M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in phosphate buffered saline (PBS) and the PAP complex prepared. The peroxidase reaction was carried out in 3,3'-diaminobenzidine tetrahydrochloride solution containing 0.01% H₂O₂ in Tris-HCl buffer (0.05 M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive cells were observed under a light microscope.

Results

According to the developmental stages, variable changes in the regional distribution and relative frequency of serotonin- and somatostatin-immunoreactive cells were observed. These changes are shown in Table 2-5.

The tadpole with hindlegs (Table 2)

A few to moderate numbers of somatostatin-immunore-

Table 2. The regional distribution and relative frequencies of immunoreactive cells in the gastrointestinal tract of the tadpole with hindlegs

Immunoreactive cell	Fundus	Pylorus	Duodenum	Ileum	Rectum
Serotonin	-	-	-	-	-
Somatostatin	+	++	-	-	-
Motilin	-	-	-	-	-

++; moderate, +; a few, -; not detected.

Table 3. The regional distribution and relative frequencies of immunoreactive cells in the gastrointestinal tract of the metamorphosed bullfrog with a tail

Immunoreactive cell	Fundus	Pylorus	Duodenum	Ileum	Rectum
Serotonin	-	-	-	-	-
Somatostatin	+	++	++	-	±
Motilin	-	-	-	-	-

++; moderate, +; a few, ±; rare, -; not detected.

active cells were detected in the epithelium of the fundus and pylorus. These cells were round to spindle shaped (Fig. 1A, B). However, serotonin- and motilin-immunoreactive cells were not observed in this stage.

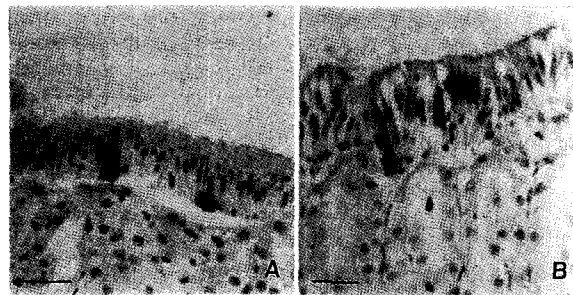


Fig. 1. Somatostatin-immunoreactive cells in the gastrointestinal tract of the tadpole with hindlegs. Spindle or spherical a shaped cells were restricted to the epithelium of the fundus (A) and pylorus (B). Scale bars=50 μm.

The metamorphosed bullfrog with tail (Table 3)

Spherical, round or spindle shaped somatostatin-immunoreactive cells were found in the entire GIT except

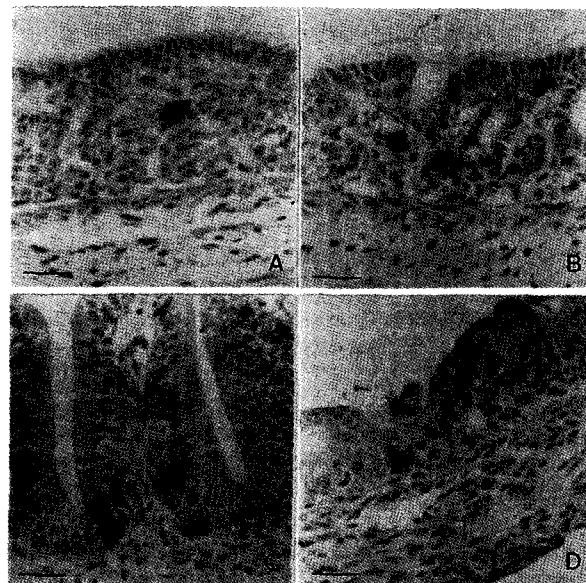


Fig. 2. Somatostatin-immunoreactive cells in the gastrointestinal tract of the metamorphosed bullfrog with a tail. Spherical to spindle shaped cells were observed in the gastric gland of fundus (A) and pylorus (B), in the basal portion of epithelium of the duodenum (C) and rectum (D). Scale bars=50 μm.

Table 4. The regional distribution and relative frequencies of immunoreactive cells in the gastrointestinal tract of the 2 wk after metamorphosed bullfrog

Immunoreactive cell	Fundus	Pylorus	Duodenum	Ileum	Rectum
Serotonin	+	++	±	+	+
Somatostatin	+	++	+	±	-
Motilin	-	-	-	-	-

++; moderate, +; a few, ±; rare, -; not detected.

for the ileum. In the stomach regions, a few number of cells were detected in the gastric gland of the fundus (Fig. 2A) and a moderate number of cells were observed in the epithelia and gastric gland of the pylorus (Fig. 2B). In the duodenum, a moderate number of immunoreactive cells were found in the basal portion of the epithelium (Fig. 2C), but seen rarely in the rectum (Fig. 2D). However, serotonin- and motilin-immunoreactive cells were not observed in this stage.

The 2 wk after metamorphosed bullfrog (Table 4)

Spindle or polymorphic shaped serotonin-immunoreactive cells were detected in the entire GIT. In the stomach regions, a few or moderate number of cells were observed in the fundus (Fig. 3A) and pylorus (Fig. 3B), respectively. They were also restricted to the gastric glands. These cells were found in the ileum and rectum in moderate numbers, but rarely in the duodenum. In these regions, they were restricted to the epithelium.

Spherical to spindle shaped somatostatin-immunoreactive cells were observed in the entire GIT, except for

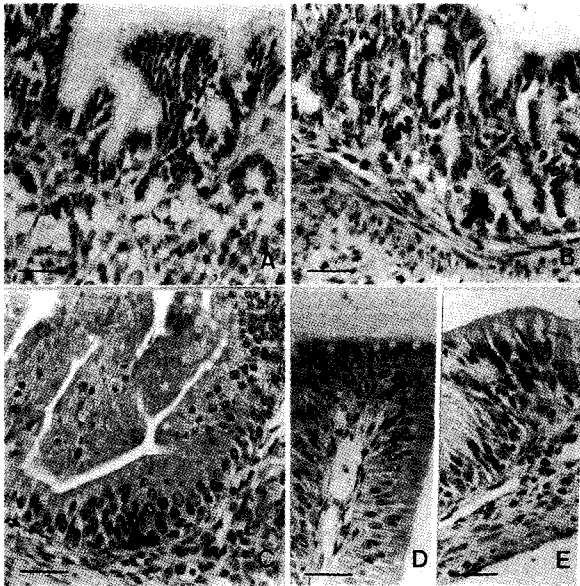


Fig. 3. Serotonin-immunoreactive cells in the gastrointestinal tract of the 2 wk after metamorphosed bullfrog. Spindle to polymorphic shaped cells were restricted to the gastric gland of the fundus (A) and pylorus (B) but spherical to spindle shaped cells were found in the basal portion of the epithelium of the duodenum (C), ileum (D) and rectum (E). Scale bars=50 µm.

Table 5. The regional distribution and relative frequencies of immunoreactive cells in the gastrointestinal tract of the adult bullfrog

Immunoreactive cell	Fundus	Pylorus	Duodenum	Ileum	Rectum
Serotonin	++	++	±	+	+
Somatostatin	++	+++	+	+	-
Motilin	-	-	-	-	-

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

the rectum. A few number of cells were detected in the fundus (Fig. 4A) and duodenum (Fig. 4C), moderate numbers in the pylorus (Fig. 4B), but rarely in the ileum (Fig. 4D). While these cells, similar to serotonin-immunoreactive cells, were found in the gastric glands of the stomach, they were observed in the epithelium of the other regions.

Motilin-immunoreactive cells were not detected again in this study.

The adult bullfrog (Table 5)

Spherical to spindle shaped serotonin-immunoreactive cells were found in the entire GIT. The regional distribution and relative frequency of these cells were similar to that of the metamorphosed bullfrog after 2 wk except for the fundus where the relative frequency increased compared to that of the 2 wk metamorphosed bullfrog (Fig. 5A-E).

The regional distribution and shapes of somatostatin cells were similar to that of the 2 wk metamorphosed bullfrog, but the relative frequency had increased throughout the GIT (Fig. 6A-D).

Motilin-immunoreactive cells were not found again in

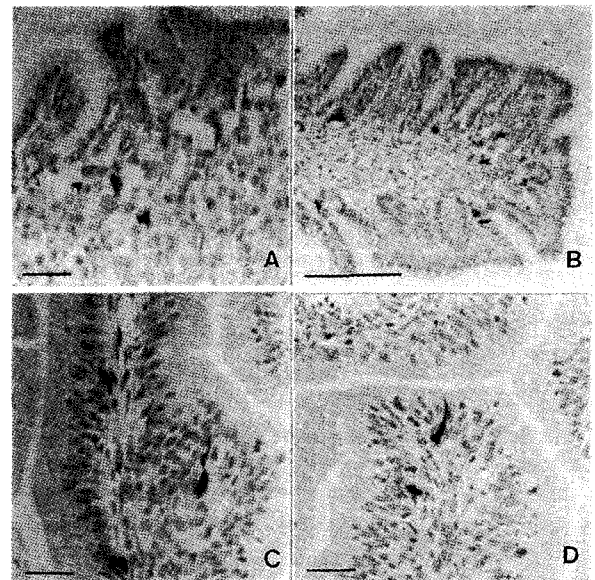


Fig. 4. Somatostatin-immunoreactive cells in the gastrointestinal tract of the 2 wk after metamorphosed bullfrog. Spherical to spindle shaped cells were observed in the gastric gland of the fundus (A) and pylorus (B) but these cells were located in the basal portion of epithelium of the duodenum (C) and ileum (D). Scale bars=50 µm (A, C, D) and 200 µm (B).

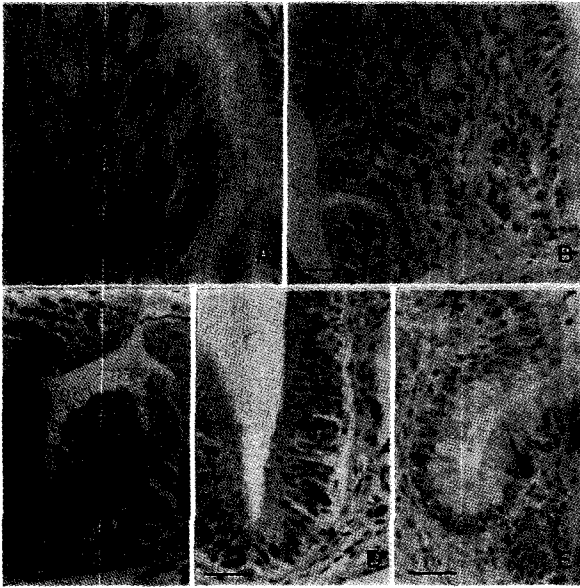


Fig. 5. Serotonin-immunoreactive cells in the gastrointestinal tract of the adult bullfrog. Spindle to polymorphic shaped cells were restricted to the gastric gland of the fundus (A) and pylorus (B) but spherical to spindle shaped cells were located in the basal portion of the epithelium of the duodenum (C), ileum (D) and rectum (E). Scale bars=50 μ m.

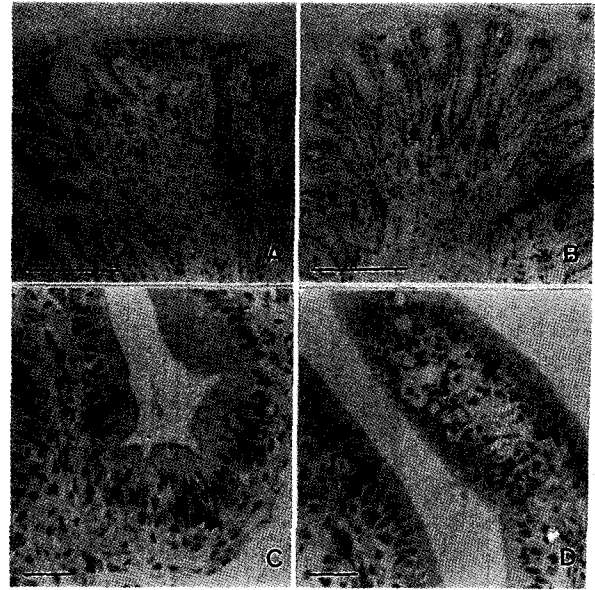


Fig. 6. Somatostatin-immunoreactive cells in the gastrointestinal tract of the adult bullfrog. Spherical to spindle shaped cells were found in the gastric gland of the fundus (A) and pylorus (B), but these cells were located in the basal portion of epithelium of the duodenum (C) and ileum (D). Scale bars=50 μ m (C, D) and 200 μ m (A, B).

this study.

Discussion

Depending on differences among species and each regional part of the GIT, gastroenteropancreatic (GEP) endocrine cells appeared remarkably different in terms of the regional distribution, relative frequency, and cell types (Gabe, 1972; Alumets et al., 1977). Yamada et al. (1989) suggested that these differences might be due to the differences in feeding behavior. In this study, variable changes in the regional distribution and relative frequency of serotonin- and somatostatin-immunoreactive cells in the GIT of the bullfrog were observed at each stag. Interestingly, motilin-immunoreactive cells were not found in this study.

Serotonin consists of monoamines and are widely distributed in the nervous system and GEP endocrine cells (El-Salhy et al., 1985). Main functions of serotonin include inhibition of gastric acid secretion and contraction of the smooth muscle in the GIT (Guyton, 1988). El-Salhy et al. (1985) reported that serotonin-immunoreactive cells are found throughout the GIT of all species and established in the GIT in the early stage of vertebrate evolution. Lee and Lee (1996, 1992) reported that serotonin-immunoreactive cells were detected in the entire GIT of *Rana dybowskii* and *Xenopus laevis*; most predominantly in the pylorus. In this study, these cells were observed from the 2 wk metamorphosed bullfrog in the entire GIT and most predominantly in the pylorus regions. Thereafter, they appeared to increase in the fundus. This result shows that the regional

distribution and relative frequency of these cells are quite similar to those of previous works (Lee and Lee, 1992, 1996). However, changes according to developmental stages were difficult to determine compared to other works because there was no report that studied the changes of serotonin-immunoreactive cells with developmental stages in other species of the Anuran amphibia.

Somatostatin consisting of 14 amino acids was isolated from the hypothalamus of the sheep for the first time. It could be divided into a straight form and cyclic form (Brazeau et al., 1973). This substance inhibits the secretion of the gastrin, cholecystokinin, secretin, glucagon, insulin, motilin, and gastric acid (Kitamura et al., 1984) and the absorption of amino acid, glucose, and fatty acid in the GIT (Brazeau et al., 1973). It is known that somatostatin-immunoreactive cells show the widest distribution in the entire GIT of all vertebrate species investigated, including the primitive agnathans (Falkmer and Van Noorden, 1983). In the Anura, these cells were detected in *Rana dybowskii* (Lee and Lee, 1996), *Rana esculenta* (Trandaburu and Nürnberger, 1995), *Xenopus laevis* (Lee and Lee, 1992), *Bufo regularis* (El-Salhy et al., 1981) and 8 species of Anura (Buchan, 1986). According to these reports, they were most predominant in the fundus, but decreased distally along the GIT of the adult Anura. In this study, somatostatin-immunoreactive cells were detected from the tadpole with hindlegs in the stomach regions. In the metamorphosed bullfrog with a tail, they were found in the entire GIT except for the ileum. Thereafter, these cells were found increasingly in each

developmental stage except for the rectum. In the rectum, these cells were not detected after that period. This result, especially for the 2 wk after metamorphosed bullfrog, were quite similar to those of previous works (El-Salhy et al., 1981; Buchan, 1986; Lee and Lee, 1992; Trandaburu and Nürnbergger, 1995; Lee and Lee, 1996). However, the changes with developmental stages, especially from the tadpole with hindlegs to the 2 wk metamorphosed bullfrog stage, were difficult to determine compared to other works because there was no report that carried out the changes of these cells with developmental stages in other species of the Anura amphibian.

Motilin-immunoreactive cells are exclusive to the small intestine, particularly to its upper parts (Pearse et al., 1977). They seem to play an important role in the modulation of gut motility, especially in the interdigestive phase (Solcia et al., 1989). Buchan (1986) reported that the motilin containing cells within the intestine of the axolotl (*Ambystroma mexicanum*). This is the first time that this peptide has been detected outside mammals, but in most amphibian species, they were not detected in the GIT (Buchan et al., 1981; El-Salhy et al., 1981; Fujita et al., 1981). As in previous works, motilin-immunoreactive cells were not found in the present study. The failure to detect motilin-immunoreactivity in lower vertebrates have previously been explained by a high degree to species specificity for motilin antisera (Bloom and Polak, 1978).

Future studies using histochemical or immunohistochemical methods will clarify the changes in regional distribution and relative frequency of other types of endocrine cells in the GIT of other Anura species according to developmental stage.

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