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An Immunohistochemical Study on the Endocrine Cells in the Gastrointestinal Tract of the Frog, *Rana ridibunda*

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Abstract: The regional distribution and relative frequency of endocrine cells was studied immuohistochemically in the gastrointestinal tract of the frog, *Rana ridibunda*, using antisera against serotonin, somatostatin-14, cholecystokinin and vasoactive intestinal polypeptide. These immunoreactive cells were located in the gastric glands of stomach and in the intestinal epithelium with variable frequencies. They were spherical or spindle shaped. The regional distributions and relative frequencies of the endocrine cells in the gastrointestinal tract of the *Rana ridibunda* were resembled to those of the other anuran species.

Key words: Endocrine cells, immunohistochemistry, gastrointestinal tract, *Rana ridibunda*

INTRODUCTION

Rana ridibunda, belonging to the family Ranidae in order Anura is widely distributed in Europa.

Gastrointestinal endocrine cells dispersed in the epithelia and mucosal glands of the alimentary tract synthesize various kinds of the gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract[1].

Many reports have dealt with the identification of the regulatory peptides of the alimentary tract in various amphibians using histochemical and either electron microscopical or immunohistochemical methods[2-4]. Altough 17 types of immunoreactive cell including serotonin, somatostatin, glucagon, Cholecystokinin (CCK)-8, Chromo Granin (CG), Pancreatic Polypeptide (PP), bombesin, neurotensin, gastrin-releasing peptide, substance-P, peptide YY, secretin, gastrin, Vasoactive Intestinal Polypeptide (VIP), motilin, met-enkephalin and ß-endorphin have been detected in *Rana dybowskii*[5], *Rana pipens*[6], *Xenopus leavis*[6-8], *Rana esculaenta*[9], *Bufo regularis*[10,11], *Rana catesbeiana*[12,13], *Rana nigromaculata*[14], *Rana rugosa*[15] there are no work on the endocrine cells of the Rana ridibunda.

In this study, the regional distributions and relative frequencies of the endocrine cells in the gastrointestinal tract of the frog, *Rana ridibunda* were investigated by an immunohistochemical method using spesific antisera against serotonin, somatostatin-14, CCK and VIP.

MATERIAL AND METHODS

Five female frog, *Rana ridibunda* were captured in Elazığ, Turkey and used in this study. The animals were anaesthetised with ethyl ether. After phlebotomize tissue samples were taken from stomach (pyloris and antrum), small and large intestine and fixed in 4% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. 7 μm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by using the Peroxidase-antiperoxsidase (PAP) method. Blocking of endogenous peroxidase was carried out with 0.008% hydrogen peroxidase (H_2O_2) in methanol for 5 min[16]. In order the block unspecific binding, an incubation with (1:10) normal goat serum in 0.1 M Phosphate Buffered Saline (PBS), pH 7.2 was performed.

PAP technique: Sections were incubated for 16-20 h at 4°C in, rabbit anti-serotonin (Zymed Lab., 18.0077, San Francisco), rabbit anti-somatostatin-14 (Chemicon, AB1976, Canada) or rabbit anti- cholecystokinin (Chemicon, AB1973, Canada) and rabbit anti-vasoactive intestinalpolypeptide (Chemicon, AB982, Canada). Antibodies were diluted to 1:200, 1:50, 1:100 and 1:50 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark), followed

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by rabbit peroxidase anti-peroxidase complex (Zymed Lab., 61.2003, San Francisco), both at dilution of 1:50 in PBS, for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation and finally immersed in glucose oxidise-DAB-nickel ammonium sulphate substrate[17] for 10 min. After washing in distilled water and counterstaining with eosin, sections were dehydrated and coverslips mounted with DPX.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger[18]. The relative frequency of occurrence of each type of Immunoreactive (-IR) cell was placed into one of five categories, not detected (-), rare (+), low (++) and moderate (+++) according to their observed numbers as seen using light microscope. Sections were examined with light microscope and photographs were taken.

RESULTS

Serotonin, somatostatin-14, cholecystokinin and vasoactive intestinal polypeptide -IR cells were observed in the gastrointestinal tract of the frog *Rana ridibunda*. The regional distribution and relative frequency of these immunoreactive cells in the gastrointestinal tract of the frog *Rana ridibunda* are shown in Table 1.

Serotonin-IR cells were located in the interepithelial cell region throughout the gastrointestinal tract at various frequencies. These immunoreactive cells were found in the gastric gland of the pylorus and antrum with a spherical to round shape (Fig. 1). These cells were observed with moderate and a few frequency in the pylorus and antrum, respectively. In the intestinal part of the tract, open typed serotonin-immunoreactive cells were situated in the interepithelial cell region with rare frequencies (Fig. 2).

Somatostatin-IR cells were restricted to pyloris with very low frequencies. These immunoreactive cells were situated in the basal portion of the epithelia. These cells were spindle shaped (Fig. 3).

CCK-immunoreactive cells were observed the antrum to the small intestine (Fig. 4). CCK containing cells were

Table 1: Distribution and frequency of gastrointestinal endocrine cells in the frog, *Rana ridibunda*

	Pyloris	Antrum	Small Intestine	Large Intestine
Serotonin	+++	++	+	++
Somatostatin	+	-	-	-
CCK	-	++	+	-
VIP	-	-	++	

CCK: Cholecystokinin, VIP: Vasoactve Intestinal Polypeptide
Relative frequencies: +++ (modeate), ++ (low), + (rare), - (not detected)

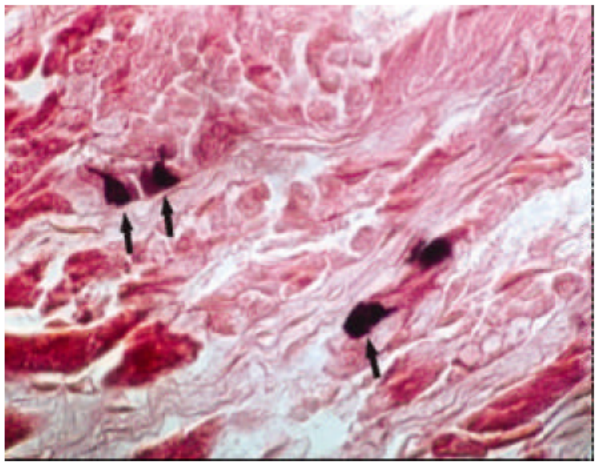

Fig. 1: Serotonin containing cells in the stomach of frog, *Rana ridibunda* (arrows). x 200

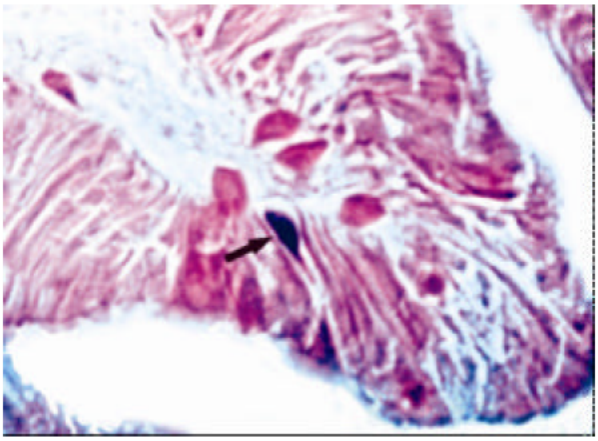

Fig. 2: Serotonin immunoreactive cell in the large intestine of frog, *Rana ridibunda* (arrow). x 200

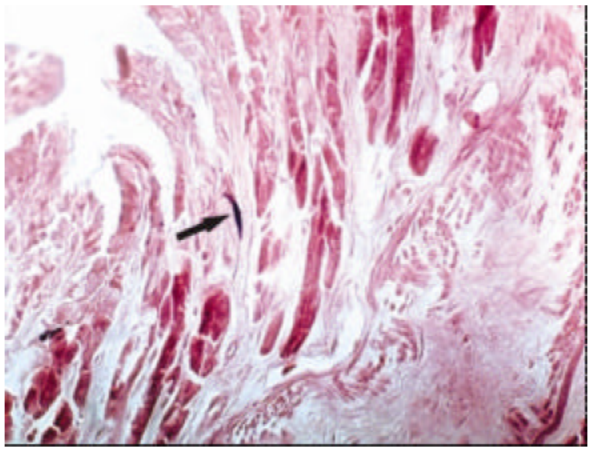

Fig. 3: Somatostatin containing cell in the stomach of frog, *Rana ridibunda* (arrow). x 100

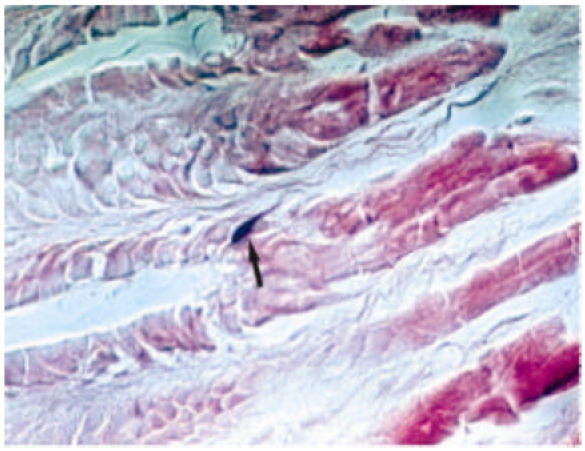

Fig. 4: Cholecystokinin containing cell in the stomach regions of frog, *Rana ridibunda* (arrow). x 100

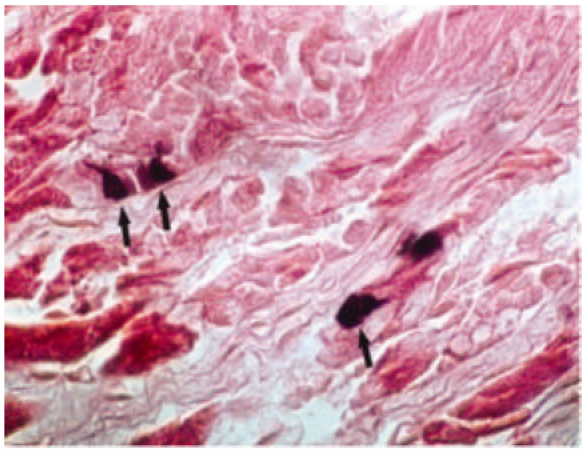

Fig. 5: Vasoactive intestinal polypeptide immunoreactive cell in the small intestine of frog, *Rana ridibunda* (arrow). x 100

usually spindle shaped and of open type with rare frequency.

VIP-IR cells were deteted only in small intestine with rare frequency. They showed spindle shape (Fig. 5).

DISCUSSION

Serotonin is a monoamine and is a videly distributed in nervous system and gastric gastroenteropancreatic endocrine cells[19]. The regional distributions and relative frequencies of serotonin-IR cells were detected whole gastrointestinal tract of *Rana dybowskii*[5], *Xenopus leavis*[7], *Rana nigromaculata*[14], *Rana catesbeiana*[13], *Bombina orientalis*[20] and *Hyla arborea japonica*[21]. According to these reports, serotonin-IR cells were most predominant in antrum except for, *Rana nigromaculata*[14]. That were most predominant in pyloris and duodenum. It is reported that this variance of relative frequencies in the anuran species might be due to sampling time or season[5]. In the present study these cells were observed throughout the gastrointestinal tract as like previous reports.

Somatostatin, which consist of 14 amino acids, was isolated from hypotalamus of sheep for the first and it could be subdivided into straight form and cyclic form[20]. In the anuran species, somatostatin-IR cells were detected in *Rana dybowskii*[5], *Rana esculenta*[9], *Xenopus leavis*[7], *Bufo regularis*[10], *Rana nigromaculata*[14] and *Bombina orientalis*[21]. According to these reports, they were most predominant in pyloris but decreased distally alond the gastrointestinal tract in adult anuran. In the present study, somatostatin-IR cells were mainly detected in pyloric region as like previous reports but the relative frequencies are somewhat different from other anuran. This may due to variation between the species.

In anuran species, CCK immunoreactive cells were restricted to the antrum, duodenum and ileum[5,15,21-23]. In the present study, these cells were detected in antrum and small intestine. These distributions well corresponded to previous reports[5,15,21-23].

VIP-immunoreactive nevre fibres have been widely described in all layers of the gut wall in amphibians[24-26]. However, VIP-immunoreactivity in endocrine cells has only been reported in a few studies[23,25,27] including this study. These results are noteworthy since the presence or absence of VIP containing endocrine cells in mammals continues to be a controversial topic.

CONCLUSION

The distribution and relative frequency of four types of immunoreactive cells observed in this study, correspond well to the previous report on anuran species.

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