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RESEARCH ARTICLE

Endocrine Cells in the Gastrointestinal Tract of *Garra rufa*

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Summary

The distribution and relative frequency of endocrine cells in the gastrointestinal tract of the *Garra rufa* were studied immunohistochemically using the peroxidase anti-peroxidase complex method. The aim was to determine the distribution of specific immunoreactivities in some regions of digestive tract of the *Garra rufa* against the antisera VIP, substance-P, histamine, glucagon, gastrin, CCK-8, somatostatin and serotonin. The samples were taken from the enlarged areas of after oesophagus, anterior intestine, middle intestine and posterior intestine. The most intensive region of immunoreactive cells were determined as the anterior intestine. Generally, eight types of endocrine cells declined in number from the enlarged areas of after oesophagus to the intestines. These immunoreactive cells were found in the posterior intestine at very low frequencies. As a result of the immunohistochemical studies, all the peptides studied were determined to be localized generally in different distribution within the stomachs of *Garra rufa* (the enlarged areas of after oesophagus) as well as within their intestines (anterior, middle and posterior intestine). Density of the peptides was concluded to be associated closely with functions in *Garra rufa*.

Keywords: *Garra rufa, Gastrointestinal tract, Endocrine cells, PAP*

Garra rufa'nın Gastrointestinal Kanalındaki Endokrin Hücreler

Özet

Peroxidase anti-peroxidase (PAP) yöntemi ile *Garra rufa* gastrointestinal kanalındaki endokrin hücrelerin dağılım ve yoğunlukları çalışıldı. Bu çalışmada VIP, substance-P, histamin, glukagon, gastrin, CCK-8, somatostatin and serotonin antiserumlarına karşı spesifik immunoreaktivitelerinin *Garra rufa* sindirim kanalının bazı bölümlerindeki dağılımlarının belirlenmesi amaçlandı. Materyal özofagustan sonraki genişlemiş bölgeden, ilk, orta ve son bağırsaktan alındı. Immunreaktif hücrelerin en yoğun olduğu bölgenin ilk bağırsak olduğu belirlendi. Genelde bu çalışmada araştırılan 8 tip immunoreaktif hücrelerin özofagustan sonraki genişlemiş bölgeden son bağırsağa doğru azaldığı gözlendi. Bu hücrelerin son bağırsakta oldukça az yoğunlukta olduğu saptandı. Yapılan immunohistokimyasal çalışmalar sonucunda *Garra rufa*'nın mide (özofagustan sonraki genişlemiş bölge) ve bağırsak bölümlerinde (ilk, orta ve son bağırsak) genel olarak çalışılan tüm peptidlerin farklı yoğunlukta lokalize oldukları tespit edildi. Peptid lokalizasyon ve yoğunluğunun fonksiyonlarına göre *Garra rufa*'da da bölgesel olarak yerleşim gösterdiği gözlendi.

Anahtar sözcükler: *Garra rufa, Gastrointestinal kanal, Endokrin hücre, PAP*

INTRODUCTION

Gastrointestinal endocrine cells dispersed throughout the epithelia and gastric glands of the alimentary tract synthesize various kinds of gastrointestinal hormones and play an important role in the physiological functions of the alimentry tract [1]. Many reports have dealt with the identification of regulatory peptides of the alimentary tract in fish species using silver-staining techniques and either radioimmunochemical or immunohistochemical methods [1-8].

Secretions of many endocrine cells act in digestion process together in fishes. The secretions, located in the

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gastrointestinal tract, are the chemicals regulating tracts structure and functions. These chemicals in gastrointestinal tract, accepted as the largest endocrine organ, are mainly secreted by endocrine cells [3,8,9]. A large number of endocrine-type secretions are produced in the gastrointestinal tract mucosa of the fish. These secretions, which are called peptide and/or amine, are detected in lamina epithelialis, glands, several connective tissue cells, mucosal nerve ganglions [10,11], and intermuscular nerve plexus [12]. These secretions assure the mobility as well as the proper functioning of the nervous system, regulation of the secretion through the cellular interaction [13], cellular proliferation [9,14], regulation of the intestinal epithelium [15], and the contraction of the smooth muscles [16].

This paper examines the distribution and localization of gut endocrine cells in the gastrointestinal tract of the *Garra rufa,* using specific immunohistochemical methods. This species is known as a doctor fish. It apparently feeds on skin scales of men and is used in the treatment of neurodermitis. The fish lives in Sivas thermal waters (28°C-34°C) and is used to treat psoriasis. It is also known as an oily fish [17]. Environmental condition of this fish are pH 7.0-7.4 [18].

MATERIAL and METHODS

Ten adult (length 8-10 cm, weight 10-20 g) *Garra rufa (Fig. 1, 2)* were used in this study without sexual distinction. After being anaesthetized, the intestinal tract of *Garra rufa* was divided into four portions from proximal to distal; the enlarged area after oesophagus and anterior, middle and posterior intestine. All samples were fixed for 12 h in Bouin's solution and embedded in paraffin. Serial, transverse 6-7 µm sections of these portions were cut. Each representative section was deparaffinized, rehydrated and immunostained using the peroxidase anti-peroxidase (PAP) method [19]. Sections were treated with methanol containing 0.3% H_2O_2 for 30 min, to block any endogenous peroxidase.

Subsequently, the sections were incubated for 1 h at room temperature in normal goat serum (1:100), then stained immunohistochemically to identify specific endocrine cells using PAP. The sections were incubated with specific antisera for individual peptides for 12 h at 4°C. Details of primary antisera used in this study are listed in *Table 1*. After rinsing with phosphate buffered saline (PBS, 0.01 mol/L, pH 7.4), the sections were incubated in secondary antiserum (ant-rabbit IgG serum raised in goats; 1:200) for 1 h at room temperature. Sections were then washed with PBS buffer and incubated with PAP complex (1:400) for 1 h at room temperature. The peroxidase reaction was carried out in 0.02% 3,3-diaminobenzidine tetrahydrochloride solution containing 0.01% H_2O_2 in Tris-HCl buffer (0.05 mol/L, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's haematoxylin and immunoreactive (IR) cells were observed with light microscopy. Light microscope with magnification 10x40 in total 4 different preparations were made VIP, substance-P, histamine, glucagon, gastrin, CCK-8, somatostatin and serotonin immunoreactive cells numbers. Total cell numbers in the 1 mm^2 area of the cross sections were

Fig 1. *Garra rufa* were used in this study (lateral view) (original)
Şekil 1. Çalışmada kullanılan *Garra rufa* (lateral görünüm) (orijinal)

Fig 2. *Garra rufa* were used in this study (ventral view) (original)
Şekil 2. Çalışmada kullanılan *Garra rufa* (ventral görünüm) (orijinal)

Table 1. *List of primary antisera used in the study*
Tablo 1. *Bu çalışmadaki primer antiserumların listesi*

Antiserum	Code	Dilution	Source
CCK-8	C2581	1:200	Sigma USA
Gastrin-1	G0785	1:200	Sigma USA
Substance P	S1542	1:200	Sigma USA
VIP	V3508	1:200	Sigma USA
Somatostatin-14	S0694	1:200	Sigma USA
Glucagon	G2654	1:200	Sigma USA
Histamine	H7403	1:200	Sigma USA
Serotonin	S5545	1:200	Sigma USA

All antisera were raised in rabbit
CCK: *Cholecystokinin,* ***VIP:*** *Vasoactive intestinal peptide*

determined. Data were subjected to analysis of variance SPSS 10 statistical software programme and analysis of variance results were significant in the region of average density immunreactive cell type were compared with ANOVA test [20].

RESULTS

Eight types of endocrine cells were detected with the antibodies against VIP, substance-P, histamine, glucagon, gastrin, CCK-8, somatostatin and serotonin. The distribution patterns and relative frequencies of these endocrine cells in the gastrointestinal tract of the *Garra rufa* are shown in *Table 2*. Generally, all antisera used showed specific immunoreactivity within endocrine cells in the mucosa, and they were higher in number in the crypt epithelia than in superficial epithelia.

VIP-positive cells were detected the whole gastro-intestinal tract and most predominant frequencies were detected anterior intestine *(Fig. 3, 4)*. The numbers of these cells appeared to be low in the middle and posterior intestine. Substance P-IR cells were demonstrated in both the enlarged area after oesophagus and gastro-intestinal tract but were more numerous in the former *(Fig. 5)*. The number of cells was decreased in the middle intestine. A great number of histamine-immunoreactive cells were detected in the enlarged area after oesophagus *(Fig. 6)*. Histamine immunoreactive cells were the most predominant cell types in the study. In intestines *(Fig. 7, 8)*, density of the cells lowered towards posterior end.

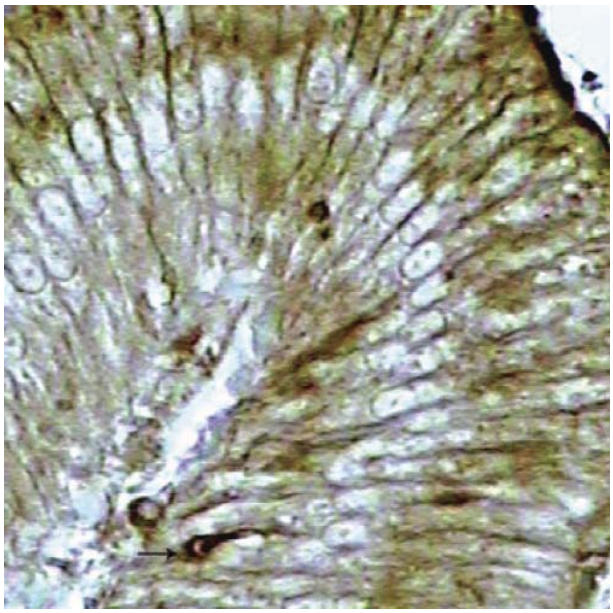

Fig 3. VIP IR cell, anterior intestine, PAP, X400
Şekil 3. VIP IR hücre, ilk bağırsak, PAP, X400

Fig 4. VIP IR cell, anterior intestine, PAP, X400
Şekil 4. VIP IR hücre, ilk bağırsak, PAP, X400

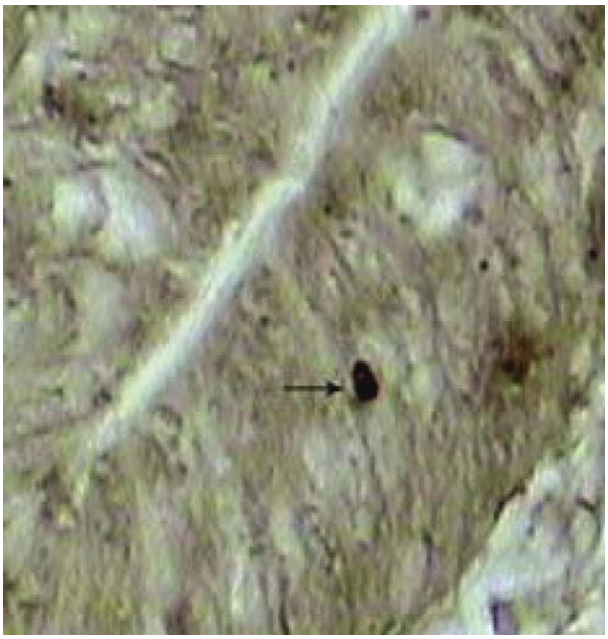

Fig 5. Sub-P IR cell, aterior intestine, PAP, X400
Şekil 5. Sub-P IR hücre, ilk bağırsak, PAP, X400

Cells immunoreactive for glucagon were numerous in the enlarged area after oesophagus. Glucagon IR cells, which were at the highest frequency in the anterior intestine, were found in the epithelia throughout the tract at various frequencies. These cells increased in number from the posterior intestine to the anterior intestine.

Gastrin immunoreactive cells decreased gradually from anterior intestine to posterior intestine. In enlarged region after oesophagus and anterior intestine, Gastrin-IR cells were denser in middle and posterior intestines. Gastrin immunoreactive cells were observed in large numbers in the anterior intestine compered to the other regions. Somatostatin immunoreactivity was observed in several cells of the middle and posterior intestines, whereas higher somatostatin immunoreactive cells were observed in the enlarged area after oesophagus and anterior intestine *(Fig. 9)*. CCK-8 positive cells were observed from area after oesophagus to intestines and

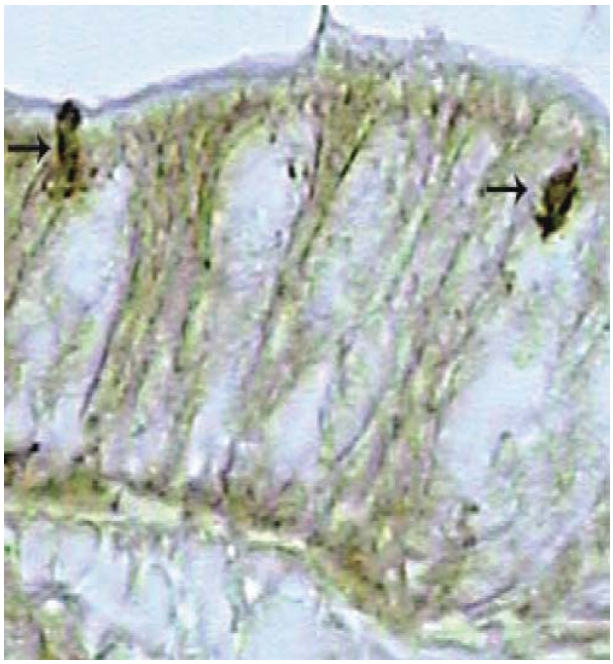

Fig 6. Histamine IR cells, the enlarged area after oesophagus, PAP, X400, *Garra rufa*

Şekil 6. Histamin IR hücreleri, özofagustan sonraki genişlemiş bölge, PAP, X400

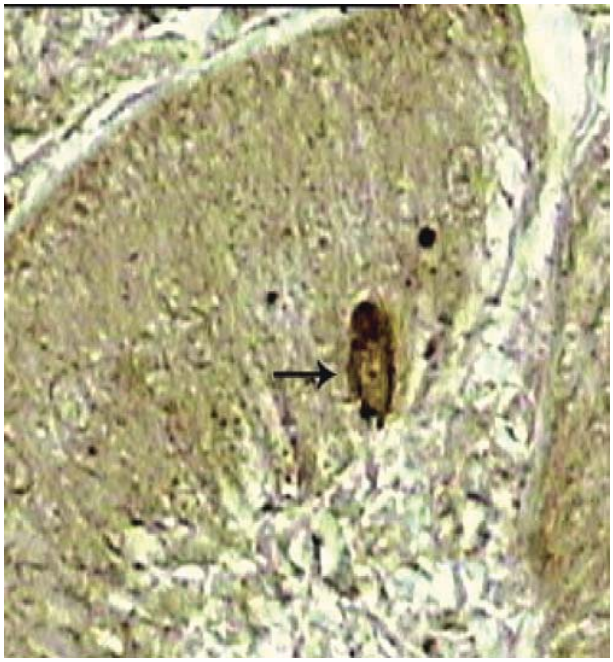

Fig 7. Histamine IR cell, anterior intestine, PAP, X400

Şekil 7. Histamin IR hücre, ilk bağırsak, PAP, X400

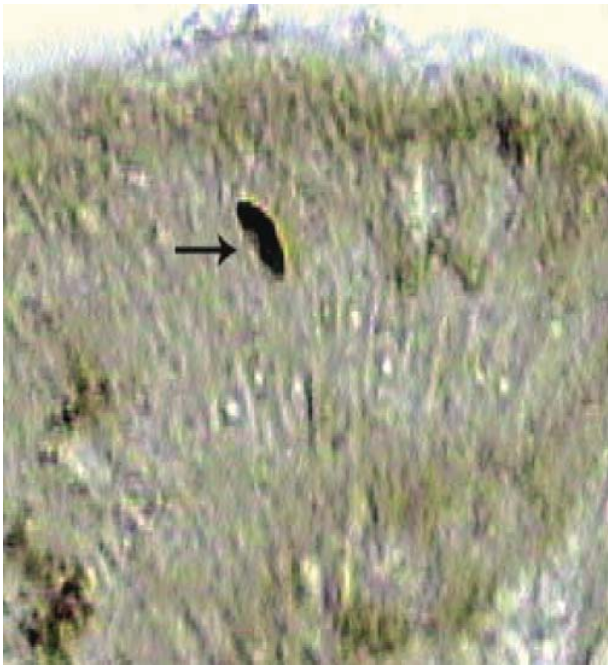

Fig 8. Histamine IR cell, anterior intestine, PAP, X400

Şekil 8. Histamin IR hücre, ilk bağırsak, PAP, X400

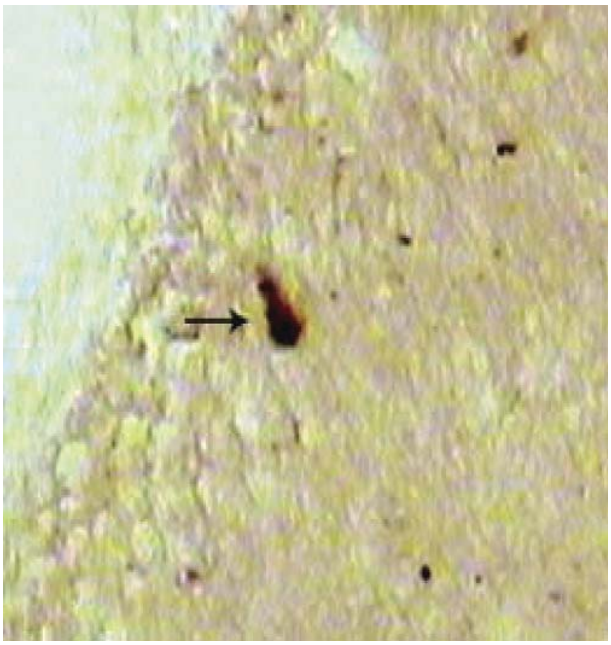

Fig 9. Somatostatin IR cell, anterior intestine, PAP, X400

Şekil 9. Somatostatin IR hücre, İlk bağırsak, PAP, X400

showed the highest frequency in anterior intestine. The numbers of serotonin immunoreactive cells in the enlarged area after oesophagus showed regional distribution to that in the anterior intestine *(Fig. 10, 11)*. These cells were found at a very low frequency in the middle and posterior intestines. Generally, eight types of endocrine cells declined in number from the enlarged area after oesophagus to the intestines. These immunoreactive cells were found in the posterior intestine at very low frequencies.

Fig 10. Serotonin IR cell, anterior intestine, PAP, X400

Şekil 10. Serotonin IR hücre, ilk bağırsak, PAP, X400

ganglion cells. The present study describes 8 types of endocrine cells were observed in the gastrointestinal tract of *Garra rufa*. The endocrine cells were immunoreactive for VIP, Substance-P, histamine, glucagon, gastrin, CCK-8, somatostatin and serotonin.

VIP immunoreactive cells were determined the whole gastrointestinal tract and most predominant frequencies were detected in anterior intestine. Similar results were reported by Çınar et al.[21], who also found that VIP-IR cells

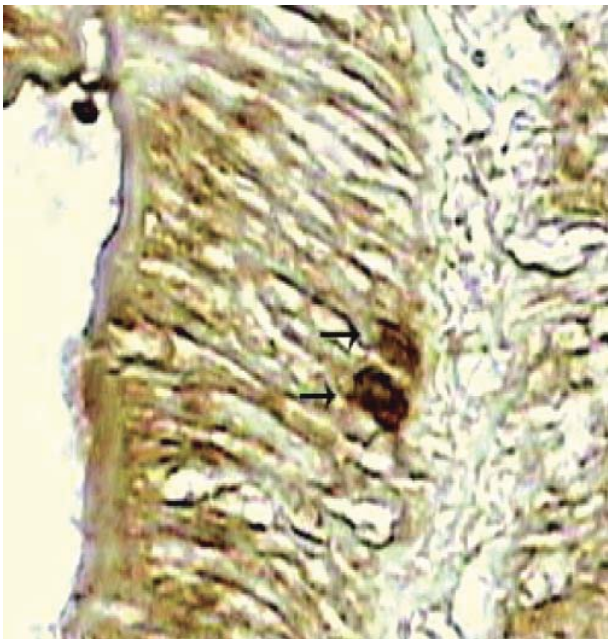

Fig 11. Serotonin IR cells, the enlarged area after oesophagus, PAP, X400

Şekil 11. Serotonin IR hücre, özofagustan sonraki genişlemiş bölge, PAP, X400

Table 2. *Mean number of endocrine cells per intestinal fold (mean ± SD) in Garra rufa*
Tablo 2. *Garra rufa (± standart sapma) bağırsağındaki endokrin hücre sayısı*

Area/Type of Endocrine Cells	VIP	Sub-P	Histamine	Glucagon	Gastrin	CCK	Somatostatin	Seratonin
The enlarged area after oesophagus	5.75±4.27	24±12.83	30±14.71	11.75±8.46	10.5±5.80	13.5±5.32	12.5±15.00	7.5±7.54
Anterior intestine	17±6.68	13±5.59	12±1.41	19.75±9.17	19±6.05	19.25±7.63	11.25±11.29	6.25±6.13
Middle intestine	1.75±2.06	0.5±0.57	3.75±2.21	2.25±0.95	2.5±0.57	6.25±2.62	1.5±1.29	1.25±0.5
Posterior İntestine	4±3.77	5.75±2.06	1±0.81	1.75±0.95	2±1.41	2±0.81	0±0.00	1.25±0.5

DISCUSSION

IR cells were generally observed in crypt, while weak or no IR cells were seen in connective tissues and were sparsely distributed in the digestive tract of *Pseudophoxinus antalyae* species. While VIP immunoreactive cells were not observed in the stomach of *Oncorhynchus mykiss* [16] and in the whole digestive tract

of *Gadus morhua* L.[22], *Oncorhynchus mykiss* [14], *Coreoperca herzi* [4] and *Zacco platypus* [1], Rajjo et al.[23] found these cells were present in all regions of *Amia calva* L. stomach. In the present study, numerous VIP immunoreactive cells were observed in the enlarged area after oesophagus and anterior intestine, except in the middle and posterior intestines. As in the present study, VIP immunoreactive cells have been determined in the intestine of many fish species, such as *Barbus conchonius* [24], *Anguilla anguilla* [25], *Stizostedion lucioperca* [26]. These results suggest that VIP immunoreactive cells provide contraction of intestinal wall and blood flow.

Çınar and Diler [26] showed that Sub-P IR cells were present throughout the intestine of *Stizostedion lucioperca*. Similar results were reported by Pan et al.[12], Domeneghini et al.[25], Rombout and Reinecke [2,3]. Sub-P immunoreactive cells were not observed in *Zacco platypus* [1], *Coreoperca herzi* [4]. In this study, numerous Sub-P immunoreactive cells were observed in the enlarged area after oesophagus and anterior intestine. These results suggest that Sub-P immunoreactive cells inhibit gastrin secretion.

Histamine is peptide functioning in contraction of smooth muscles and stimulation of acide secretion in stomach [1,4,27]. Like *Oncorhynchus mykiss* [28], *Salmo salar* [29], *Dicentrarchus labrax* [30] gastrointestinal Histamine IR cells were found densely. Dezfuli et al.[26] reported that Glucagon-like immunoreactive ECs were found in proximal intestine of uninfected and parasitised *Salmo trutta*. These findings agree with Bosi et al.[31] reports. Glucagon IR cells were observed in the digestive tract of *Coreoperca herzi* [4], *Zacco platypus* [1] and teleost fishes [12]. Similar results were determined in this study. These results imply that Histamine and Glucagon immuno-reactive cells are kept a certain level of acid secretion in stomach and intestines.

Gastrin secreted by the intestinal G cell promoted gastric acid secretion. In this study, gastrin immuno-reactive cells were detected from the enlarged area after oesophagus to the posterior intestine. These results corresponded with other reports on the fish [12,26,31,32]. These results suggest that Gastrin immunreactive cells are most predominant frequencies in the enlarged area after oesophagus in order to increase gastric acid secretion. CCK immunoreactive cells are distributed from the segment II of the gut to the segment V of the gut in the *Zacco platypus* [1]. As in the present study CCK immunoreactive cells have been determined in the gastrointestinal tract of many fish species, such as *Coreoperca herzi* [4], *Salmo trutta* L. [27], *Pseudophoxinus antalyae* [21,32], *Anguilla anguilla* [25], *Stizostedion lucioperca* L.[33]. These results imply that CCK and Gastrin peptides are affecting each other.

Somatostatin, which consist of 14 amino acids, was isolated from the hypothalamus of sheep for the first time and it could be subdivided into straight form and cyclic form [1]. As in the present study, somatostatin -14 immunoreactive cells have been determined in the gastrointestinal tract of many fish species, such as *Oncorhynchus mykiss* [16], *species of the Osteoglossomorpha* [34], *Zacco platypus* [1], *Coreoperca herzi* [4]. These results suggest that Somatostatin immunoreactive cells inhibit gastrin secretion. Serotonin immunoreactive cells were determined the whole gastrointestinal tract and most predominant frequencies were detected the enlarged area after oesophagus and anterior intestine. Similar results were reported by Ku et al.[1] who also found that serotonin IR cells were sparsely distributed in the *Zacco platypus* segments of the gut. As in the present study, serotonin immunoreactive cells have been determined in the intestine of many fish species, for instance *Coreoperca herzi* [4], *Stizostedion lucioperca* [33], *Anguilla anguilla* [25]. These results imply that Serotonin immunoreactive cells regulate gastro-intestinal motility.

In conclusion, the regional distribution and relative frequency of immunoreactive cells in the *Garra rufa,* are essentially similar to those of other fish. However, some characteristic differences are observed in this species, which may be due to differences in the antisera tested, the methods used and/or the species investigated in the various studies. Density of the peptides was concluded to be associated closely with functions in *Garra rufa*.

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