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An Immunohistochemical Study on the Endocrine Cells in the Stomach and Intestine Regions of the *Dicentrarchus labrax*, L., 1758

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The distribution and relative frequency of endocrine cells was studied in the stomach and intestine regions of the *Dicentrarchus labrax*, by immunohistochemistry using antisera against bombesin, cholecystokinin (CCK)-8, histamine, neurotensin, secretin, somatostatin-14, Trk A, Trk B, Trk C and vasoactive intestinal peptide (VIP).

As a result of immunohistochemical observations, immunoreactive cells studied were detected to be localized in different distribution and relative frequency in the stomach and intestine regions of *Dicentrarchus labrax*. Histamine, Trk B and Trk C immunoreactive cells were detected in the fundus and pylorus regions. Trk A immunoreactive cells were found in the anterior and posterior intestine. Cells reactive for CCK-8 and somatostatin-14 were demonstrated only in the anterior intestine.

Keywords: *Dicentrarchus labrax, endocrine cells, digestive tract, immunohistochemistry.*

Dicentrarchus labrax, L., 1758 Mide ve Bağırsak Bölgelerindeki Endokrin Hücreler Üzerine İmmunohistokimyasal Çalışma

Dicentrarchus labrax' ın mide ve bağırsak bölümlerindeki endokrin hücrelerin dağılım ve yoğunlukları bombesin, kolesistokinin (CCK)-8, histamin, neurotensin, sekretin, somatostatin-14, Trk A, Trk B, Trk C and vazoaktif intestinal polipeptid (VIP)' e karşı hazırlanmış antiserum kullanılarak imunohistokimyasal metodlar ile araştırıldı.

İmmunohistokimyasal çalışmalar sonucunda, balıkların mide ve bağırsak bölümlerinde çalışılan immunoreaktif hücrelerin dağılım ve yoğunluklarında farklılık olduğu gözlendi. Fundus ve pilorus bölgelerinde histamin, Trk B ve Trk C immunoreaktif hücreler gözlendi. Anterior ve posterior bağırsak bölgelerinde Trk A immunoreaktif hücrelerin bulunduğu belirlendi. CCK-8 ve somatostatin-14' e reaktif hücreler sadece anterior bağırsakta gösterildi.

Anahtar kelimeler**:** *Dicentrarchus labrax*, *endokrin hücre, sindirim kanalı, immunohistokimya.*

Introduction

Dicentrarchus labrax investigated in this study is a member of the Moronidae family (1). It is found from the Atlantic Ocean to the Mediterranean Sea. The grey body is covered by scales. Two separate dorsal fins; the first with 8 to 10 spines; the second with 1 spine and 10 or 14 soft rays. It can grow to a total length of over 1 m and 15 kg of weight. A diffuse spot on the edge of opercle. (1, 2). Young with some dark spots on upper part of body. *Dicentrarchus labrax* is an euryhaline and carnivore fish (3, 4). The gastrointestinal tract in the *Dicentrarchus labrax* comprises buccopharynx, esophagus, stomach, anterior intestine, posterior intestine and rectum (5). Although the carnivore fish have well-developed stomach, the intestinal tract is relatively short (6).

Gastrointestinal endocrine cells are distributed in the mucosa of the gastrointestinal tract and they synthesize various kinds of gastrointestinal hormones. They play important functions in the regulation of physiological functions of the gastrointestinal tract (7).

The existence of endocrine cells has been immunohistochemically demonstrated in the gastrointestinal tract mucosa of different fish species (8-18).

In the present study, in order to characterize the regional distribution and the relative frequency of the endocrine cells in the stomach and intestinal regions of the *Dicentrarchus labrax*, endocrine cells were investigated by immunohistochemical method using 10 types of specific antisera, bombesin, cholecystokinin (CCK)-8, histamine, neurotensin, secretin, somatostatin-14, Trk A, Trk B, Trk C, vasoactive intestinal peptide (VIP).

Bombesin is a tetradecapeptide originally isolated from the skin of the amphibian Bombina bombin (19). Endocrine function of bombesin regulates the secretion of gastric acid and its motility. (20).

In vertebrates, CCK-8 plays in important role in the control of gut motility, stimulation of pancreatic secretion and inhibition of gastric emptying (21, 22).

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Histamine is a peptide which assures the smooth muscle contraction of the gastrointestinal tract and stimulates the stomach acid (23).

Neurotensin is a tridecapeptide widely distributed in the nervous system and intestine. Neurotensin regulates several biological processes, such as intestinal motility, secretion, vascular smooth muscle activity, and intestinal epithelial cell proliferation, but recent evidence indicates that in neurotensin there is also a potent neuroimmunomodulator (24).

Secretin is a 27-amino acid peptide hormone belonging to the structurally related peptides of pituitary adenylate cyclase-activating polypeptide/glucagon superfamily (25). Secretin stimulates the secretion of bicarbonate-rich pancreatic fluid (26).

Somatostatin, which consisted of 14 amino acids, was isolated from the hypothalamus of sheep for the first time and it could be divided into a straight form and cyclic form (27), inhibited the secretion of gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid and absorption of amino acid, glucose and fatty acid in the gastrointestinal tract (27, 28).

Trk-like (A-B-C) proteins which are secreted by the cells making up the sub-population of the endocrine cells carry out the neurotrophin synthesis, amine and/or peptide storage as well as the regulation of the blood circulation of the gastrointestinal tract. (29)

VIP is a peptide consisting of 28 amino acids. The main function of this peptide appears to be as a modulator or co-transmitter (30).

Material and Methods

In this study ten adult *Dicentrarchus labrax* were obtained from a fish farm Ege-Mar Su Ürünleri Ltd. Şti. in Akbük/Aydın. Fish were killed by decapitation. The digestive tract was rapidly removed and samples from stomach (fundus, pylorus) and intestine (anterior, posterior) regions were fixed in Bouin solution for 12 hours. After routine histological tissue process, they were embedded in parafin. Five µm thick sections were obtained and immunohistochemical staining was carried out by using the PAP method (31). Blocking of endogenous peroxidase was carried out with 0.08% hydrogen peroxide (H_2O_2) in methanol for 5 minutes. Subsequently, the sections were incubated with normal goat serum in order the block unspecific binding. Sections were incubated for 16-20 hours at 4^{o}C with primary antisera, the name and the dilutions of which are given in Table 1. Sections were then incubated in goat anti-rabbit IgG, followed by rabbit peroxidase anti-peroxidase complex for 1 hour at room temperature. The immunoreactions were visualized using DAB (3.3' diaminobenzidine tetrahydrochloride) solution for 10 minutes. After washing in distilled water, sections were dehydrated and cover slips mounted with aqueous permanent mounting medium. After immunostaining, the immunoreactive cells were observed under a light microscope, the relative frequencies of each type of immunoreactive cells was placed into one of five categories: (-), absent; (+), rare; (++), moderate; (+++), numerous and photographs were taken.

Table 1. List of primary antisera used in the study.

Antisera	Code	Dilution	Source
Bombesin	NCL-BOMp	1: 200	Nova Castra Lab.
CCK-8	C2581	1: 200	Sigma
Histamine	H7403	1: 200	Sigma
Neurotensin	sc-20806	1: 200	Santa Cruz Biotec.
Secretin	sc-20938	1: 200	Santa Cruz Biotec.
Somatostatin-14	S0694	1: 200	Sigma USA
Trk A	sc-118	1: 200	Santa Cruz Biotec.
Trk B	sc-12	1: 200	Santa Cruz Biotec.
Trk C	sc-117	1: 200	Santa Cruz Biotec.
VIP	NCL-VIPp	1: 200	Nova Castra Lab.

Results

Immunohistochemical results are summarized in Table 2.

CCK-8 immunoreactive cells: While these immunoreactive cells were in high numbers in the l. epithelialis of the anterior intestine (Figure 1), no CCK-8 immunoreactive cells found in the fundus, pylorus and posterior intestine.

Histamine immunoreactive cells: Numerous histamine immunoreactive cells were detected in the fundus and in that region they were dispersed in the l. epithelialis. In the pylorus, they were dispersed in the l. epithelialis with moderate occurrences (Figure 2). No histamine immunoreactive cells were demonstrated in the anterior and posterior intestine.

Somatostatin-14 immunoreactive cells: Somatostatin-14 immunoreactive cells were detected in both the anterior (Figure 3) and posterior intestine l. epithelialis but were more numerous in the former. These cells were not observed in the fundus and pylorus.

Trk A immunoreactive cells: Trk A immunoreactive cells were demonstrated in the l. epithelialis of the anterior (Figure 4) and posterior intestine (Figure 5) at medium intensity, but not in the fundus and pylorus.

Trk B immunoreactive cells: Trk B immunoreactive cells were moderate in the l. epithelialis of the pylorus, but their number decreased in the fundus. We never found them in the intestinal regions.

Trk C immunoreactive cells: Trk C immunoreactive cells were demonstrated in the l. epithelialis of the stomach regions, but they were moderate frequency in the fundus (Figure 6) and rare frequency in the pylorus. These cells were not identified in the intestine regions.

No bombesin, secretin, neurotensin and VIP immunoreactive cells were detected in the studied regions.

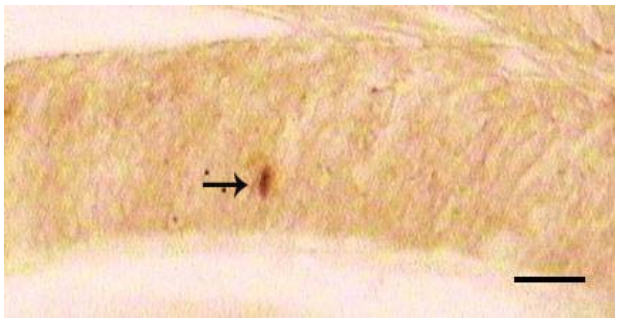

Figure 1. CCK-8 immunoreactive cell (arrow), Anterior intestine, PAP method, 50 µm.

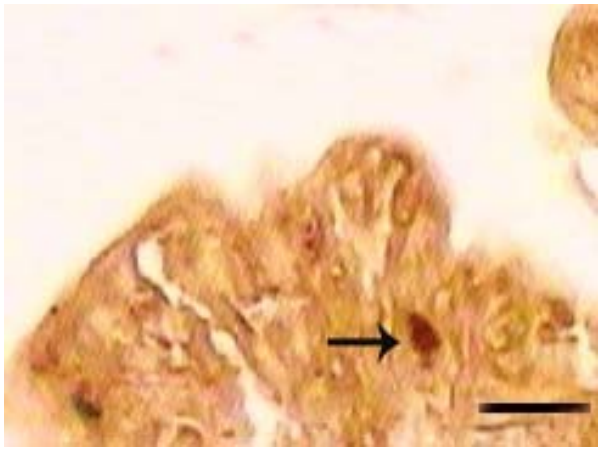

Figure 2. Histamine immunoreactive cell (arrow), Pylorus, PAP method, 50 µm.

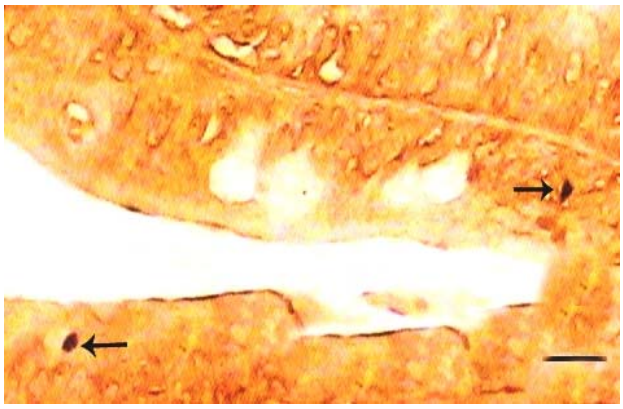

Figure 3. Somatostatin-14 immunoreactive cells (arrows), Anterior intestine, PAP method, 50 µm.

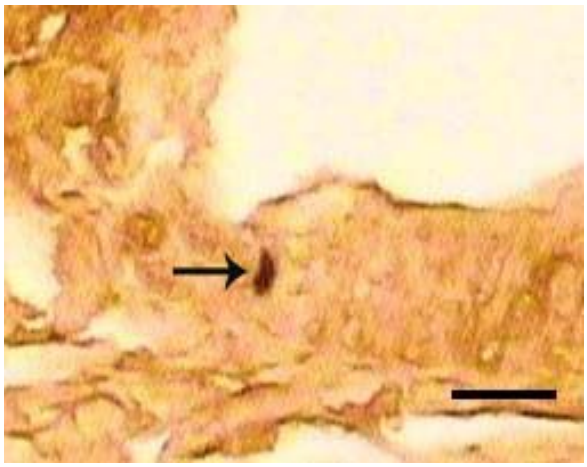

Figure 4. Trk A immunoreactive cell (arrow), Anterior intestine, PAP method, 50 µm.

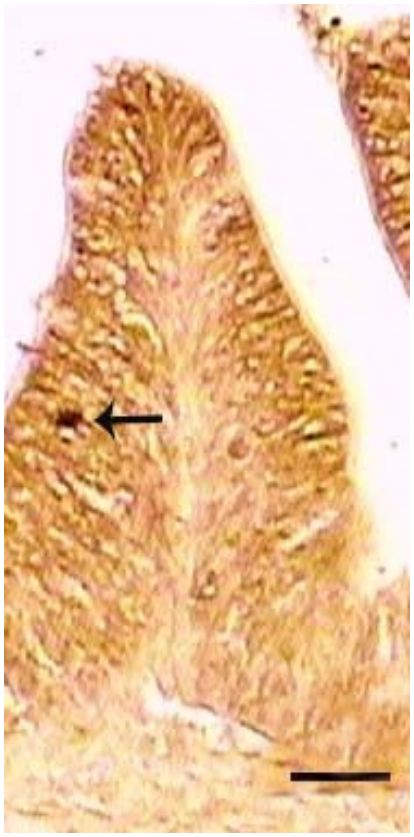

Figure 5. Trk A immunoreactive cell (arrow), Posterior intestine, PAP method, 50 µm.

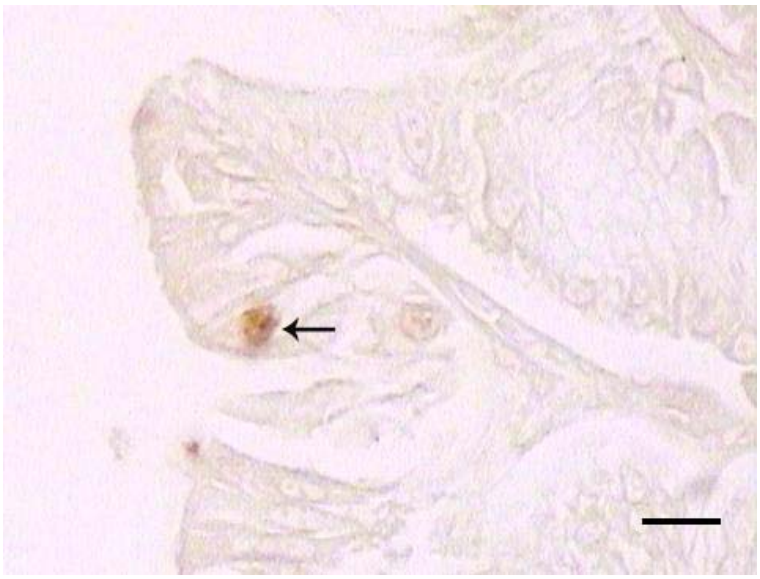

Figure 6. Trk C immunoreactive cell (arrow), Fundus, PAP method, 50 µm

Table 2. The regional distributions and relative frequencies of the immunoreactive cells in the fundus, pylorus, anterior and posterior regions of the *Dicentrarchus labrax.*

IR cells / Region	Bombesin	CCK-8	Histamine	Neurotensin	Secretin	Somatostatin-14	Trk A	Trk B	Trk C	VIP
Fundus	-	-	+++	-	-	-	-	+	++	-
Pylorus	-	-	++	-	-	-	-	++	+	-
Anterior intestine	-	+++	-	-	-	+++	++	-	-	-
Posterior intestine	-	-	-	-	-	+	++	-	-	-

Relative frequencies of immunoreactive cells: +++, numerous; ++, moderate; +, rare; -, absent

Discussion

Bombesin immunoreactive cells found in the stomach of the *Salmo trutta* (11) and *Oncorhynchus mykiss* (32) and intestine of *Pseudopxinus antalyae* (16), on the contrary, authors did not detect them in the stomach of the Korean aucha perch (13) and intestinal regions of the *Salmo trutta* (11), *Zacco platypus* (12), Korean aucha perch (13) and *Barbus conchonius* (33). We did not observe bombesin immunoreactivity in the studied regions.

As in the present study, cells immunoreactive for CCK were determined in the anterior intestine by authors (8, 11, 15, 17, 34). No CCK immunoreactive cells were identified in the stomach (11, 13, 17, 34, 35) and posterior intestine (8, 11, 17, 34). Similar results were observed in the *Dicentrarchus labrax*. On the other hand, these cells were identified in the stomach of the *Stizostedion lucioperca* (8) and posterior intestine of the *Pseudopxinus antalyae* (15).

In the *Pseudopxinus antalyae* (16), cells that immunoreactive for histamine were demonstrated throughout the whole gastrointestinal tract but they were not observed in the intestinal regions in this study.

In the present study, neurotensin immunoreactive cells were not determined in the studied regions. Similar results were reported by authors (34, 36, 37). Contrary to these results, they were observed in the intestine (15) and stomach (36).

In this study, secretin immunoreactive cells were not observed in the stomach and intestine regions of the *Dicentrarchus labrax*. These results agree well with those of (11-13, 32-37), but differ from results of authors who demonstrated the secretin immunoreactive cells in the stomach (11, 36) and intestine regions (15).

Several authors (13, 34, 36, 38-40) were demonstrated cells that immunoreactive for somatostatin in the stomach of the different species. In this study, somatostatin immunoreactive cells were not identified in the stomach of the *Dicentrarchus labrax.* Similar results were reported by Youson et al., Gençer Tarakçı et al., Min et al. The result that somatostatin immunoreactive cells, which were present in the intestine of the *Dicentrarchus labrax* in this study was similar to that of the study on *Ictalurus punctatus* (18) and *Tilapia nilotica* (41). These cells were demonstrated in the entire intestine except for posterior intestine of the *Zacco*

platypus (12) and *Pseudophoxinus antalyae* (15), but Gençer Tarakçı et al. reported that somatostatin immunoreactive cells found only in the posterior intestine of the *Oreochromis niloticus*. In addition, it is also demonstrated that no somatostatin immunoreactive cells were found in the intestinal regions of different species by several authors (13, 33, 34, 42, 43).

Although Trk A immunoreactive cells were identified in the intestine by Lucini et al. and Çınar et al. did not find them in the intestinal regions. In this study, we observed them in the anterior and posterior intestine.

It was reported that Trk B immunoreactive cells were present in the intestine (15) and stomach (44). In the present study, they were observed only in two stomach regions.

Lucini et al. determined that Trk C immunoreactive cells were present both in the stomach and the intestine. On the other hand, they were found in the intestine regions by Çınar et al.. In the present study, we observed them in the stomach regions, not in the intestine.

Authors (11, 13, 32, 34-37) reported that cells immunoreactive for VIP were not present in the stomach of different species and these cells were not found in the intestine of the *Salmo trutta* (11), *Zacco platypus* (12), Korean aucha perch (13), *Sparus auratus* (34) and *Mugil saliens* (37). Similar results were obtained in the present study. On the other hand, these cells were demonstrated in the stomach of the zander (9) and *Ictalurus punctatus* (18) and intestine of the zander (9), *Pseudophxinus antalyae* (15) and *Ictalurus punctatus* (18).

In conclusion, the regional distribution and relative frequency of immunoreactive cells in the stomach and intestine of the *Dicentrachus labrax* were essentially similar to those of different species. However, some differences were determined in this species.

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