Immunohistochemical study of the endocrine cells in the stomach and pyloric caeca of the mountain trout, *Salmo trutta macrostigma*

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Abstract

The endocrine cells of *Salmo trutta macrostigma* stomach and pyloric caeca have been investigated using immunohistochemical techniques. 8 antisera were tested and 6 of them were detected in the endocrine cells; serotonin, somatostatin, substance P, galanin, CCK and neuropeptide Y. These immunoreactive cells are described for the first time in the stomach and pyloric caeaca of *Salmo trutta macrostigma*. Neurotensin and VIP immunoreactivity were not detected in these regions. The regional distribution and relative frequency of the endocrine cells in the stomach and pyloric caeca of *Salmo trutta macrostigma* were resembled (with respect to serotonin, somatostatin, CCK and substance P immunoreactivity) and showed also some particularities (presence of galanin and NPY positive cells) to those of the other salmonid species.

Keywords: Endocrine cell, Stomach, Pyloric caeca, *Salmo trutta macrostigma*, Immunohistochemistry

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Introduction

Gastrointestinal endocrine cells dispersed through the epithelia and gastric glands of the alimentary tract, synthesize various kinds of gastrointestinal hormones and play in fish, as in mammals, important roles in coordinating various processes such as motility, blood flow and secretion/absorption (Bell, 1979; Chang et al., 1998; Olsson et al., 1999). The presence of large variety of endocrine cells in the digestive tract of fish has been reported by many authors (Rombout, 1977; Beorlegui et al., 1992; Barrenechea et al., 1994; Reinecke et al., 1997; Pan et al., 2000; Bosi et al., 2004; Gencer et al., 2005).

Among the salmonids, most studied species is the rainbow trout, *Oncorhynchus mykiss* (Walbaum) and many immunohistochemical studies have been carried out on the presence of several peptides in neurons and endocrine cells of the gut (Dubois et al., 1979; Beorlegui et al., 1992; Barrenechea et al., 1994; Gencer and Köprücü, 2002), whereas the informations about other species of the taxonomic group are lacking.

Salmo trutta macrostigma (Dumeril, 1858) is a salmonid species occurring in inland water habitats of Southern Europe, Western Asia, Northern Africa and Anatolia (Geldiay and Balık, 1988). In the present study, the regional distribution and relative frequency of the endocrine cells in the proximal regions of gastrointestinal tract (stomach and pyloric caeca wall) of *S. trutta macrostigma* were investigated by immunohistochemistry using 8 antisera against serotonin, somatostatin, substance P, galanin, cholecystokinin (CCK), neuropeptide Y, neurotensin, and vasoactive intestinal polypeptide (VIP). Because, until now, no information knowledge concerning the occurrence of endocrine cells in any part of gastrointestinal tract in the mountain trout, *Salmo trutta macrostigma* is available. When possible, the results here obtained will be compared with the data collected on the other salmonids species, thus providing further information for a better knowledge of the gut in diverse species of trouts, all of the of high commercial interest.

Materials and methods

Animals and Tissue Samples

Adult of *S. trutta macrostigma* were collected by seine net at Tunceli (River Munzur, Eastern Anatolia, Turkey). Ten fish ranging from 25 to 30 cm in total length were used. Tissue samples were immediately collected from stomach and pyloric caeca after the sacrifice by a blow to the head. The small pieces were fixed in 4% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. Seven µm-thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemistry

Immunohistochemical staining was carried out by using the peroxidase-antiperoxidase (PAP) method. Blocking of endogenous peroxidase was carried out with 3% hydrogen peroxidase (H_2O_2) in methanol for 10 minutes. In order to block

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unspecific binding, an incubation with normal goat serum in 0.1 M phosphate buffered saline (PBS), pH 7.2 (dilution 1:10) was performed.

Sections were incubated with primary antibodies for 16-20 hours at 4°C, as detailed in Table 1. The primary antibodies were diluted in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin (BSA). Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark) followed 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 minutes after each incubation and finally immersed in glucose oxidise-DAB-nickel ammonium sulphate substrate (Shu et al., 1988) for 10 minutes. After washing in distilled water and counterstaining with hematoxylin, sections were dehydrated and coverslips mounted with aqueous permanent mounting medium.

Table 1: Details of antibodies used in this study

Primer Antibodies	Dilution	Trade Name	Cat. No.
Serotonin	1:500	Zymed (Invitrogen), UK	18-0077
SOM	1:1000	Chemicon (Millipore), Canada	AB1976
SP	1:500	Chemicon (Millipore), Canada	AB1566
Galanin	1:100	Chemicon (Millipore), Canada	AB5909
CCK	1:100	Chemicon (Millipore), Canada	AB1973
NPY	1:100	Chemicon (Millipore), Canada	AB1915
Neurotensin	1:50	Chemicon (Millipore), Canada	AB5496
VIP	1:100	Chemicon (Millipore), Canada	AB982

All antisera were raised in rabbit (polyclonal). *SOM*, somatostatin; *SP*, substance P; *CCK*, cholecystokinin; *NPY*, neuropeptide Y; *VIP*, vasoactive intestinal polypeptide.

The control for the specificity of immunhistochemical reactions were performed by the pre-absorption of each antiserum with the corresponding antigen (Sternberger, 1979). Sections were examined with Olympus BX-51 microscope and photographs were taken.

Evaluation of the distribution and frequency of immunopositive cells in stomach and pyloric caeca was based on subjective estimates after the examination of 5 randomly selected sections per stomach and pyloric caeca for each antibody used in this study. The density of distribution was subjectively rated into 5 grades, not detected (-), rare (+), a few (++), moderate (+++) and numerous (++++) (for details see Table 2).

Results

Serotonin-, somatostatin-, substance P-, galanin-, CCK- and neuropeptide Y-immunoreactive cells were observed in the stomach and pyloric caeaca region of gastrointestinal tract of mountain trout (*S. trutta macrostigma)* and neurotensin and

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VIP immunoreactivity were not detected in these regions. Endocrine cells immunohistochemically detected were either roundish to ovoidal (close type cells) or elongated (open type cells) in shape. The relative densities of immunoreactive endocrine cells observed in the stomach and pyloric caeca are summarized in Table 2.

Table 2: Regional distribution and relative frequencies of immunoreactive endocrine cells in the stomach and pyloric caeca of the mountain trout

	Stomach	Pyloric caeca
Serotonin	+ + + +	+
SOM	+ + +	+ + +
SP	+	+ + + +
Galanin	+ +	+ + + +
CCK	+	+ + +
NPY	+	+ + +
Neurotensin	-	-
VIP	-	-

Mean number of detected immunoreactive endocrine cells in one 10x40 field: - = not detected; + (rare) = < 1; + + (a few) = 1-4; + + + (moderate) = 5-10; + + + + (numerous) = > 10

Serotonin-immunoreactive cells were mostly evidenced in the stomach and scarcely in the pyloric caeca. Most of these cells were shown spindle shape (Figure 1A and B). Somatostatin-immunoreactive cells were usually observed in elongated shape and they were located in the stomach and pyloric caeca (Figure 1C) with a moderate frequency. They appeared near the gastric gland and long cytoplasmic processes reaching parallel to the basal membrane and sometimes runing to lumen of organs have been observed.

Substance P-, galanin-, CCK- and neuropeptide Y-immunoreactive cells were observed in stomach with a low frequency. Whereas, these immunoreactive cells were found numerous in the pyloric caeca. These cells were open or closed type cells (Figure 2A-D).

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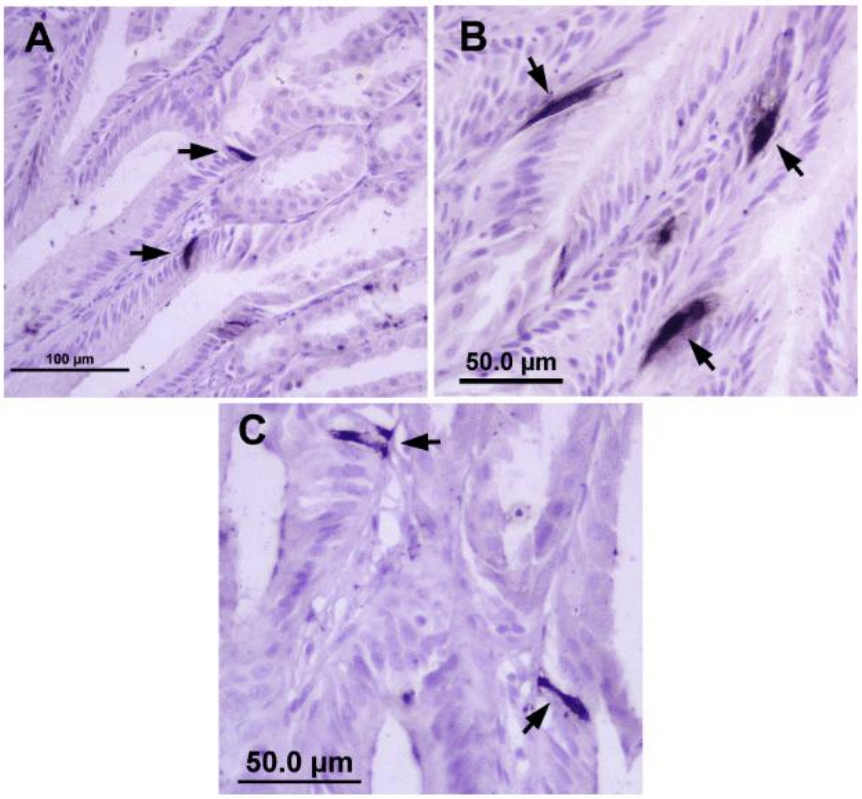

Fıgure 1: In the stomach. Serotonin (A, B) and somatostatin (C) immunoreactivity are detected in the epitelial endocrine cells (arrows).

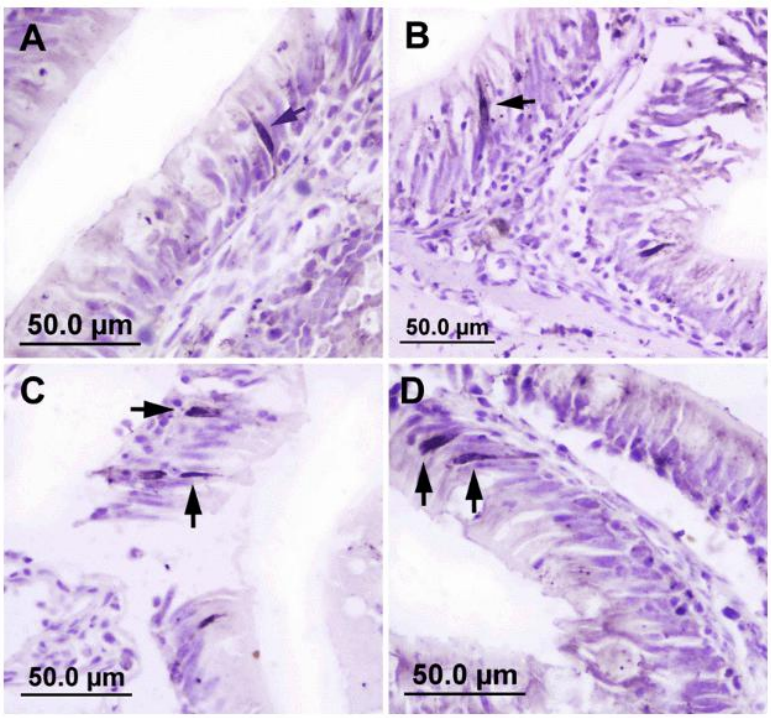

Fıgure 2: In the pyloric caeca. SP (A), galanin (B), NPY (C) and CCK (D) immunoreactivity are detected in the epitelial endocrine cells (arrows).

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Discussion

Gastrointestinal endocrine cells have previously been observed in some species of salmonids (Dubois et al., 1979; Holmgren et al., 1982; Beorlegui et al., 1992; Barrenechea et al., 1994; Gencer and Köprücü, 2002; Bosi et al., 2004). We have here shown that immunohistochemistry of regulatory peptides within the stomach and pyloric caeca endocrine cells reveals a structural pattern in *Salmo trutta macrostigma*, which only in some part resembles that of the most studied species in salmonids, *Oncorhynchus mykiss* and other species (Dubois et al., 1979; Holmgren et al., 1982; Beorlegui et al., 1992; Barrenechea et al., 1994; Gencer and Köprücü, 2002; Bosi et al., 2004).

The occurrence of serotonin immunoreactivity has been described for gastrointestinal endocrine cells and nerve fibres of several teleosts. In some species, serotonin immunoreactivity restricted to stomach which corresponds to their absence in stomachless fish (Rombout et al., 1986; Abad et al., 1987). Present study showed serotonin immunoreactviy mainly in the endocrine cells of stomach in the *S. trutta macrostigma*. Immunoreactivity was seen rarely in the pyloric caeca. Barrenechea et al. (1994) have also reported the occurrence of serotonin-immunoreactive endocrine cells in the stomach of rainbow trout, *Salmo trutta*. The main functions of serotonin were inhibition of gastric acid secretion and contraction of smooth muscle in the gastrointestinal tract (Guyton, 1988).

Several authors (Langer et al., 1979; Holmgren et al., 1982; Abad et al., 1987) have described somatostatin-immunoreactive cells exclusively in the stomach of teleost. Somatostatin is known to be paracrine inhibiting factor in gastrin release (Larsson et al., 1979). The localization of long stoplazmic processes of somatostatin containing endocrine cells parallel to basal lamina suggest its paracrine behaviour. In addition, some of containing cells laying to the lumen of stomach may be involved in the regulation of gastric cell proliferation as a response to luminal stimulous as has been suggested in cartilaginous fishes (Tagliafierro et al., 1989).

Substance P is a peptide of the tachykinin family that have an aminoacid sequence very conversed through phylogeny (Cimini et al., 1989). Our results show very numerous population of substance P-containing cells in trout pyloric caeca. Substance P-immunoreactive cells have been described in the stomach and intestine of *Salmo gairdneri* (Holmgren et al., 1982; Beorlegui et al., 1992) and in the intestine of some fish species (Langer et al., 1979; Rombout et al., 1984; Rombout et al., 1986).

Galanin is an originally porcine peptide, which displays a number of physiological actions upon mammalian gut, which are various and frequently depend upon species and different parts of the alimentary canal (Yağcı et al., 1990; Rattan, 1991). Although galanin immunoreactivity was only localized in nervous element of the digestive tract of fish (Karila et al., 1993; Karila and Holmgren, 1997; Bosi et al., 2004),

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galanin immunoreactive endocrine cells were found in the pyloric caeca of the mountain trout *Salmo trutta macrostigma* and to our knowledge this is the first report about this peptide in salmonids endocrine cells. This discrepancy may due to differences between the species and subspecies.

In the present study, CCK-immunoreactive endocrine cells were found numerous in the pyloric caaeca. A similar situation has been observed in the some teleost species (Elbal et al., 1988; Barrenechea et al., 1994; Reinecke et al., 1997; Bosi et al., 2004). In fish, CCK influences stomach motility (Olsson et al., 1999) and acts in the control of food intake (Le Bail and Roeuf, 1997).

NPY is a peptide belonging to the pancreatic polypeptide family and has a stimulate effect on insulin secretion in pancreas (Adeghate et al., 2001; Conlon, 2002). Although limited data were reported with regard to NPY immunoreactivity in endocrine cells of gastrointestinal tract of fish, we have observed numerous immunoreactive cells in the pyloric caeca of *Salmo trutta macrostigma*

No endocrine cells showing neurotensin and VIP immunoreactivity were detected in the stomach and pyloric caeca of *Salmo trutta macrostigma*. These results are in agreement with studies reported in the gastrointestinal tract of some salmonid species (Holmgren et al., 1982; Beorlegui et al., 1992; Bosi et al., 2004).

The present study is the first report of localization and relative frequency of the some endocrine cells in the stomach and pyloric caeca of *Salmo trutta macrostigma*. The distribution and relative frequency of the 4 types (serotonin, somatostatin, CCK and substance P) of immunoreactive cells observed in the stomach and pyloric caeca of *Salmo trutta macrostigma* well correspond to previous reports on trout species. However, present study suggests that the distribution of the different endocrine cell type in the pyloric caeca of *S. trutta macrostigma* exhibites some particularities (presence of galanin and NPY positive cells).

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