

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

Gençer Tarakçı B. *; Bayrakdar A.; Yaman M.

Received: March 2012 Accepted: July 2012

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 caeca 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

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 Köprüci, 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 (H₂O₂) in methanol for 10 minutes. In order to block

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 oxidase-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 immunohistochemical 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 caeca region of gastrointestinal tract of mountain trout (*S. trutta macrostigma*) and neurotensin and

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 running 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).

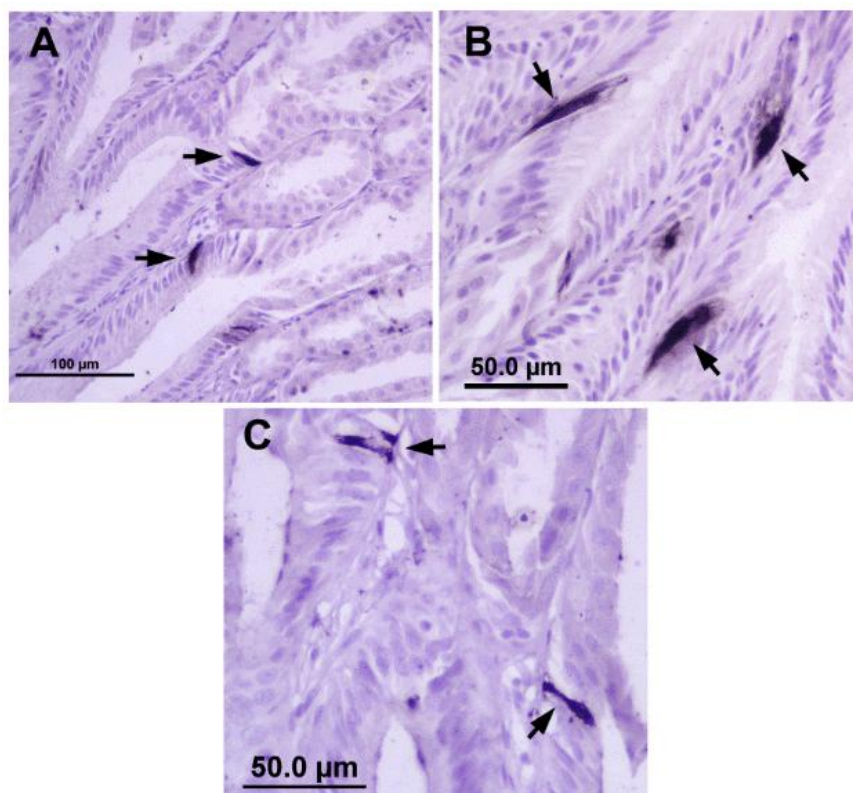


Figure 1: In the stomach. Serotonin (A, B) and somatostatin (C) immunoreactivity are detected in the epithelial endocrine cells (arrows).

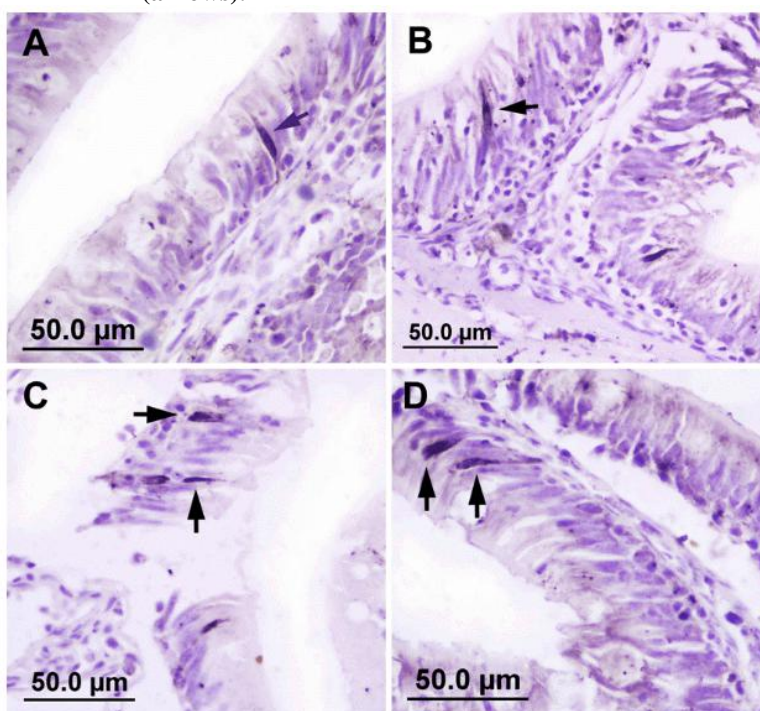


Figure 2: In the pyloric caeca. SP (A), galanin (B), NPY (C) and CCK (D) immunoreactivity are detected in the epithelial endocrine cells (arrows).

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 Köprüçü, 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 Köprüçü, 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 immunoreactivity 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 cytoplasmic 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 stimulus as has been suggested in cartilaginous fishes (Tagliafierro et al., 1989).

Substance P is a peptide of the tachykinin family that have an amino acid sequence very conserved 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),

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 be due to differences between the species and subspecies.

In the present study, CCK-immunoreactive endocrine cells were found numerous in the pyloric caeca. A similar situation has been observed in 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 Roef, 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* exhibits some particularities (presence of galanin and NPY positive cells).

References

- Abad, M. E., Peeze Binkhorst, F. M., Elbal, M. T. and Rombou, J. H. W. M., 1987.** A comparative immunocytochemical study of the gastroenteropancreatic (GEP) endocrine system in a stomachless and stomach containing teleost. *General and Comparative Endocrinology*, 66, 123-136.
- Adeghate, E., Ponery, A. S., Pallot, D. J. and Singh, J., 2001.** Distribution of vasoactive intestinal polypeptide, neuropeptide Y and SP and their effects on insulin secretion from the *in vitro* pancreas of normal and diabetic rats. *Peptides*, 22, 99-107.
- Barrenechea, M. A., Lopez, J. and Martinez, A., 1994.** Regulatory peptides in gastric endocrine cells of the rainbow trout *Oncorhynchus mykiss*: general distribution and colocalizations. *Tissue and Cell*, 26, 309-321.
- Bell, F. R., 1979.** The relevance of the new knowledge of gastrointestinal

- hormones to veterinary science. *Veterinary Science Communications*, 2, 305-314.
- Beorlegui, C., Martinez, A. and Sesma, P., 1992.** Endocrine cells and nerves in the pyloric caeca and intestine of *Oncorhynchus mykiss*: an immunocytochemical study. *General and Comparative Endocrinology*, 86, 483-495.
- Bosi, G., Di-Giancamillo, A., Arrighi, S. and Domeneghini, C., 2004.** An immunohistochemical study on the neuroendocrine system in the alimentary canal of the brown trout, *Salmo trutta*, L., 1758. *General and Comparative Endocrinology*, 138, 166-181.
- Chang, C. H., Chey, W. Y., Erway, B., Coy, D. H. and Chang, T. M., 1998.** Modulation of secretin release by neuropeptides in secretin-producing cells. *American Journal of Physiology*, 275, 192-202.
- Cimini, V., Van Norden, S. and Polak, J. M., 1989.** Co-localization of substance P-, bombesin-, and peptide histidine isoleucine (PHI)-like peptides in gut endocrine cells of the dogfish *Scyliorhinus stellaris*. *Anatomy and Embryology*, 179, 605-614.
- Conlon, J. M., 2002.** The origin and evolution of peptide YY (PYY) and pancreatic polypeptide (PP). *Peptides*, 23, 269-278.
- Dubois, M. P., Billard, R., Breton, B. and Peter, R. E., 1979.** Comparative distribution of somatostatin, LH-RH, neurophysin, and alpha-endorphin in the rainbow trout: an immunocytological study. *General and Comparative Endocrinology*, 37, 220-232.
- Elbal, M. T., Lozano, T. and Agulleiro, B., 1988.** The endocrine cells in the gut of *Mugil saliens Risso, 1810 (Teleostei)*: an immunohistochemical and ultrastructural study. *General and Comparative Endocrinology*, 70, 231-246.
- Geldiy, R. and Balık, S., 1988.** Türkiye tatlı su balıkları (Freshwater fish in Turkey). *Ege Üniversitesi Fen Fakültesi Kitaplar Serisi*, 97, 519 (in Turkish).
- Gençer, B. T. and Köprücü, S., 2002.** Regulatory peptides in gastroenteropancreatic cells of the rainbow trout (*Oncorhynchus mykiss* Walbaum 1792). *Ege University Journal of Fisheries and Aquatic Sciences*, 19, 157-162.
- Gençer, B. T., Köprücü, S. and Köprücü, K., 2005.** Immunohistochemical identification of peptide hormones in the endocrine cells of the gastrointestinal tract of the *Oreochromis niloticus*. *Turkish Journal of Veterinary and Animal Sciences*, 29, 207-210.
- Guyton, A.C., 1988.** Secretory functions of the alimentary tract. In: Textbook of Medical Physiology. 8 th. edn. W. B. Saunders, Philadelphia. 801p.
- Holmgren, S., Vaillant, C. and Dimaline, R., 1982.** VIP-, substance P-, gastrin/CCK-, bombesin-, somatostatin-, and glucagon-like immunoreactivities in the gut of the rainbow trout, *Salmo gairdneri*. *Cell and Tissue Research*, 223, 141-153.

- Karila, P. and Holmgren, S., 1997.** Anally projecting neurons exhibiting immunoreactivity to galanin, nitric oxide synthase and vasoactive intestinal peptide detected by confocal laser scanning microscopy in the intestine of the Atlantic cod, *Gadus morhua*. *Cell and Tissue Research*, 287, 525-533.
- Karila, P., Jönsson, A. C., Jensen, J. and Holmgren, S., 1993.** Galanin-like immunoreactivity in extrinsic and intrinsic nerves to the gut of the Atlantic cod *Gadus morhua* and the effect of galanin on the smooth muscle of the gut. *Cell and Tissue Research*, 271, 537-544.
- Langer, M., Van Noorden, S., Polak, J. M. and Pearse, A. G. E., 1979.** Peptide hormone-like immunoreactivity in the gastrointestinal tract and endocrine pancreas of eleven teleost species. *Cell and Tissue Research*, 199, 493-508.
- Larsson, L. I., Goltermann, N., De Magistris, L., Rehfeld, J. F. and Schwartz, T. W., 1979.** Somatostatin cell processes as pathways for paracrine secretion. *Science*, 205, 1393-1395.
- Le Bail, P. Y. and Roef, G., 1997.** What hormones may regulate food intake in fish. *Aquatic Living Resources*, 10, 371-379.
- Olsson, C., Aldman, G., Larsson, A. and Holmgren, S., 1999.** Cholecystokinin affects gastric emptying and stomach motility in the rainbow trout *Oncorhynchus mykiss*. *The Journal of Experimental Biology*, 202, 161-170.
- Pan, Q. S., Fang, Z. P. and Huang, F. J., 2000.** Identification, localization and morphology of APUD cells in gastroenteropancreatic system of stomach-containing teleost. *World Journal of Gastroenterology*, 6, 842-847.
- Rattan, S., 1991.** Role of galanin in the gut. *Gastroenterology*, 100, 1762-1768.
- Reinecke, M., Muller, C. and Segner, H., 1997.** An immunohistochemical analysis of the ontogeny, distribution and coexistence of 12 regulatory peptides and serotonin in endocrine cells and nerve fibres of the digestive tract of the turbot, *Scophthalmus maximus* (Teleostei). *Anatomy and Embryology*, 195, 87-101.
- Rombout, J. H. W. M., 1977.** Enteroendocrine cells in the digestive tract of *Barbus conchonioides*. *Cell and Tissue Research*, 185, 435-450.
- Rombout, J. H. W. M. and Reinecke, M., 1984.** Immunohistochemical localization of (neuro) peptide hormones in endocrine cells and nerves of the gut of a stomachless teleost fish, *Barbus conchonioides* (Cyprinidae). *Cell and Tissue Research*, 237, 57-65.
- Rombout, J. H. W. M., Van Den Grinten, C. P., Binkhorst, F. M. P., Taverne-Thiele, J. J. and Schooneveld, H., 1986.** Immunocytochemical identification and localization of peptide hormones in the gastroenteropancreatic (GEP) endocrine system of the mouse and a

stomachless fish, *Barbus conchoniuus*. *Histochemistry*, 84, 471-483

Sternberger, L. A., 1979. The unlabelled antibody peroxidase-antiperoxidase (PAP) method, in: Sternberger L.A., (ed.) Immunocytochemistry. J. Wiley, Sons, New York, 104.

Shu, S., Ju, G. and Fan, L., 1988. The glucose oxidase -DAB-nickel method in peroxidase histochemistry of the nervous system. *Neuroscience Letters*, 85, 169-171.

Tagliaferro, G., Farina, L., Faraldi, G., Rossi, G. G. and Vacchi, M., 1989.

Distribution of somatostatin and glucagons immunoreactive cells in the gastric mucosa of some cartilaginous fishes. *General and Comparative Endocrinology*, 75,1-9.

Yagci, R. V., Alpetkein, N., Rossowski, W. J., Brown, A., Coy, D. H. and Ertane, A., 1990. Inhibitory effect of galanin on basal and pentagastrin-stimulated gastric acid secretion in rats. *Scandinavian Journal of Gastroenterology*, 25, 853-858.