E.Ü. Su Ürünleri Dergisi 2002
E.U. Journal of Fisheries & Aquatic Sciences 2002
Cilt/Volume 19, Sayı/Issue (1-2): 157 – 162

ISSN 1300 - 1590
http://jfas.ege.edu.tr/

Regulatory Peptides in Gastroenteropancreatic Endocrine Cells of the Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792)

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Özet: ***Gökkuşağı alabalığı (*Oncorhynchus mykiss *Walbaum, 1792)'nın gastroenteropankreatik endokrin hücrelerindeki düzenleyici peptidler.*** Gökkuşağı alabalığının gastroenteropankreatik endokrin hücreleri, parafin bloklarda immunohistokimyasal tekniklerin peroksidaz-anti-peroksidaz ve avidin-biotin-peroksidaz kompleks yöntemleri kullanılarak araştırıldı. Gastrin- ve substance P-immunoreaktif hücrelere pylorik mide bölümünde rastlanıldı. Substance P-immunoreaktif hücreleri ayrıca pylorik seka ve intestinal mukozada tesbit edildi. Glukagon içeren hücreler sadece bağırsakların başlangıç kısımlarında ve seyrek olarak da pylorik seka da gözlendi. Gastroenteropankreatik endokrin hücrelerinde calbindin immunoreaktivitesi bulunamadı. Pankreasda ise glukagon-ve insülin-immunoreaktivitesi tesbit edildi.

Anahtar Kelimeler: Gökkuşağı alabalığı, immunohistokimyasal teknikler, endokrin hücreler, düzenleyici peptidler, mide, bağırsak, pankreas.

Abstract: Gastroenteropancreatic endocrine cells of rainbow trout have been investigated using the immunohistochemical techniques of peroxidase-anti-peroxidase and avidin-biotin-peroxidase complexes on parafin section. Gastrin- and substance P-immunoreactive cells were identified in the pyloric stomach region. Substance P-immunoreactive cells were also observed in intestinal mucosa and pyloric seca. Glucagon-like containing cells were restricted to the anterior intestine and rarely seen in pyloric seca. Calbindin-immunoreactivity was not find in gastroenteropancreatic endocrine cells. In pancreas, glucagon- and insulin-immunoreactivity were demonstrated.

Key Words: Rainbow trout, immunocytochemical techniques, endocrine cells, regulatory peptides, stomach, intestine, pancreas.

Introduction

The presence of endocrine cells in the gut of teleost fishes has been demonstrated histochemically (Fange, 1962; Read and Burnstock, 1968; Rombout, 1977), and the cells have been classified according to the ultrastructural characteristics of their secretory granules (Ling and Tan, 1975; Rombout, 1977; Noalillac-Depeyre and Gas, 1982).

On the other hand their hormone content has not been clearly established. Several immunocytochemical studies using antisera raised against mammalian hormones have been carried out (Larsson and Rehfeld, 1977; Langer *et al*., 1979; Reinecke *et al*., 1980; Van Norden *et al*., 1980; Yoshida *et al*., 1983; Rombout and Reinecke, 1984; Abad *et al*., 1987). They showed that the different endocrine cells types were related to the fish species studied and the source of the antisera used.

The aim of present work is to study, immunocytochemically regulatory

substances present in the gastroenteropancreatic endocrine cells of the trout using a panel of antisera. This study would be the first report in Turkey using immunocytochemical techniques in fishes.

Materials and Methods

15 specimens of *O. mykiss* measuring 20-25 cm, were used in this study. The fishes were killed by decapitation. Samples of stomach, pyloric seca, anterior and posterior intestine and pancreas were fixed in 4% neutral-buffered formaldehyde, for 24 hour. They were then dehydrated through graded ethanol and embedded in parafin. 7 mm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by using the peroxidase-antiperoxsidase (PAP) method or the peroxidase linked avidin-biotin complex (ABC) method. Blocking of endogenous peroxidase was carried out with 0.008 % hydrogen peroxidase (H_2O_2) in methanol for 5 minutes (Sternberger 1986). 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.

a) ABC technique; Sections were incubated for 16-20 hours at 4 ^{o}C in mouse anti-calbindin (Sigma), mouse anti-glucagon (Sigma) or mouse anti-insulin (Sigma). Antibodies were diluted to 1:200, 1:1500 and 1:1000 in PBS containing 0.25 % sodium azide and 2.5 % bovine serum albumin respectively. Sections were then incubated in biotinylated sheep anti-mouse IgG (Sigma), followed by streptavidin-biotinylated horseradish peroxidase complex (Sigma), both at dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation. Sections were then immersed in glucose oxidise-DAB-nickel ammonium sulphate (GDN) substrate (Shu *et al*., 1988) for 10 minutes, washed in distilled water and counterstained with eosin. Sections were examined with light microscope (Leitz Dialux 20). Photographs were taken with Kodak film, ASA 50. b) PAP technique. Sections were incubated for 16-20 hours at 4^{o}C in rabbit anti-gastrin (Sigma) or rabbit anti-substance P (Sigma). Antibodies were diluted to 1:10.000 and 1:20.000 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in goat anti-rabbit IgG (DAKO), followed by rabbit peroxidase anti-peroxidasecomplex (Sigma), both at dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation and finally immersed in GDN substrate (Shu *et al*., 1988) for 10 minutes. After washing in distilled water and counterstaining with eosin, sections were dehydrated and coverslips mounted with DPX. Sections were examined with light microscope and photographs were taken.

Results

The cardia part of stomach mucosa of rainbow trout has two regions: an anterior part (corpus or cardiac region) containing tubular glands that open in the bottom of the mucosecretory crypts (Fig. 1a) and a posterior part (pyloris or pyloric region) that present no gland and in this case, with a mucosecretory epithelium infolded forming crypts longer than those found in the body (Fig. 1b).

Gastrin-immunoreactive cells have only been detected in the pyloric region of cardiac mucosa (Fig. 2) where they are the most abundant cell population. No immunoreactivity was observed in the intestine.

Glucagon-immunoreactive cells were not identified in the stomach. Glucagon containing cells were only appeared in the anterior intestine and pancreas (Fig. 3 and 4).

Substance P-immunoreactive cells were found in the pyloric stomach region (Fig. 5) and in the basal part of the folds throughout the intestine being more numerous in the pyloric seca and first part of intestine than in the posterior intestine.

No calbindin- and insulin-immunoractive cells were identified. Insulin containing cells were only seen in the pancreas (Fig. 6).

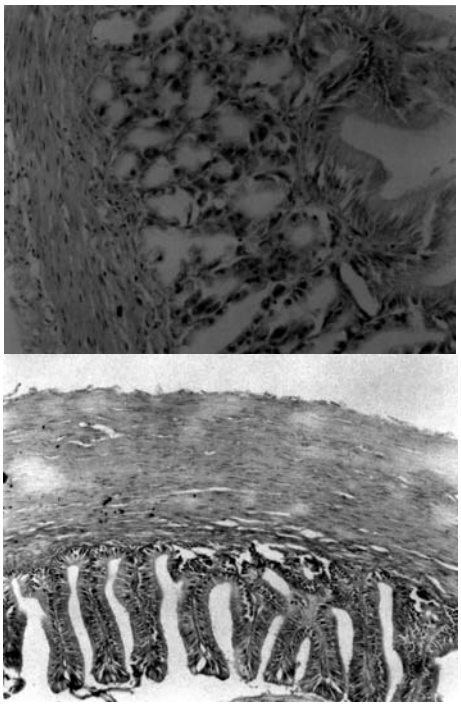

Figure 1. Gastric mucosa of rainbow trout. In the body (a) tubular glands that open in the bottom of crypts are present, while the pyloric mucosa (b) is devoided of glands. Hematoxylin. X200**.**

Discussion

Substance P is a peptide of the tachykinin family that have an aminoacid sequence very converged through phylogeny and shares the C-terminal end with bombesin (Cimini *et al*., 1989). By now, two tachykinins have been isolated and sequenced from the elasmobranchian fish, *Scyliorhinus canicula*: scyliorhinin I and II (Conlon *et al*., 1986). Their physiological effects are very similar to those caused by substance P (Jensen *et al*. 1987) mainly release of serotonin from enteric neurons (Holmgren *et al*., 1985). Our result show a very numerous population of substance P containing cells in trout stomach confirming previous data (Holmgren *et al*., 1982).

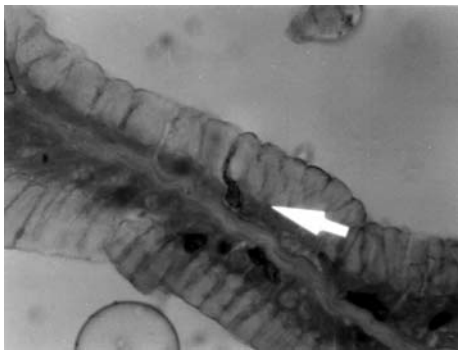

Figure 2. Gastrin-immunoreactive cells in the pyloric region of cardiac mucosa (arrow). 400x.

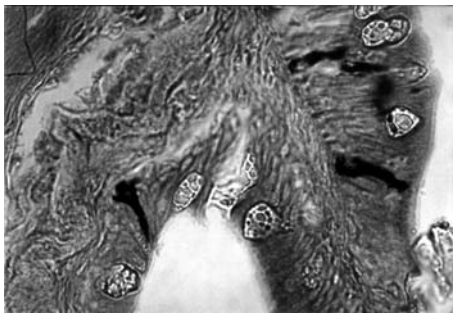

Figure 3. Glucagon-immunoreactive cells in the anterior intestine. 400x.

Pancreatic and gut glucagon are members of secretin family their

spectrums of biologic action are relatively distinct (Mutt and Jorpes, 1971). Pancreatic glucagon is a metabolic hormone inducing glycogeneolysis, lipolysis, gluconeogenenesis and ketogenesis. Pancreatic glucagon also inhibits gut motility, intestinal absorption, gastrointestinal motility, pancreatic secretion and lower esophagial sphincter tone (Walsh, 1987). Enteroglucagon likely function as trophic hormone stimulating growth of the small intestine (Buchan *et al*., 1985).

Glucagon immunoreactivity was found in pancreatic islets and in the anterior intestine. Immunoreactive cells were not observed in the stomach. *Mugil saliens* as known that teleost where glucagon positive cells have been detected in the upper part of the gastric glands using other antisera (Elbal *et al*. 1988). However this data have also been shown that glucagon positive cells are present in anterior intestine of rainbow tout.

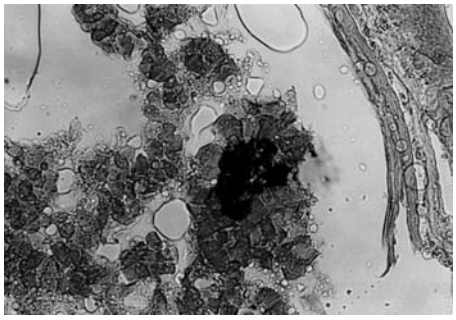

Figure 4. Glucagon-immunoreactive cells in the pancreas. 400x.

The general distribution pattern for glucagon immunoreactive cells is different for teleost and cartilaginous fishes, in teleosts they appear in the intestine (Abad *et al*., 1987, Beorlegui *et al*., 1992), while cartilaginous fishes they are located in the gastric mucosa (Tagliaefierro *et al*., 1989).

Figure 5. Substance P-immunoreactive cells in the pyloric stomach (arrow). 400x.

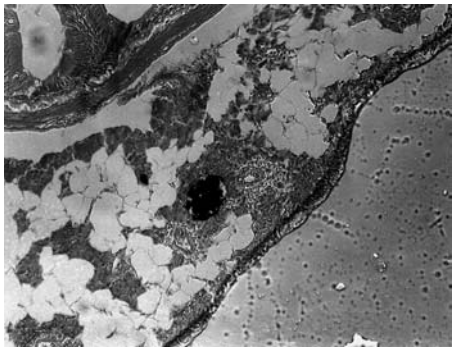

Figure 6. Insulin containing cells in the pancreas. 200x.

Gastrin immunoreactive cells appear exclusively in the pyloric region of rainbow trout stomach. This limitation has been described in *Gadus morhua* (Larsson and Rehfeld 1978, Jonsson *et al*. 1987) while in *Mugil saliens* this cell type was also found in the cephalic region among the gastric glands (Elbal *et al*. 1988). In an earlier work in trout cholecystakinin/gastrin immunoreactivity was not detected in stomach, but it was found in the endocrine cells of intestinal mucosa (Holmgren *et al*., 1982). Cholecystakinin, caerulin and the different gastrins together form a family of regulatory peptides that share same terminal pentapeptide. This small region is responsible for the biological activities

while the rest of molecule selects the target cells and modulate activity (Larsson and Rehfeld, 1977).

In the latest study by Barrenechea *et al*. (1994) three of five antibodies raised against members of this family which gave positive results. In all cases the antigen included the terminal pentapeptide, and when another region of the molecule was tested no staining was obtained. These result suggest that teleost molecule is similar to its mammalian counterpart in the carboxyl-terminal region but not in the other end.

Although detection of insulin immunoreactivity in the pancreatic islet of many teleost, this is the first time that immunoreactivity for insulin is described in pancreatic islet cells of *O. mykiss*.

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