

IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLECYSTOKININ  
AND HISTAMINE IN GASTROINTESTINAL TRACT IN FLOWER FISH  
(*Pseudophoxinus antalyae*)

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**Abstract:** In this study, our aim was to determine the distribution of specific immunoreactivities against the antisera Cholecystokinin (CCK) and Histamine in some regions of the digestive tract of the flower fish (*Pseudophoxinus antalyae*). The samples were taken from the enlarged area after oesophagus and anterior, middle and posterior part of intestine, and the Avidin-Biotin-Immunoperoxidase method was applied. Histamine and Cholecystokinin immunoreactive cells were found to be more frequent in enlarged region after oesophagus than in gut. There immunoreactive cells were distributed among surface epithelium, epithelium between surface crypt, and crypts proper. Despite determination of Histamine immunoreactivity in enlarged region after oesophagus, connective tissue of intestine and ganglion cells, CCK immunoreactivity was not be detected in the same regions and cells.

**Key words:** *Pseudophoxinus antalyae*, gastrointestinal tract, endocrine cells, immunohistochemistry

KOLESİSTOKİNİN VE HİSTAMİN' NİN ÇİÇEK BALIĞI (*Pseudophoxinus antalyae*) SİNDİRİM KANALINDA İMMUNOHİSTOKİMYASAL LOKALİZASYONU

**Özet:** Bu çalışmada, Cholecystokinin (Kolesistokinin=CCK) ve Histamine (Histamine) antiserumlarına karşı spesifik immunreaktivitelerinin Çiçek Balığı (*Pseudophoxinus antalyae*) sindirim kanalının bazı bölümlerindeki dağılım ve lokalizasyonlarının belirlenmesi amaçlandı. Sindirim kanalının özofagustan sonraki genişlemiş bölgesi (mide) ile ilk, orta ve son bağırsaktan alınan materyale Avidin-Biotin-İmmunoperoksidaz yöntemi uygulandı. Histamin ve Kolesistokinin immunreaktif hücrelerinin özofagustan sonraki genişlemiş bölgede (mide) bağırsaklara oranla daha fazla buldukları saptandı. Bu immunreaktif hücrelerin sindirim kanalının yüzey, yüzey-kript arası ve kript epiteline yer aldığı belirlendi. Histamin immunreaktivitesinin özofagustan sonraki genişlemiş bölgenin ve bağırsakların bağ dokusu ve gangliyon hücrelerinde nadir olarak saptanmasına karşın CCK immunreaktivitesine belirtilen bölgelerde ve aynı hücrelerde rastlanmadı.

**Anahtar kelimeler:** *Pseudophoxinus antalyae*, gastrointestinal kanal, endokrin hücre, immunohistokimya

## INTRODUCTION

Secretions of many endocrine cells coact in digestion process in fishes. The secretions, located in the gastrointestinal (GI) tract, are the chemicals regulating tracts structure and functions. These chemicals in GI tract, accepted as the largest endocrine organ, are mainly secreted by the endocrine cells. Classically, a chemical secreted by a particular affecting organs through blood is called a hormon. Recently these chemicals have been explained to be short chains of peptides, and aminoacide compositions have also been determined. In studies on fish gastrointestinal tracts, for the last 15 years more than 45 GI peptide have been determined (REIFEL 1988, SCHEUERMANN et al. 1991, HIMICK & PETER 1994, D' ESTE et al. 1995, KILIAAN et al. 1997, GIROLAMO et al. 1999, LUCINI et al. 1999, KIM et al. 2000, DOMENEGHINI et al. 2000, PAN et al. 2000a, YOUSON et al. 2001, BURRIN et al. 2003).

GI hormones and neuropeptides are collectively called as peptide families due to structural and functional resemblances. There are three general families in GI peptides, namely Gastrin-Cholecystokinin, Secretin, Pancreatic polypeptide. Furthermore, there is another group including somatostatin, bombesin, neurotensin, and substance-P acting in control of gut functions. As newer peptides are discovered with new techniques, the list is to be lengthened (ABAD et al. 1987, PAN et al. 2000b, YOUSON et al. 2001).

One of the peptides studied here namely cholecystokinin (CCK) has three different receptors; CCK-A, CCK-B and gastrin. CCK-A receptors are known from cells in pancreas, bile duct, colon and central nervous system (CNS). CCK-B receptors are localised in CNS neurons, while gastrin-type receptors are found in stomach and other regions of the gut (BOUERLEGUI et al. 1992).

CCK is similar to pancreozamin. Carboxy-terminal tetrapeptide amide is essential for biological activity and this part is identical with gastrin (JÖNSSON et al. 1987, BARRENECHEA et al. 1994, BOUERLEGUI et al. 1992). CCK has different variants such as CCK-39, CCK-33, CCK-25, CCK-22, CCK-18, CCK-8, CCK-7, CCK-5. In human being the main form is CCK-58. Later, CCK-33 & CCK-8 have been found. In intestines mostly CCK-4 is found. CCK-4 strongly stimulates insulin and glucagon secretion of pancreas, having no considerable effect on outer secretions, whereas CCK-8 acts exactly in the adverse way (TELATAR & ŞİMŞEK 1993, HIMICK & PETER 1994).

CCK secretuar cells (I cells) in mammals are more frequent in duodenum and jejunum, with ileum becoming scarcer. In fish, CCK is more frequent in cardial and pylorus regions of stomach and proximal part of the intestines. Most effective agents involving in the CCK secretions are lipid and protein content in the food (MAAKE et al. 2001, DEZFULI et al. 2003, RONNESTAD et al. 2003).

Histamine, the other peptide to be studied by means of localization in GI tract mucosa is a decarboxylated form of histidine and a strong vasodilator in animal tissues. H1, H2 and H3 are the receptors to histamine. H1 receptor is localised in gut, bronch and smooth muscle of blood-vessel, H2 receptor is found in stomach parietal cells and central nervous system, while H3 receptor is localised in brain and peripheral nervous system (LEHNINGER et al. 1993, YANAI et al. 1998, HALPERT et al. 2002).

Basically Histamine is formed by ECL cells. Functionally, important cells within endocrine cells to refer oxyntic mucosa. Histamine secreted by those cells stimulates acide secretion in parietal cells. It also regulates gastrointestinal acidity, thus prevent ing gastric and duodenal ulcer. This peptide in turn increases intestinal smooth muscle contractions and help digestion (BHAGAVAN 1992, MATHEWS & VAN HOLDE 1996).

## MATERIAL AND METHODS

Twenty five adult Flower fish (*Pseudophoxinus antalyae*) were used for this study (length 15 – 20 cm, weight 40 – 50 g). Samples were taken from the enlarged area after oesophagus and anterior, middle and posterior intestine. All samples were fixed for 12 h in Bouin' s fluid. After dehydration by passing tissues through a series of alcohol solutions, the samples were vacuum-embedded in paraffin, and sagittally sectioned at 6-7µm. One section of each specific tissue was mounted on an albuminized slide and dried overnight at 56 ° C. After dewaxing with xylol and subsequent rehydration by a series of alcohol solutions, sections were rinsed in 0.01M phosphate buffered saline (PBS, pH 7.2), and used for immunohistochemistry.

The immunohistochemical reactions were carried out using avidin-biotin-peroxidase complex (ABC), an immunoperoxidase method modified by HSU et al (1981). Prepared slides were repeatedly rinsed in hydrogen peroxidase solution (5 ml 30% H<sub>2</sub>O<sub>2</sub> Per 300ml methanol) and in tris buffer (0.05 M Tris-HCl pH 7.6, 0.9% NaCl w/w) containing 5% normal sheep serum (S-3772 Sigma). Finally the sections were treated with normal sheep serum. The sections were individually incubated at 4 ° C for 18-24 h in one of two antisera (Table 1), and treated with biotin conjugated goat anti-rabbit IgG (B-8895 Sigma-1/500), and avidin peroxidase complex (A-3151 Sigma-1/2500) for 30 min respectively. After incubating with Hanker-Yates reagent (7.5 mg Hanker-Yates Per 5 ml Tris buffer containing 80 µl H<sub>2</sub>O<sub>2</sub> ), the sections were repeatly rinsed in tris buffer, alcohol, xylol and mounted in entellan.

**Table 1.** Antisera used to test immunohistochemical reactions of *Pseudophoxinus antalyae* digestive system tissues

Antisera used	Source and code no	Working dilution
Rabbit Anti-Cholecystokinin	C-2581 (Sigma)	1:500
Rabbit Anti-Histamine	H-7403 (Sigma)	1:500

## RESULTS

All sections obtained from the material were formed by tunica mucosa, tunica muscularis (circular inwards, longitudinal outwards) and tunica serosa layers. Neither lamina propria-submucosa separation nor any glands couldn' t be observed in tunica mucosa. In enlarged region after oesophagus mucosal projections (villi) were detected

to be rather decreasing in length towards posterior intestine. Except for length of the villi, no mucosal differences between regions were observed. Widely distributed in all portions studied, Goblet cells were most densely distributed in posterior intestine.

Despite the supplement by simple columnar epithelial cells, Goblet cells were determined to constitute a much larger reserve at digestive tract mucosa.

Immunohistochemical studies showed that histamine immunoreactive cells were more frequent in enlarged region after oesophagus than in intestines and less frequent towards the distal of the intestines.

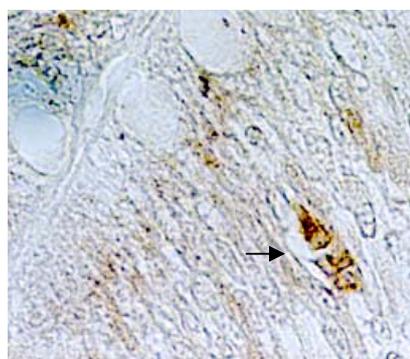
In enlarged region after oesophagus, histamine immunoreactive (HIR) cells were found frequently, especially between surface-crypt, but rarely in surface epithelia. HIR cells were also found less in crypt as compared to surface epithelia. Also the cells were rarely observed in connective tissues and ganglion cells.

In anterior intestine surface epithelia HIR cells density was higher in comparison to epithelia between surface-crypt. In crypts, these cells turned to be of lesser amount than in epithelia between surface-crypt. In connective tissue, HIR cells were seldomly observed. In middle gut enlarged region after oesophagus, HIR cells are found most abundantly and followed by surface epithelia and crypts (Figure 1, 2). As with anterior intestine low frequency, HIR cells were observed, also with weak ganglion cells. HIR cells were observed in posterior gut in low number, denser in surface epithelia, but quite rare in epithelia between surface-crypt and crypts. No immunoreactivity in connective tissue and ganglion cells were observed.

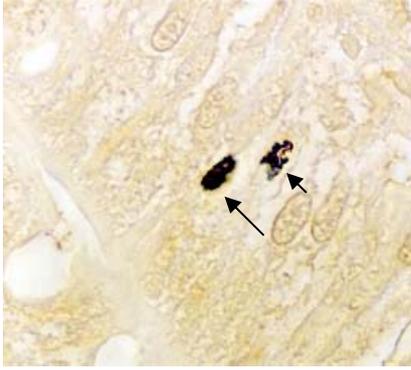
Cholecystokinin immunoreactive (CCK IR) cells, like HIR cells, decreased gradually from enlarged region after oesophagus to intestines. In intestines, density lowered towards posterior end. In enlarged region after oesophagus and middle gut, CCK IR cells were denser in epithelia between surface-crypt, followed by surface epithelia and crypts (Figure 3, 4). In anterior gut, CCK IR cells were found more frequently in epithelia between surface-crypt, but there was no significant differences between surface epithelia and crypts. On the contrary, in posterior gut epithelia between surface-crypt CCK IR cell was rarely observed. High density was observed in surface epithelia followed by crypts. No CCK IR could be observed in connective tissue and ganglion cells.



**Figure 1.** Histamine IR cell, middle intestine, 1500 X



**Figure 2.** Histamine IR cell, middle intestine, 1500 X



**Figure 3.** CCK IR cells, enlarged region after oesophagus, 1500 X



**Figure 4.** CCK IR cell, middle intestine, 600 X

## DISCUSSION

IR cells, the antisera used in the study, was generally observed in surface epithelia, epithelia between surface-crypt and crypt, while weak or no IR cells were seen in connective tissues and ganglion cells. Endocrine cells in gastrointestinal tract are known to have two forms, open and closed. In open type cells apex directly opens to lumens, but in closed type cells it is covered by lumen epithelia (TELATAR & ŞİMŞEK 1993, LIU et al. 2003). Determined CCK and Histamine IR cells in this study were completely in the closed form.

The CCK IR cells were found in epithelia between surface-crypt in enlarged region after oesophagus of *Pseudophoxinus antalyae*, similarly localised in stomachs of *Salmo gairdneri* (VIGNA 1983), *Gadus morhua* (JÖNSSON et al. 1985) and *Oncorhynchus mykiss* (BARRENECHEA et al. 1994). CCK, which stimulates pancreatic enzyme secretions, was determined densely in cardia part of *Salmo salar* (EINARSSON & DAVIES 1996) stomach. RAJJO et al. (1988) argued that no IR cells were present in *Amia calva* and *Lepomis macrochirus*.

CCK IR cells in intestine were found densely throughout the tract, as in *Sparus auratus* (ABAD et al. 1987), *Amia calva* and *Lepomis macrochirus* (RAJJO et al. 1988), *Scyliorhinus stellaris* (CIMINI et al. 1989), *Oncorhynchus mykiss* (BARRENECHEA et al. 1992), *Anguilla anguilla* (DOMENEGHINI et al. 2000), differing from *Carassius carassius* (HIMICK & PETER 1994), in which these are restricted to anterior part of intestine.

Some secretions in endocrine cells are assumed to be acting in immunity response to parasitory infections. CCK IR cells in parasite-infected and non-infected specimens of *Salmo trutta* determined differently by DEZFULI et al. (2000, 2002, 2003), were more densely found in especially non-infected fishes. Our observations were similar in that CCK IR was higher pyloric caeca and anterior gut. CCK IR cells numerically increase in relation with the developmental stages. A study in *Hippoglossus hippoglossus* larvae, CCK IR cells observed in 7 th day after the start of external feeding numerically increased in proportion to growth and size of the intestine (RONNESTAD et al. 2003).

In spite of presence of CCK IR cells in *Scyliorhinus stellaris* (CIMINI et al. 1989), *Carassius carassius* (HIMICK & PETER 1994), *Anguilla anguilla* (DOMENEGHINI et al. 2000) ganglion cells, no such IR was observed in this study.

Histamine is peptide functioning in contraction of smooth muscles and stimulation of acide secretion in stomach (KÖSE & HALL 2000). Similar to results of FAIRGRIEVE et al. (1994), *Oncorhynchus mykiss*, Histamine IR was most observed in Lamina epithelialis of enlarged region after oesophagus. Similar to a study on *Gadus morhua* (Bomgren et al. 1998), this IR cells were also observed in connective tissue.

Like *Oncorhynchus mykiss* (ELLIS 1985), *Salmo salar* (REITE 1997) *Dicentrarchus labrax* (PALEOLOGOS et al. 2004), intestinal Histamine IR cells were found densely. In these species parasite-infected specimens showed marked high Histamine IR. Consequently, CCK & Histamine IR in *Pseudophoxinus antalyae* were found through the tract, most frequently in enlarged region after oesophagus and gradually lowering towards posterior gut.

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