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Research Article

# Immunohistochemical Identification of Peptide Hormones in the Endocrine Cells of the Gastrointestinal Tract of the *Oreochromis niloticus*

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**Abstract:** The endocrine cells of gastrointestinal tract of the *Oreochromis niloticus* were investigated using immunohistochemical techniques. 8 antisera were tested and 3 immunoreactivities were detected: Serotonin, glucagon and somatostatin immunoreactive cells. Substance P, insulin, gastrin, calcitonin gene related peptide (CGRP) and calbindin immunoreactive cells were not found.

**Key Words:** *Oreochromis niloticus*, immunohistochemical techniques, endocrine cells, gastrointestinal tract

## *Oreochromis niloticus*'un Gastrointestinal Kanalındaki Endokrin Hücrelerinin Peptid Hormonlarının İmmunohistokimyasal Olarak Tespit Edilmesi

**Özet:** *Oreochromis niloticus*'un gastrointestinal kanalındaki endokrin hücreler immunohistokimyasal teknikler kullanılarak araştırıldı. 8 antiserum kullanıldı ve 3 immunoreaktivite tesbit edildi: Serotonin, glukagon ve somatostatin immünoreaktif hücreler. Substans P, insülin, gastrin, calsitonin gene related peptide (CGRP) ve calbindin immünoreaktif hücreler bulunamadı.

**Anahtar Sözcükler:** *Oreochromis niloticus*, immünohistokimyasal teknikler, endokrin hücreler, gastrointestinal kanal

## Introduction

The existence of endocrine cells has been histochemically demonstrated in the digestive tract of some species of teleost (1-3). Using electron microscopic techniques, classification has been based mainly on ultrastructural characteristics of the secretory granules (3-5) without the hormonal content having been clearly stated. Some cell types have, however, been identified with immunocytochemical techniques using several antisera against mammalian hormones which cross react with endocrine cells of the digestive tract of teleost (6-11).

Gastrointestinal endocrine cells have previously been observed in different species of tilapia (*Oreochromis mosambicus*) (7). In the present paper the gastrointestinal tract of teleost, Nile tilapia (*Oreochromis niloticus*), has been studied using peroxidase-antiperoxidase (PAP) and the peroxidase linked avidin-biotin complex (ABC) techniques. The results obtained have been compared with those indicated by other authors studying different fish species using various antisera and amplify our knowledge of the endocrine cells of digestive tract of *Oreochromis niloticus* whose gastrointestinal endocrine cells have been studied recently (11).

## Materials and Methods

15 specimens of *Oreochromis niloticus* measuring 20-25 cm were used in this study. The fishes were killed by decapitation. Samples of stomach, anterior and posterior intestine were fixed in 4% neutral-buffered formaldehyde for 24 h. They were then dehydrated

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through graded ethanol and embedded in paraffin. 7 µm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by using the peroxidase-antiperoxsidase (PAP) method and the peroxidase linked avidin-biotin complex (ABC) methods. Blocking of endogenous peroxidase was carried out with 0.08% hydrogen peroxide ($H_2O_2$) in methanol for 5 minutes (12). In order to 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 h at 4 °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 a dilution of 1:50 in PBS, for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation. Sections were then immersed in glucose oxidase-DAB-nickel ammonium sulphate (GDN) substrate (13) for 10 minutes, washed in distilled water and counterstained with eosin.

**b. PAP technique.** Sections were incubated for 16-20 h at 4 °C in rabbit anti-gastrin (Sigma), rabbit anti-substance P (Sigma), rabbit anti-somatostatin (Sigma), rabbit anti-serotonin (Sigma) or rabbit anti-calcitonin gene related peptide (Sigma). Antibodies were diluted to 1:200, 1:200, 1:200, 1:200 and 1:100 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-peroxidase complex (Sigma), both at dilution of 1:50 in PBS, for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation and finally immersed in GDN substrate (13) for 10 min. After washing in distilled water and counterstaining with eosin, sections were dehydrated and coverslips mounted with DPX.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (14), including the replacement of specific antiserum pre-incubated with its corresponding antigen.

Sections were examined with light microscope and photographs were taken.

## Results

The positive reaction of serotonin immunoreactive endocrine cells were only found in the stomach by using PAP technique (Figure 1). No immunoreactivity was observed in the intestine by using PAP technique.

Glucagon-immunoreactive cells were not identified in the stomach. Glucagon containing cells were observed in the anterior and posterior intestine by using ABC technique (Figure 2). Somatostatin containing cells were only seen in the posterior intestine by using PAP technique (Figure 3).

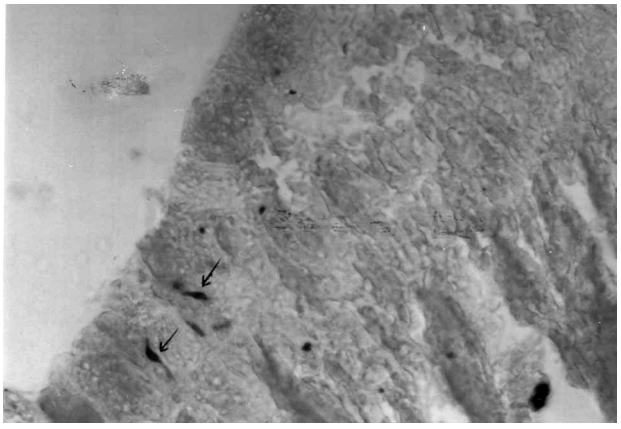

Figure 1. Serotonin-immunoreactive cells in the stomach of *Oreochromis niloticus* (arrows) by using PAP technique. x400

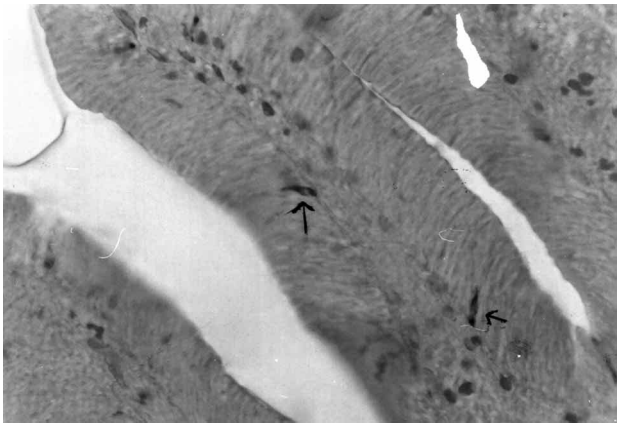

Figure 2. Glucagon-immunoreactive cells in the anterior intestine of *Oreochromis niloticus* (arrows) by using ABC technique. x400

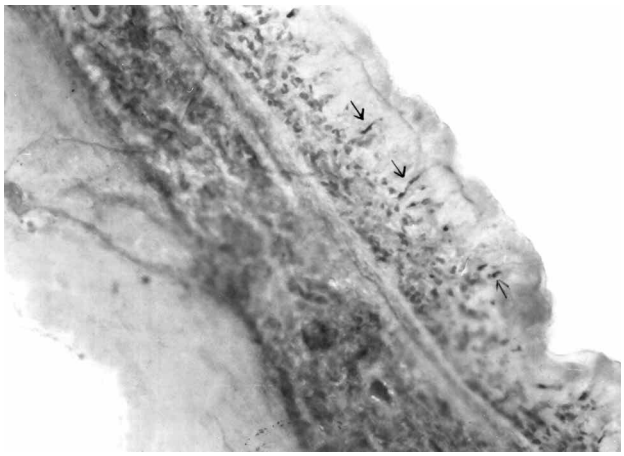

Figure 3. Somatostatin-immunoreactive cells in the posterior intestine of *Oreochromis niloticus* (arrows) by using PAP technique. x200

No calbindin, insulin, substance P, calcitonin gene-related peptide or gastrin immunoreactive cells were identified.

## Discussion

The occurrence of serotonin immunoreactivity has been described for gastrointestinal endocrine cells and nerve fibres of several teleosts. In some species, serotonin immunoreactive endocrine cells seem to be restricted to the stomach (15), which corresponds to their absence in stomachless fish (16). The present study also showed serotonin immunoreactivity only in the endocrine cells of tilapia stomach. Immunoreactivity was not observed in the intestine. Our result also confirms previous observations in the same species (11). The excitatory effect of serotonin on gut motility has been well established in *Gadus morhua* (17) and a mechanism involving its action on cholinergic neurones has been proposed (18).

In the present study, glucagon immunoreactive cells were only found in the intestine of *Oreochromis niloticus*. The general distribution pattern for glucagon immunoreactive cells is different for teleosts, they appear in the intestine (6,15), while in cartilaginous fishes they are located in the gastric mucosa (19). *Mugil saliens* is the only teleost in which glucagon positive cells have been detected in the upper part of the gastric gland (20) using other antisera against pancreatic glucagon.

Several authors (15,21-23) have described somatostatin immunoreactive cells exclusively in the stomach of teleosts. However, in the present study, somatostatin immunoreactive cells are found only in the intestine. This discrepancy is likely to be due to species differences. As somatostatin is known to be the paracrine inhibiting factor in gastrin release (24), its absence in the stomach also supports the non-existence of gastrin cells in the stomach of *Oreochromis niloticus*. These results confirm previous data (11).

The absence of positive staining for some of these hormones (insulin, substance P, CGRP and calbindin) does not necessarily indicate their absence, but possibly that the fish hormones do not cross react with antibodies of their mammalian counterparts.

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