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Characterization of cholecystokinin-producing cells and mucus-secreting goblet cells in the blacktip grouper, *Epinephelus fasciatus*

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ABSTRACT

The characteristics and distributions of cholecystokinin (CCK)-producing cells and mucus-secreting goblet cells were investigated in the digestive tract of the blacktip grouper (*Epinephelus fasciatus*). CCK-producing cells were scattered throughout the digestive tract. The highest frequency of CCK-producing cells was observed in the anterior intestine portion and pyloric ceca, with a very small number of cells distributed as far as the rectum. Mucus-secreting goblet cells were found to differ remarkably in their regional distributions and relative frequencies. High frequencies of mucus-secreting goblet cells were found in the digestive tract, mainly in the anterior intestine portion and pyloric ceca, but not the esophagus; the frequency decreased slightly toward the rectum. Our result suggests that food digested by gastric acid in the stomach moves on the anterior (including the pyloric ceca) and mid intestine portion, thereby ensuring effective stimulation of the CCK-producing cells. In addition, the distribution pattern of the CCK-producing cells closely resembled that of mucus-secreting goblet cells. In *E. fasciatus*, CCK-producing cells and mucus-secreting goblet cells seem to be well adapted to promoting optimal control of the digestive process.

1. Introduction

The digestive system comprises the largest endocrine organ in the vertebrate body (Holst et al., 1996). The wide diversity and amount of secreted hormones and signaling molecules secreted by numerous types of endocrine cells rapidly and reversibly alter the characteristics of the digestive system and other organ systems (Buddington and Krogdahl, 2004). Energy and nutrients are made available by a sequential process. Complex polymers are hydrolyzed into small molecules that are absorbed across the apical membrane of epithelial cells and transferred into the systemic circulation (Buddington et al., 1987; Collie and Stevens, 1985; Collie and Ferraris, 1995).

Digestive system functions are regulated by several digestive hormones and substances. Among these materials, cholecystokinin (CCK) and mucus-secreting goblet cells play important physiological roles in the intestines of vertebrates including fish. CCK, one of the gastrointestinal hormones, is the most abundant neurotransmitter peptide in the brain and the intestines. CCK plays a crucial role in the regulation of pancreatic enzymes secretion (Jensen and Holmgren, 1985; Einarsson et al., 1997; Johnsen, 1998), gallbladder contraction (Liddle, 1997; Aldman et al., 1992; Einarsson et al., 1997), amino acid and sugar transport regulation (Verspohl and Ammon, 1987), and intestinal peristalsis regulation (Olsson et al., 1999). The mucus-secreting goblet cells in the fish digestive tract produce a lubricant for the mucosal surface to protect it against damage induced by physical or chemical substances as well as digestive enzymes (Allen et al., 1986). The mucus secreted by the goblet cells in vertebrates including fish plays important roles in the absorption of easily digestible substances (Osman and Caceci, 1991; Domeneghini et al., 2005).

The blacktip grouper, *Epinephelus fasciatus* is one of the most commercially important marine aquaculture species in Korea. As mentioned above, CCK and mucus-secreting goblet cells are the main regulators of the digestive processes in fish. To identify the characteristics of CCK and mucus-secreting goblet cells in *E. fasciatus*, we investigated the distribution and characteristics of CCK-producing cells and mucus-secreting goblet cells in order to

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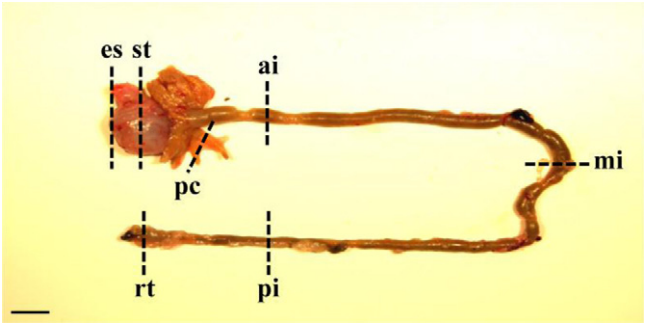

Fig. 1. Anatomical feature of *Epinephelus fasciatus* digestive tract divided into seven segments. Abbreviations: ai, anterior intestine portion; es, esophagus; mi, mid intestine portion; pc, pyloric ceca; pi, posterior intestine portion; rt, rectum; st, stomach. Scale bar: 1 cm.

provide a basis for understanding the digestive physiology and biology of *E. fasciatus*.

2. Materials and methods

2.1. Specimens

E. fasciatus (<3 years old) for the present study were reared in an indoor tank at the Marine and Environmental Research Institute of Jeju National University (Jeju, South Korea). Fish were kept in natural photoperiod and natural water temperature conditions. Fish were fed commercial pellets (moisture, 10.00%; crude protein, 52.00%; crude fat, 8.00%, crude fiber, 1.30%; ash, 11.90%; and phosphorous, 1.70%; Le Gouessant, France) once a day.

For distribution and characteristic studies of CCK-producing cells and mucus-secreting goblet cells, ten specimens of *E. fasciatus* (average body weight of 280 ± 8.7 g) were used in this study. Tissue samples were collected after the fish were anesthetized with 2-phenoxyethanol (Sigma, MO, USA). The entire digestive tract, from the esophagus to the rectum, was removed from the body cavity (Fig. 1).

2.2. Immunohistochemistry

CCK-producing cells were visualized using the avidin–biotin complex (ABC) method (Hsu et al., 1981). Microscope slides were coated with poly-L-lysine to promote tissue section adherence.

Sampled digestive tracts were divided into seven parts (the esophagus, stomach, anterior intestine portion, mid intestine portion, posterior intestine portion, rectum, and pyloric ceca), fixed in Bouin's solution, dehydrated in a graded series of ethanol, embedded in paraffin, and then cut into 6-μm cross sections (Fig. 1). After the sections were deparaffinized and rehydrated, they were incubated in 0.5 mM periodic acid to block the endogenous peroxidase. After 3 rinses in 0.1 M phosphate-buffered saline (PBS; pH 7.2), nonspecific binding was blocked with 10% normal goat serum in PBS for 15 min. The solution was blotted off from the slides, primary CCK-8 antiserum (1:1000, Sigma, Israel) was added, and the slides were incubated for 26 h at 4 °C in a moist chamber. After 3 rinses in PBS, the sections were incubated for 50 min at room temperature (around 20 °C) in anti-rabbit goat serum (IgG; Vector, USA) diluted to 1:200 with PBS. After 3 more rinses in PBS, the sections were incubated for 1 h at room temperature (around 20 °C) with streptavidin-labeled peroxidase diluted to 1:100. After 3 rinses in PBS, the DAB substrate system was added for the peroxidase reactions. All samples were prepared on a clean bench and incubated in a moist chamber. After immunostaining, the sections were mounted in Canada balsam (Junsei, Japan). CCK-producing cells from different regions of the digestive tract were observed and counted using light microscope (HBO 50; Carl Zeiss) with Image scope 2.3 (Image Line, Inc.) software.

Table 1
Numbers of CCK-producing cells and goblet cells in different digestive tract regions of *Epinephelus fasciatus*.

	Numbers/tissue section	
	CCK-producing cells (mean ± S.E.)	Goblet cells (mean ± S.E.)
Esophagus	ND	2078 ± 105
Stomach	ND	ND
Anterior intestine portion	12 ± 1	1237 ± 86
Mid intestine portion	8 ± 1	1056 ± 91
Posterior intestine portion	5 ± 2	859 ± 67
Rectum	3 ± 1	719 ± 78
Pyloric ceca	12 ± 2	214 ± 24

"ND" indicates not detected.

2.3. Histochemistry

Tissue samples from seven regions of the digestive tract (the esophagus, stomach, anterior intestine portion, mid intestine portion, posterior intestine portion, rectum, and pyloric ceca) were fixed in Bouin's solution, dehydrated in a graded series of ethanol, embedded in paraffin, and then cut into 5-μm cross-sections (Fig. 1). The slides were stained with Alcian blue (AB) at pH 2.5 and periodic acid-Schiff (PAS) for the observation of mucus-secreting goblet cells.

Microscopy of the mucus-secreting goblet cells was carried out using a light microscope (Carl Zeiss, HBO 50) with Image scope 2.3 (Image Line, Inc.) software. The characteristics and the number of mucus-secreting goblet cells from different regions of the digestive tract were noted.

3. Results

3.1. Characteristics of CCK-producing cells

CCK-producing cells of *E. fasciatus* were not detected in the esophagus or stomach but were found at varying frequencies in the anterior intestine and extended to the rectum. The numbers of CCK-producing cells were recorded from the anterior intestine portion (12 ± 1), mid intestine portion (8 ± 1), posterior intestine portion (5 ± 2), rectum (5 ± 1), and pyloric ceca (12 ± 2) (Table 1). Thus, the highest frequency was observed in the anterior intestine portion and pyloric ceca (Table 1). The CCK-producing cells were typical endocrine-like cells, with a characteristic elongated spindle shape with a narrow apex pointing toward the intestinal lumen (Fig. 2). Spindle-shaped CCK-producing cells were dispersed among the epithelial cells of the mucosal folds of the intestine and pyloric ceca (Fig. 2).

3.2. Characteristics of mucus-secreting goblet cells

Mucus-secreting goblet cells of *E. fasciatus* were not detected in the stomach. They were, however, found with varying frequencies in the esophagus and extended to the rectum. The frequencies of the mucus-secreting goblet cells were recorded from the esophagus (2078 ± 105), anterior intestine portion (1237 ± 86), mid intestine portion (1056 ± 91), posterior intestine portion (859 ± 67), rectum (719 ± 78), and pyloric ceca (214 ± 24) (Table 1). Mucus-secreting goblet cells in the esophagus were very densely distributed within the epithelium of the mucosal folds and were mainly large and oval in shape (Fig. 3A). From the anterior intestine portion to the posterior intestine portion, the cells were mainly spindle shape and were distributed throughout the mucosal folds. In the posterior