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Comparative Biochemistry and Physiology, Part A 146 (2007) 124–130
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Effects of exogenous cholecystokinin and gastrin on the secretion of trypsin and chymotrypsin from yellowtail (*Seriola quinqueradiata*) isolated pyloric caeca

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Received 27 October 2005; received in revised form 19 September 2006; accepted 25 September 2006
Available online 4 October 2006

Abstract

The humoral control of secretion of the proteolytic enzymes trypsin and chymotrypsin was studied in yellowtail (*Seriola quinqueradiata*). *In vitro* trials were performed to investigate the effects of cholecystokinin (CCK) and two commercially available gastrin peptides. Isolated preparations of pyloric caeca/pancreas release trypsin and chymotrypsin when incubated with cholecystokinin (CCK) at 10 μM and gastrin I (G1) at 50 μM after 15 min of incubation. On the other hand, G1 at 10 μM and gastrin-related peptide (G2) did not enhance trypsin and chymotrypsin secretion. The studies concerning the CCK effects at different incubation temperatures have shown that trypsin and chymotrypsin secretion at 25 °C was stimulated by CCK after 15 min, while at 10, 15 and 20 °C the stimulatory effects of CCK were observed only after 30 min of incubation. The CCK effects were increased at higher incubation temperatures and longer incubation periods.

Keywords: Cholecystokinin; Gastrin; Pancreas; Pyloric caeca, trypsin; Chymotrypsin; *In vitro* assays; Proteolytic enzyme secretion; Temperature; Yellowtail (*Seriola quinqueradiata*)

1. Introduction

In vertebrates, the secretion of pancreatic enzymes is controlled by both neuronal and humoral factors. Humoral control is mediated by hormones, such as cholecystokinin (CCK) and gastrin, secreted by mucosal cells present in the digestive tract (Holmgren et al., 1983). In mammals, the peptide CCK was found to stimulate the secretion of pancreatic enzymes (Scheele and Palade, 1975; Tartakoff et al., 1975), the contraction of smooth muscle in the small intestine (Mutt and Jorpes, 1968; Ranirez and Farrar, 1970), and gallbladder discharge (Behar and Biancani, 1980; Shiratori et al., 1986). Gastrin was shown to increase gastric acid secretion (Broccardo et al., 1975; Von Schrenk et al., 1989), gastric motility (Mayer et al., 1982; Fox and McDonald, 1984), gallbladder contractility (Liu et al., 1995; Carbone et al., 1997) and pancreatic release of amylase (Iwamoto et al., 1983; Howard et al., 1985; Hildebrand et al., 1992).

In fish, Einarsson et al. (1997a,b) demonstrated that injected porcine CCK stimulates the dose-dependent secretion of trypsin and chymotrypsin, and gallbladder discharge. Olsson et al. (1999) suggested that sulfated CCK involved in the control of gastric emptying in rainbow trout. In isolated pyloric caeca of Atlantic salmon, porcine CCK at pharmacological concentrations has also stimulated trypsin and chymotrypsin secretion *in vitro*, independently from the central nervous system (Einarsson et al., 1997a). Moreover, indirect evidence for the presence of CCK has come from immunohistochemical studies in bluegill and killifish (Rajjo et al., 1988), rainbow trout (Holmgren et al., 1982), Atlantic halibut (Kamisaka et al., 2001), bluefin tuna (Kamisaka et al., 2002), ayu (Kamisaka et al., 2003), and turbot (Bermúdez et al., in press). Peptides homologous to mammalian CCK, and their corresponding cDNAs, have been isolated and sequenced from some fish species (Jensen et al., 2001; Kurokawa et al., 2003; Kamisaka et al., 2005; Murashita et al., 2006). Furthermore, the presence of CCK and strong evidence for its participation in the regulation of protein digestion were observed in goldfish (Himick et al., 1996), Japanese flounder larvae (Kurokawa et al., 2000),

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doi:10.1016/j.cbpa.2006.09.017

Atlantic halibut larvae (Rojas-Garcia and Ronnestad, 2002) and Japanese eel larvae (Kurokawa et al., 2004).

Although no report of the gastrin effects on pancreatic enzyme secretion in fish has been found, there is evidence for its function as a regulator of acid secretion in cartilaginous fish (Koelz, 1995). Furthermore, peptides with gastrin-CCK immunoreactivity extracted from cod (*Gadus morhua*) muscle have been found to stimulate amylase release from pancreatic AR4-2J cells (Ravallec-Ple and Van Wormhoudt, 2003). Similar affinities of CCK and gastrin peptides to the same binding sites were found in binding studies performed in bullfrogs (Vigna et al., 1984), sharks (Oliver and Vigna, 1996) and goldfish (Himick et al., 1996), suggesting that only a single receptor is expressed for CCK and gastrin in those animals.

Based on the importance of CCK and gastrin on the humoral control of digestive functions, this study was conducted to elucidate the *in vitro* effects of CCK and gastrin on trypsin and chymotrypsin secretion from the pyloric caeca/pancreatic tissue of yellowtails. Trypsin was chosen due to its key role in the digestion process, activating several other proteases, including chymotrypsin. Moreover, the activity ratio of these two enzymes has been suggested as an indicator of the nutritional status and the growth efficiency of the fish (Rungruangsak Torrissen and Male, 2000; Sunde et al., 2001).

The rate of dietary protein digestion in the intestinal system limits the uptake of nutrients and can potentially limit the growth, as has been suggested in Atlantic cod, *G. morhua* (Lemieux et al., 1999), and Atlantic salmon (Rungruangsak Torrissen and Male, 2000). In winter, lower growth performance in consequence of lower protein digestibility is found in yellowtail culture (Watanabe et al., 1990; Kofuji et al., 2005), therefore, it is important to study the factors involved in these lower apparent protein digestibility values. One factor that could affect yellowtail protein digestion at low water temperatures is the possible decrease in protease secretion, which could be due to the inhibition of CCK action. Thus, the present study also aimed to clarify the effects of temperature on the CCK and gastrin functions.

2. Material and methods

2.1. Experimental conditions and fish

Yellowtail (*Seriola quinqueradiata*) fingerlings were obtained from a commercial fish farm in Kochi prefecture, Japan and maintained for approximately 1 year in 2000-l concrete tanks at Usa Marine Biological Institute, Kochi University. Fish were fed daily a commercial fish diet (Yamaha Nutreco Aquatec Co. Ltd., Fukuoka, Japan) and they were starved for 2 days before the experiments.

Three experiments were performed. Experiment 1 was elaborated to evaluate the effects of two concentrations of CCK at three periods of tissue incubation; Experiment 2 aimed to examine the effects of CCK at four different incubation temperatures and Experiment 3 which was performed to investigate the effects of two commercially available gastrin peptides on pancreatic enzyme secretion. Four or five fish were used in each experiment and the mean body weights in the three experiments were 763.2 g, 604.3 g and 628.0 g respectively. In Experiment 1, samples were taken in May and June, 2001 when the rearing water temperature was about 21 °C. In Experiments 2 and 3, samples were taken between December and March, 2003/2004 when the rearing water temperature was of about 16 °C. Fish were taken from the tank and sacrificed; the pyloric caeca was removed and rapidly used in the assays. The pyloric caeca was isolated together with the surrounding fat tissue, which contains the pancreatic tissue.

2.2. Experimental procedure

2.2.1. Experiment 1

Sections of pyloric caeca (300 mg) were cut into small pieces (2 to 3 mm), using surgical scissors and placed in the cells of a net well plate (3482, Corning, USA) containing 2 ml of the experimental incubation solutions, in duplicate. A commercial synthetic product of CCK-octapeptide (non-sulfated form) (4087-v Peptide Institute, Inc., Osaka, Japan) was diluted in DMSO (10%). The incubation solutions were Tris buffer with DMSO (10%) (control), Tris buffer with 1 μM of CCK and Tris buffer with 10 μM of CCK. The plates were shaken in a microplate shaker (Iwaki Glass Co., Ltd, model MPX-96, Japan) and incubated at 25 °C in an incubator (Wakenyaku, model 9100, Japan). A sample of 100 μl was taken from the incubation solutions at 45, 60 and 150 min after treatment for trypsin activity determinations. A simplified outline of the *in vitro* assay protocol is shown in Fig. 1.

2.2.2. Experiment 2

Similarly to the procedures described for the Experiment 1, in Experiment 2 sections of pyloric caeca were incubated with: Tris buffer with DMSO (10%) (control) and Tris buffer with 10 μM of CCK. The plates were placed in the shakers (Iwaki Glass Co., Ltd, model MPX-96, Japan) and incubated at 10, 15, 20 and 25 °C in incubators (Wakenyaku, model 9100, Japan). Samples of 100 μl and 30 μl were taken from the incubation solutions at 15, 30 and 45 min after treatment for trypsin and chymotrypsin activity determinations, respectively (Fig. 1).

2.2.3. Experiment 3

G1, gastrin I (Pyr- Gly- Pro- Trp- Leu- Glu- Glu- Glu- Glu- Glu- Ala- Tyr- Gly- Trp- Met- Asp- Phe- NH_2) (human 4143-v Peptide Institute, Inc., Osaka, Japan) was diluted in distilled water (DW). G2, gastrin-related peptide (*t*- Amyloxycarbonyl- Trp- Met- Asp- Phe- NH_2) (4004 Peptide Institute, Inc., Osaka, Japan), a short synthetic peptide with the same C-terminal sequence of CCK and G1 was diluted in DMSO (100%). A control was prepared for each hormonal treatment, consisting of the dilution solution without the hormones. The isolated pyloric caeca sections were incubated with: Tris buffer with DMSO (10%) (control), Tris buffer with 10 μM of CCK, Tris buffer with DW (control-G1), Tris buffer with 10 μM of G1, Tris buffer with 50 μM of G1, Tris buffer with DMSO (control-G2), Tris buffer with 10 μM of G2, Tris buffer with 50 μM of G2 and Tris buffer with 100 μM of G2. The plates were shaken in