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Circulating levels of cholecystokinin and gastrin-releasing peptide in rainbow trout fed different diets

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Abstract

Cholecystokinin (CCK) and gastrin-releasing peptide (GRP) are gastrointestinal peptides thought to be important regulators of intake and digestion of food in vertebrates. In this study, pre- and postprandial plasma levels of CCK and GRP were measured in rainbow trout (*Oncorhynchus mykiss*) by the establishment of homologous radioimmunoassays, and the hormonal levels assessed in relation to dietary lipid:protein ratio and food intake. Fish were acclimated to either a high protein/low lipid diet (HP/LL diet; 14.1% lipids) or a normal protein/high lipid diet (NP/HL diet; 31.4% lipids). On three consecutive sampling days, radio-dense lead-glass beads were included in the diets for assessment of feed intake. Fish were terminally sampled for blood and stomach contents prior to feeding at time 0, and at 0.3, 1, 2, 4, 6, and 24 h after feeding. There was a postprandial elevation of plasma CCK levels, which was most evident after 4 and 6 h. Fish fed the NP/HL diet had higher plasma CCK levels compared with those fed the HP/LL diet. Plasma CCK levels were not affected by the amount of food ingested. GRP levels in plasma were not influenced by sampling time, diet, or feed intake. The results indicate that the endocrine release of gastrointestinal CCK is increased during feeding and may be further influenced by the dietary lipid:protein ratio in rainbow trout. Plasma GRP levels, on the other hand, appear not to be influenced by feeding or diet composition.

Keywords: Postprandial response; Gastrointestinal peptides; CCK; GRP; Feed intake; Dietary lipid; *Oncorhynchus mykiss*

1. Introduction

Feeding induces a cascade of endocrine events which regulate and synchronize the processing of food, the use and partitioning of nutrients following absorption, as well as subsequent feeding behaviour. The presence of food in the gastrointestinal tract stimulates the release of a number of neuroendocrine peptides belonging to the gut–brain regulatory axis, which may act both through endocrine and neural pathways. While having important roles for the function of the gastrointestinal tract, several of these neuroendocrine peptides may also act as short-term satiety factors, signaling back to the brain to reduce further food intake.

In vertebrates, one of the most studied gastrointestinal peptides is cholecystokinin (CCK), which is present in the gastrointestinal tract and the central and peripheral nervous system of both mammalian and non-mammalian species. CCK occurs in several different molecular forms, all of which have the C-terminus Trp-Met-Asp-Phe-NH_2, which they share with gastrin (Johnsen, 1998). In salmonids, immunohistochemical studies have indicated the presence of CCK endocrine cells in the intestine (Beorlegui et al., 1992; Bosi et al., 2004; Holmgren et al., 1982; Vigna et al., 1985). This was recently confirmed, when three cDNAs that encode peptides that are homologous to mammalian CCK were identified in rainbow trout (*Oncorhynchus mykiss*) (Jensen et al., 2001). At the peptide level,

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two of these, denoted CCK-N and CCK-L were both expressed in the brain, whereas in the gut, only CCK-L was expressed. The peptides were purified using an antibody directed against the C-terminus common for both gastrin and CCK. Multiple sequence alignment showed that the trout CCKs are more similar to one another than other members of the gastrin/CCK family for a number of species. Therefore, the current opinion is that all these peptides are CCKs, although it is not excluded that one of them may be gastrin (Jensen et al., 2001).

Similar to mammals, CCK has a major role in coordinating and synchronizing the digestive process in rainbow trout (Aldman and Holmgren, 1987, 1995; Aldman et al., 1992; Olsson et al., 1999). In addition, CCK has been called 'the prototypic gastrointestinal hormonal satiety agent' (Morley, 1995), as it potently suppresses food intake across species including fish (see Jensen, 2001; Moran and Kinzig, 2004; Volkoff et al., 2005). Supporting this, CCK mRNA expression increases after a meal in brain areas important for regulating food intake in goldfish (*Carassius auratus*) (Peyon et al., 1999). Also, in rainbow trout, CCK may suppress food intake, as the administration of CCK receptor antagonists increases feeding (Gélineau and Boujard, 2001). However, to be defined as a satiety factor, a substance should be released in response to feeding. In mammals, CCK is released when nutrients pass through the gastrointestinal tract, the major CCK-releasing factors being dietary fat and proteins (see Moran and Kinzig, 2004). Consistent with the ability of lipids to stimulate CCK release, endogenous CCK appears to mediate the appetite-suppressive effect of lipid ingestion that has been indicated in some mammalian species (Choi et al., 2000; Matzinger et al., 1999). In the Burmese python (*Python molurus*) there is an up to 25-fold increase in plasma CCK after a meal (Secor et al., 2001). In fish, however, the effect of food consumption and composition on the gastrointestinal release of CCK is unknown as homologous immunoassays have not been available for measuring plasma CCK levels in fish.

Gastrin-releasing peptide (GRP) consists of 27 amino acid residues in most species, sharing a region of structural similarity at the COOH-terminal region with bombesin (BBS). The current view is that the two peptides are not homologous, although they have been shown to have similar physiological effects in a range of species. Thus, GRP, but not BBS, is considered as the peptide expressed in the gut of vertebrates (see Merali et al., 1999; Volkoff et al., 2005). Consistently, the GRP-like peptides which have been isolated from rainbow trout and goldfish are both more closely related to GRP than to BBS (Jensen and Conlon, 1992; Volkoff et al., 2000). Exogenous GRP/BBS stimulates gastric acid secretion in fish, but several other functions have been indicated, including stimulation of gastrointestinal motility and inhibition of gastric emptying (see Jensen, 2001; Jensen and Holmgren, 1994).

Studies on a variety of species have provided evidence that GRP/BBS may act as satiety agents from the gut (see Jensen, 2001; Merali et al., 1999; Volkoff et al., 2005). In goldfish, BBS injections dose-dependently suppress food intake within 30–45 min (Himick and Peter, 1994), whereas in carp (*Cyprinus carpio*) BBS treatment delays the onset of feeding (Beach et al., 1988). A single meal induces an increase in the levels of a BBS-like peptide in the stomach of rats (Kateb and Merali, 1992). However, a postprandial endocrine release of biologically significant amounts of GRP/BBS-like peptide has not yet been proven (see Merali et al., 1999). Similar to CCK, the effect of food intake and composition on the release of gastrointestinal GRP has not been investigated in fish, as methods for assessing endogenous GRP levels in fish plasma have not been available.

The salmonids represent an interesting group of fish in terms of teleost evolution, but also a group of great commercial importance. For salmonid aquaculture, lipid and protein content is a major aspect of diet formulation. However, little is known about the endocrine responses to diets of different composition. The aim of the present study was to establish homologous radioimmunoassays to enable the study of circulating CCK and GRP levels in rainbow trout, and to elucidate their relationship to dietary lipid:protein ratio and food intake in a salmonid species.

2. Materials and methods

2.1. Fish and holding conditions

The study was conducted at the Tervo Fisheries Research Station of the Finnish Game and Fisheries Research Institute in central Finland. The fish used were two-year-old rainbow trout, all-female strain (SELEC-II-generation) from a Finnish selective breeding program. The fish were kept in a land-based raceway and were fed with commercial dry feed (Royal Plus, 5 mm pellets; 40% protein and 30% fat, Rehuraisio Ltd., Finland) prior to the acclimation period and experimental days.

On 9th of May, fish were netted and distributed among six indoor test tanks, where they were kept throughout the study. The study consisted of two parts: (1) an acclimation period from 9th of May to 17th, 18th, or 19th June depending on which day sampling started and (2) the experimental sampling period that lasted for 24 h for each tank. The average body weight was 667 g, and the initial biomass was set to be the same in all six test tanks (from 41.5 to 42.0 kg; 60–65 fish in each tank). The water volume of each tank was 3000 L, and water oxygen levels were monitored daily from tank outlets (means ± SD: 11.3 ± 0.3 mg O_2 L^{-1}). All tanks of fish were subjected to a set photoperiod regime (18L:6D) with lights on from 03:00 to 21:00 h.

From the start of acclimation on May 9th until the termination of the experiment, triplicate tanks of fish were fed two different diets; a normal protein/high lipid diet (NP/HL) or a high protein/low lipid diet (HP/LL), see Table 1 for diet composition. During the acclimation, food was supplied to all tanks by automatic feeders 10×/day between 04:00 and 09:00 h. Fish were fed to satiation (food was left on the bottom of the tanks). The feed consumption was temperature-dependent, increasing from 0.5 to 1.4% bwt^{-1} day^{-1}, as the ambient water temperature rose from 3.3 to 13.5 °C (mean temperature ± SD: 9.7 ± 0.43 °C) during the acclimation period (Lake Nilakka surface water). During the week before the experimental sampling, mean water temperature ± SD was 13.0 ± 0.73 °C and similar in all tanks. On the three sampling days, mean water temperature ± SD was 13.5 ±0.05 °C, and similar between tanks. There was no mortality during the study.