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Long-term treatment of ghrelin stimulates feeding, fat deposition, and alters the GH/IGF-I axis in the tilapia, *Oreochromis mossambicus*

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Abstract

Recently, a new peptide, ghrelin, which specifically stimulates growth hormone (GH) release from the pituitary, was identified in the rat and human stomach. Ghrelin has been shown to stimulate GH release by acting through a growth hormone secretagogue receptor. We have identified two ghrelins (ghrelin-C8 and -C10) in the stomach of tilapia, a euryhaline fish. The current study was aimed at investigating the in vivo effect of the two tilapia ghrelins on feeding, fat deposition, and on the GH/IGF-I axis. Tilapia were implanted with micro-osmotic pumps containing either ghrelin-C8, ghrelin-C10 or saline (control). Ghrelin was delivered at a continuous rate of 10 ng/h for 21 days. Food consumption increased significantly in those animals that received ghrelin-C10 but not ghrelin-C8. Treatment with ghrelin-C10 increased body weight significantly without altering body length. Hence, the condition factor was significantly higher in the ghrelin-C10 group compared with the control. Liver weight and total fat content in the liver were also elevated significantly in the fish treated with ghrelin-C10. There was no effect of either ghrelin on plasma GH levels, whereas plasma IGF-I levels were reduced significantly in the ghrelin-C10 group. These findings demonstrate that ghrelin plays a role in feeding and fat metabolism in the tilapia, and suggest that the two forms of ghrelin may be acting through different receptors.

Keywords: Ghrelin; Feeding; Fat metabolism; GH/IGF-I axis; Tilapia

1. Introduction

Ghrelin, recently identified as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R), possesses a unique *n*-octanoyl modification on the third amino acid residue (Kaiya et al., 2001, 2002, 2003a,c; Kojima et al., 1999; Unniappan et al., 2002). This modification is suggested to be necessary for biological activity (Hosoda et al., 2000b). In the tilapia, two forms of ghrelin have been identified, *n*-decanoic (ghrelin-C10) and *n*-octanoic (ghrelin-C8). The decanoyl-modified form appears to be the major form of circulating ghrelin in the tilapia (Kaiya et al., 2003b). In mammals, ghrelin is produced predominantly in the stomach, whereas the central nervous system, kidney, placenta, testis, ovary, small intestine, lung, and lymphocytes produce substantially less ghrelin (Gualillo et al., 2003; Horvath et al., 2001). We have also reported low levels of ghrelin gene expression in the brain and gills in the tilapia (Kaiya et al., 2003b).

Ghrelin was first shown to be a potent stimulus of growth hormone (GH) release from the pituitary in mammals (Kojima et al., 1999; Muller et al., 2002; Peino et al., 2000; Seone et al., 2000; Takaya et al., 2000), chicken (Kaiya et al., 2002), bullfrog (Kaiya et al., 2001), tilapia (Kaiya et al., 2003b; Riley et al., 2002), and eel (Kaiya et al., 2003c). Since then, ghrelin has been shown

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to possess several actions that are tied to regulating metabolic balance and managing the neuroendocrine and metabolic response to starvation (Gualillo et al., 2003; Horvath et al., 2001; Wang et al., 2002). Indeed, stomach-derived ghrelin is the first peripheral orexigenic hormone identified. Both intracerebroventricular and intraperitoneal administration of ghrelin stimulated appetite in rats (Nakazato et al., 2001; Wren et al., 2000) and goldfish (Unniappan et al., 2004). It has been demonstrated that ghrelin crosses the blood–brain barrier (Banks et al., 2002), suggesting that stomach-derived ghrelin may play a role in driving the orexigenic actions of ghrelin (Wren et al., 2000).

Ghrelin possesses anabolic actions that appear to be mediated through GH. Growth hormone, in turn, acts to an important extent through insulin-like growth factor-I (IGF-I) in exercising a variety of anabolic and lipolytic actions (Moller and Norrelund, 2003). Ghrelin, on the other hand, has been found to promote fat deposition in mice and rats by reducing fat utilization (Tschöp et al., 2000). Inasmuch as GH is lipolytic, it would appear that ghrelin's adipogenic actions are independent of its stimulation of GH release (Gualillo et al., 2003; Horvath et al., 2001; Petersenn, 2002). In the current study, we demonstrate that systemic ghrelin is a potent orexigenic factor that stimulates feeding, fat deposition, and suppresses the GH/IGF-I axis in the tilapia.

2. Materials and methods

2.1. Animals and treatment

Mature male tilapia, weighing 60–75 g, were maintained (eight fish per tank) under natural photoperiod observed in October and November of 2003 in flow-through freshwater tanks at the Hawaii Institute of Marine Biology, University of Hawaii, at 25 °C. They were fin clipped to identify individuals and acclimated to the tank six weeks prior to experimentation to ensure that all animals exhibited similar appetite and growth. Fish were surgically implanted with a micro-osmotic pump (Model # 1002; Alzet, Palo Alto, CA), containing 100 μg/ml of tilapia ghrelin-C8 (*n*-octanoic), ghrelin-C10 (*n*-decanoic) dissolved in sterile saline (0.9% NaCl) or sterile saline alone (control), total volume was 100 μl per pump. Briefly, fish were anesthetized in tricaine methanasulfanate (0.15 g/L) buffered with $NaHCO_3$ (0.15 g/L). A small incision was made on the lateral side of the intraperitoneal cavity just dorsal and anterior of the genital pore. The pump was inserted into the intraperitoneal cavity and the incision was sutured with two stitches. The entire procedure took less than 5 min. Fish were returned to the tank containing Maracyn antibiotic (Mardel Laboratories, Harbor City, CA) for five days following manufacturer's instruction. No mortality was observed. The flow rate of the micro-osmotic pumps was calculated to release ghrelin at a rate of 10 ng/h at 25 °C with a predicted life of 28 days. The experiment was terminated after 21 days to ensure that animals received ghrelin for the entire length of the experiment.

Fish were fed an excess of known weight of feed (Silver Cup, Nelson and Sons, Murray, Utah) twice a day (08:00–09:00 and 14:00–15:00). After 30 min of feeding, the feed remaining in the tank was collected and the total weight of feed consumed was calculated. Body weight and standard length were measured weekly. Condition factor was calculated at the end of the experiment. Condition factor (CF) can be defined as the growth of tissues and the storage of energy in muscle and liver causing an individual to have a greater weight relative to a particular length ($CF = \text{weight}/\text{length}^3 \times 100$). Upon termination, blood was collected in heparinized (200 U/ml, sodium heparin, Sigma) syringes. Plasma was separated by centrifugation at 10,000*g* for 10 min and stored at −20 °C. Liver was weighed, and a small portion of liver and muscle tissue as well as whole pituitary were stored in TRI-Reagent (MRC, Cincinnati, OH) at −80 °C until RNA extraction. The remainder of the liver and a fillet of white muscle were snap-frozen in liquid N_2 and stored at −80 °C until total fat analysis. The experiment was conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, University of Hawaii.

2.2. Radioimmunoassays

Plasma GH levels were measured by homologous radioimmunoassay (RIA) according to Ayson et al. (1993) and Yada et al. (1994). Plasma IGF-I levels were analyzed using a commercially available RIA kit (Gro-Pep, Adelaide, Australia) as modified by Shimizu et al. (1999).

2.3. Northern dot blot and RNase protection assay

Total RNA was extracted from individual pituitary, liver, and muscle by acid guanidinium thiocyanate–phenol–chloroform extraction following the method of Chomczynski and Sacchi (1987). Quantification of GH mRNA was carried out by Northern dot blot hybridization (Riley et al., 2002). We have demonstrated that the homologous GH probe hybridizes to a single mRNA species (data not shown). GH mRNA values were normalized to β-actin mRNA values to account for any unequal loading of RNA among samples.

A homologous RNase protection assay was used to quantify liver IGF-I mRNA. cRNA probes for IGF-I and β-actin were prepared according to Kajimura et al. (2003). The RNase protection assay was performed using RPA III kit (Ambion, Austin, TX). Briefly, 20 μg of total liver RNA was hybridized with 2.0×10^4 cpm of