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Regulation of feeding behavior and food intake by appetite-regulating peptides in wild-type and growth hormone-transgenic coho salmon

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ABSTRACT

Survival, competition, growth and reproductive success in fishes are highly dependent on food intake, food availability and feeding behavior and are all influenced by a complex set of metabolic and neuroendocrine mechanisms. Overexpression of growth hormone (GH) in transgenic fish can result in greatly enhanced growth rates, feed conversion, feeding motivation and food intake. The objectives of this study were to compare seasonal feeding behavior of non-transgenic wild-type (NT) and GH-transgenic (T) coho salmon (*Oncorhynchus kisutch*), and to examine the effects of intraperitoneal injections of the appetite-regulating peptides cholecystokinin (CCK-8), bombesin (BBS), glucagon-like peptide-1 (GLP-1), and alpha-melanocyte-stimulating hormone (α-MSH) on feeding behavior. T salmon fed consistently across all seasons, whereas NT dramatically reduced their food intake in winter, indicating the seasonal regulation of appetite can be altered by overexpression of GH in T fish. Intraperitoneal injections of CCK-8 and BBS caused a significant and rapid decrease in food intake for both genotypes. Treatment with either GLP-1 or α-MSH resulted in a significant suppression of food intake for NT but had no effect in T coho salmon. The differential response of T and NT fish to α-MSH is consistent with the melanocortin-4 receptor system being a significant pathway by which GH acts to stimulate appetite. Taken together, these results suggest that chronically increased levels of GH alter feeding regulatory pathways to different extents for individual peptides, and that altered feeding behavior in transgenic coho salmon may arise, in part, from changes in sensitivity to peripheral appetite-regulating signals.

Introduction

Genetic modification or transgenesis has been employed in fish research to modify phenotypic traits for basic research and for applied purposes to enhance traits for aquaculture, such as modifying cold tolerance, disease resistance, reproduction, growth, and feed conversion efficiency (Devlin et al., 2006; Fletcher and Davies, 1991). Since the production of the first transgenic fish (goldfish, *Carassius auratus*) in the 1980′s (Zhu et al., 1985), this technology has been applied to many other fish species, including salmonids (Devlin et al., 1994, 2001; Du et al., 1992; Shears et al., 1991). In addition to growth enhancement, elevated levels of growth hormone (GH) in fish have been shown to induce a wide range of physiological, morphological, and behavioral responses (Devlin et al., 2006; Hu and Zhu, 2010) including significant changes in feeding behavior. GH-transgenic fish can exhibit increased feed motivation, appetite, and increased competitive ability, reductions in prey discrimination, *anti*-predator, and shoaling behaviors, as well as alterations to reproductive performance (Abrahams and Sutterlin, 1999; Bessey et al., 2004; Cao et al., 2014; Devlin et al., 1999; Fitzpatrick et al., 2011; Johnsson and Björnsson, 1994; Leggatt et al., 2014; Moreau et al., 2011; Sundström et al., 2003, 2004a, 2004b; Vandersteen Tymchuk et al., 2005).

Growth in fishes is regulated through complex interactions among multiple organs and hormones that form the somatotrophic axis. This axis consists of GH, insulin-like growth factors (IGFs) and their corresponding membrane receptors and binding proteins. GH influences somatic growth, but also plays an important regulatory role in reproduction, ionic balance and food intake (Björnsson, 1997; Björnsson et al., 2002; Canosa et al., 2013). In many fish species, injections of GH increases food intake [reviewed in (Donaldson et al., 1979; Johnsson and Björnsson, 1994)]. In addition, salmon containing a GH transgene display increases in both food consumption and feed conversion efficiency (Cook et al., 2000; Devlin et al., 1999, 2004). GH-transgenic coho salmon have elevated circulating protein and tissue mRNA levels for both GH and IGF-1 (Raven et al., 2008), but the effects of this up-regulation on other metabolic systems, as well as the mechanisms of action involved, are not fully understood.

In fishes, as in mammals, energy homeostasis, feeding behavior and food intake are regulated by the interaction of central (in the brain, particularly the hypothalamus) and peripheral (*e.g.* intestine, liver, *etc.*)

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endocrine pathways that respond to the body's energy status and requirements (Gorissen et al., 2006). Endocrine signals can either stimulate (orexigenic factors) or inhibit (anorexigenic factors) food intake (Gorissen et al., 2006; Volkoff et al., 2005). Neuropeptide Y (NPY) and agouti-related protein (AgRP) are examples of central orexigenic signals, whereas cocaine and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC) are examples of central anorexigenic signals. Cholecystokinin (CCK), bombesin/gastrin-releasing peptide (BBS/GRP), and glucagon and glucagon-like peptides (GLP) are important peripheral appetite signals, generated mainly by the gastrointestinal (GI) tract in response to ingested food. These hormones play a role in meal termination, creating a sensation of fullness and causing a reduction in food intake (Lin et al., 2000; Mommsen, 2000; Nelson and Sheridan, 2006; Volkoff et al., 2005, 2009; Woods, 2004). Although levels of metabolites (*e.g.* glucose Polakof et al., 2012) can be directly perceived by the brain, these peripheral appetite signals relate information on energy status to central feeding areas either by crossing the blood brain barrier to act directly through central receptors, or by binding to peripheral receptors on the vagus nerve, or both (Brightman and Broadwell, 1976). For example, the actions of CCK on appetite are mediated by CCK receptors expressed on fibers of vagus nerve which project into the hindbrain (which in turn have hypothalamic neuronal projections, (Grill and Hayes, 2012)), as well as by binding directly to brain CCK receptors (Raybould, 2007; Vigna, 2000). To date, only one peripherally acting orexigenic peptide, ghrelin, has been identified (Nelson and Sheridan, 2006; Volkoff et al., 2009). In addition to their involvement in appetite regulation, many of these factors [*e.g.* NPY, leptin, CCK, BBS and ghrelin (Canosa et al., 2013; Volkoff et al., 2010)] also act as GH secretagogues. For example, in catfish (*Ictalurus punctatus*) and goldfish, NPY stimulates growth and the secretion of GH (Himick and Peter, 1994a; Mazumdar et al., 2006) and in goldfish, BBS treatment increases GH secretion and decreases somatostatin (SS, a neuroendocrine regulating hormone) gene expression (Canosa et al., 2005).

CCK, BBS/GRP and GLP-1 are major "classical" satiety factors in fish and other vertebrates. Central and peripheral administration of CCK induces a reduction in food intake in goldfish (Himick and Peter, 1994b; Volkoff et al., 2003), and oral administration of a CCK receptor antagonist increases food intake in wild-type rainbow trout (Gelineau and Boujard, 2001), and in both wild-type and GH-T coho salmon (Lõhmus et al., 2008). Similarly, in goldfish, central or peripheral administration of BBS causes a significant reduction in food intake (Himick and Peter, 1994a). Injections with GLP-1 induce a decrease in feeding in catfish (Silverstein et al., 2001) and increases in plasma glucose levels in goldfish (Polakof et al., 2011).

In fish, as in other vertebrates, peptides and receptors of the melanocortin system are also involved in the regulation of food intake. The melanocortin system in fishes consists of α-, β-, γ- melanocyte-stimulating hormones (MSH) and adrenocortin peptides, all derived from the gene pro-opiomelancortin (POMC). These peptides interact with five G-protein-coupled receptors, MCR1–MCR5 (Metz et al., 2006). In addition, two endogenous melanocortin antagonists, agouti and agouti-related protein (AgRP) have been shown to modulate the activity of these melanocortin peptides (Gantz and Fong, 2003; Metz et al., 2006). This system regulates a diverse array of physiological functions, including appetite and energy homeostasis. In fishes, α-MSH regulates appetite by inhibiting feeding, mediated through its binding to the melanocortin 4 receptor (MC4R), with AgRP acting as an endogenous antagonist of α-MSH at MC4R (Canosa et al., 2013; Gantz and Fong, 2003; Metz et al., 2006). In juvenile rainbow trout, intracerebroventricular (ICV) injections of a melanocortin receptor agonist (MTII) decrease food intake, whereas injections of melanocortin receptor antagonists (HS024 and SHU9119) increase food intake (Schjolden et al., 2009). In goldfish, ICV injections of a melanocortin agonist inhibit food intake in a dose dependent manner (Cerdá-Reverter et al., 2003b), whereas treatment with a MC4R antagonist (HS024) increased food intake (Cerdá-Reverter et al., 2003a).

GH-transgenic (T) salmon provide a useful model to examine feeding regulation in a condition where abnormally high growth rates are associated with increased appetite and feed conversion (Dalmolin et al., 2015; Devlin et al., 1999; Sundström et al., 2004a). However, to date, our understanding of the endocrine mechanisms regulating the enhanced feeding behavior and food intake in T fish is limited. A study by Lõhmus et al. (2008) suggests that disruptions of seasonal feeding in T coho salmon might be in part due to alteration in peripheral CCK signaling, but Raven et al. (2008) reported no significant changes of CCK or NPY mRNA levels in the hypothalamus or telencephalon of T coho salmon compared to wild-type. In T zebrafish, both CCK and NPY mRNA expression is lower than in wild-type fish (Dalmolin et al., 2015). In common carp (*Cyprinus carpio* L.), T fish display a two-fold elevation in hypothalamic AgRP 1 mRNA expression levels (Zhong et al., 2013), implicating the MC4R pathway in mediating the GH-induced increase in feeding behavior. These data suggest that altered GH levels in T fish might affect the expression levels of appetite regulators, which in turn might drive changes in feeding behavior.

The aim of the present study was to examine the effects of peripheral administration of peripheral anorexigenic peptides (CCK, BBS, GLP-1, α-MSH) in both wild-type (NT) and GH-T coho salmon in order to evaluate the role of these peptides in food intake and feeding behavior in normal fish and in animals with greatly elevated GH signaling. Understanding appetite control and feeding motivation have important applications for risk assessments of potential ecosystem effects of transgenic fish in natural ecosystems (Devlin et al., 2015).

Materials and methods

Fish and culture conditions

This study was conducted at Fisheries and Oceans Canada (DFO), Centre for Aquaculture and Environmental Research (CAER), West Vancouver, BC, Canada, which houses a physical containment facility designed to prevent the escape of transgenic fish to nature. Fish were maintained and experiments conducted under Guidelines of the Canadian Council for Animal Care and were approved by the DFO Pacific Animal Care Committee.

Non-transgenic wild-type (NT) coho salmon were offspring of parents collected at the Chehalis River (BC, Canada) hatchery and reared from fertilization. NT fish reared at the Chehalis River hatchery were used in summer 2012 for CCK and BBS trials, whereas other experiments used NT fish reared at CAER facility (2012 and 2013 groups). Transgenic (T) coho salmon (M77 strain) contained the GH gene construct OnMTGH1 (Devlin et al., 1994). This strain originated from the same Chehalis River stock a NT fish, and has been maintained by backcrosses at each generation to wild-type Chehalis River hatchery fish. Stock groups were reared separately under replicated culture conditions (with the exception of the NT fish raised in large communal raceways at the Chehalis River hatchery) in fresh aerated well water (10 ± 1 °C) at a density of <5 kg/m^3 and with simulated natural lighting and photoperiod (Vancouver, BC, Canada, 49°15′N, 123°10′W). Prior to, and throughout the trials, fish were fed stage-appropriate artificial salmonid diets (Skretting, Canada) twice daily at 10 am and 2 pm. To produce fish with different growth potentials of comparable size for experimentation, the amount of food that satiated the NT fish was provided to the T fish (called Tr, pair fed to NT-satiating ration levels). This feeding approach eliminates developmental stage (size) effects when examining T and NT fish at the same age, but introduces the variable of ration restriction for T fish receiving less than their desired energy intake. Importantly for the present study, T salmon raised in this manner retain their heightened feeding motivation as seen in fully-fed transgenic animals (Sundström et al., 2007). For part of this study there was a limitation in the number of available size-matched Tr fish from the 2012 cross. Consequently, fully-fed transgenic coho salmon (Tf) produced in 2013 (same strain as the 2012 group, but one year younger)