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Effects of chronic growth hormone overexpression on appetite-regulating brain gene expression in coho salmon

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ABSTRACT

Organisms must carefully regulate energy intake and expenditure to balance growth and trade-offs with other physiological processes. This regulation is influenced by key pathways controlling appetite, feeding behaviour and energy homeostasis. Growth hormone (GH) transgenesis provides a model where food intake can be elevated, and is associated with dramatic modifications of growth, metabolism, and feeding behaviour, particularly in fish. RNA-Seq and qPCR analyses were used to compare the expression of multiple genes important in appetite regulation within brain regions and the pituitary gland (PIT) of GH transgenic (fed fully to satiation or restricted to a wild-type ration throughout their lifetime) and wild-type coho salmon (*Oncorhynchus kisutch*). RNA-Seq results showed that differences in both genotype and ration levels resulted in differentially expressed genes associated with appetite regulation in transgenic fish, including elevated *Agrp1* in hypothalamus (HYP) and reduced *Mch* in PIT. Altered mRNA levels for *Agrp1*, *Npy*, *Gh*, *Ghr*, *Igf1*, *Mch* and *Pomc* were also assessed using qPCR analysis. Levels of mRNA for *Agrp1*, *Gh*, and *Ghr* were higher in transgenic than wild-type fish in HYP and in the preoptic area (POA), with *Agrp1* more than 7-fold higher in POA and 12-fold higher in HYP of transgenic salmon compared to wild-type fish. These data are consistent with the known roles of orexigenic factors on foraging behaviour acting via GH and through MC4R receptor-mediated signalling. *Igf1* mRNA was elevated in fully-fed transgenic fish in HYP and POA, but not in ration-restricted fish, yet both of these types of transgenic animals have very pronounced feeding behaviour relative to wild-type fish, suggesting IGF1 is not playing a direct role in appetite stimulation acting via paracrine or autocrine mechanisms. The present findings provide new insights on mechanisms ruling altered appetite regulation in response to chronically elevated GH, and on potential pathways by which elevated feeding response is controlled, independently of food availability and growth.

1. Introduction

The mechanisms controlling food intake are complex and involve many organ systems, endocrine pathways, and neuronal circuits that integrate environmental signals with endogenous physiological states. Appetite regulation is crucial to appropriate growth and energy homeostasis for an organism. A major pathway controlling metabolic rate, growth, and food intake is the growth hormone (GH)/insulin-like growth factor (IGF) axis. In fish as in mammals, GH is secreted into circulation by the pituitary (PIT) and acts through the growth hormone receptor (GHR) to stimulate IGF1 production in hepatic and other tissues, which induces somatic growth and exerts a negative feedback on GH secretion. Somatostatins [SST, produced by the hypothalamus (HYP)] inhibit both GH secretion and *Igf1* gene expression whereas ghrelin (GHRL, produced by stomach and intestine) stimulates GH secretion (Won and Borski, 2013). In addition to regulating growth, GH is a pleiotropic hormone involved in many functions including appetite, stress response, energy homeostasis, and reproduction (Björnsson et al., 2002). *Gh* genes have been overexpressed or knocked out to examine physiological responses in species with determinate growth (i.e. grow to a final body size), and effects on multiple

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appetite-regulating genes have been observed (Arora and Anubhuti, 2006; Bohlool-Y et al., 2005; Kopchick et al., 1999). In fish, model transgenic strains overexpressing GH have also been developed for species that possess determinate growth (Figueiredo et al., 2007), as well as for those with indeterminate growth (i.e. growing throughout their entire life), including carp (*Cyprinus carpio*) (Wan et al., 2012; Zhong et al., 2013), tilapia (*Oreochromis niloticus*) (Lu et al., 2009; Rahman et al., 1998), loach (*Misgurnus mizolepis*) (Nam et al., 2001), and several salmonid species (Devlin et al., 1994, 2004a; Du et al., 1992). GH transgenic (T) fish can show highly elevated feeding behaviour, growth, and metabolic rate, and possess modifications of other physiological processes at the levels of gene expression, enzyme activities, and the whole animal (Devlin et al., 2001, 2004a; Lõhmus et al., 2008; Raven et al., 2008). Despite this body of literature, the mechanisms by which GH influences fish appetite regulation are not yet fully understood, in part because control of food intake in fish tends to differ among species to a greater extent than in mammals (Hoskins and Volkoff, 2012).

Feeding and energy balance are regulated by centres in the brain, which produce and are affected by appetite-regulating peptides. Examples of these peptides are orexigenic factors such as agouti related neuropeptide 1 (AGRP1), neuropeptide Y (NPY), and orexin (HCRT), and anorexigenic peptides such as cocaine and amphetamine regulated transcript (CART), cholecystokinin (CCK), and α-melanocyte stimulating hormone (α-MSH, processed from proopiomelanocortin, POMC). In mammals, the paraventricular nucleus (PVN) and arcuate nucleus (ARC) in the hypothalamic region of the brain are recognized as command centres for controlling energy balance. The preoptic area (POA) was recently defined as the PVN-homologous region in the HYP of fish (Herget et al., 2014), and has previously been found to display altered mRNA levels for *Npy* in hungry fish (Silverstein et al., 1998). In teleost fish, hypothalamic AGRP and POMC neurons associated with appetite and growth project directly into the PIT (Zhang et al., 2012) and the HYP/POA/PIT axis is thought to play a pivotal role in multiple pathways including appetite regulation, feeding behaviour, and energy use. These actions are regulated in part by production and release of, and response to, GH (Forlano and Cone, 2007; Herget et al., 2014; Zhang et al., 2012).

Due to the magnitude of phenotypic changes seen in T fish, they provide a useful model organism to understand the relationship between appetite regulation, growth, and behaviour. The pleiotropic effects of GH are believed to be largely mediated by IGF1 produced in liver and other tissues in response to GH stimulation (de Azevedo Figueiredo et al., 2007; Frago et al., 2002). It is well established that GH overexpression in fish elevates *Igf1* gene expression in multiple tissues and increases circulating IGF1 protein levels (Beckman, 2011), and this is correlated with strongly elevated feeding behaviour and food intake in animals fed *ad libitum*. However, T fish reared on a wild-type (restricted) ration level have normal levels of *Igf1* gene expression and IGF1 circulating hormone, yet possess the same heightened feeding motivation seen in fully-fed transgenic fish (Devlin, 2011; Raven et al., 2008). These data show that IGF1 production is influenced by nutritional state (Beckman, 2011), and suggest that elevated appetite in T fish is not directly mediated either by peripheral IGF1 levels, or by increased nutrient utilization signals associated with elevated somatic growth. Rather, appetite is likely elevated by direct stimulation of central feeding centres by GH or by other peripheral signals affected by GH independently of IGF1. Although the mechanisms ruling the central effects of GH on feeding behaviour and growth are not fully understood, recent studies in T fish suggest important roles for appetite-related neuropeptides. For example, T coho salmon (*Oncorhynchus kisutch*) have lower telencephalic expression of *Npy* and winter levels of *Cck* relative to wild-type fish (Lõhmus et al., 2008; Raven et al., 2008), whereas in T carp, both the hypothalamic and telencephalic expression of *Agrp1* is 2-fold higher relative to wild-type fish, although transgenesis does not seem to affect *Npy*, *Hcrt*, *Pomc*, *Cck*, or *Cart* expressions (Zhong et al., 2013).

To further understand the mechanisms controlling appetite and growth in fish, the current study has undertaken a comprehensive examination of mRNA levels of appetite-regulating genes producing orexigenic, anorexigenic, and metabolic effects, in the HYP (with POA separately) and the PIT of wild-type (NT), fast-growing GH transgenic (TF), and ration-restricted GH transgenic (TR) coho salmon.

2. Materials and methods

2.1. Experimental animals

The experiment was performed September 23–27, 2013 at the Centre for Aquaculture and Environmental Research (CAER), Fisheries and Oceans Canada (DFO), West Vancouver, Canada. This facility contains specific containment measures to prevent the escape of genetically modified fish to the natural environment. All experimental procedures were carried out in compliance with the Canadian Council for Animal Care guidelines under permit from DFO's Pacific Regional Animal Committee. Three size-matched groups of coho salmon (*O. kisutch*; 95.8 ± 15 g) were examined: (i) wild-type coho salmon (non-transgenic, NT), (ii) GH transgenic coho salmon fully fed to satiation throughout their lifespan (TF) and growing 2–3-fold faster than wild-type fish (Devlin et al., 2004b), and (iii) GH transgenic salmon that were ration-restricted to the NT satiety ration level throughout their lifespan (TR). All fish were of the same genetic background (Chehalis River hatchery coho salmon from Fisheries and Oceans Canada Chehalis River Enhancement Facility Agassiz, BC). Transgenic coho salmon (T) contained the OnMTGH1 gene construct (Devlin et al., 1994) (strain M77), and were produced at CAER (Devlin et al., 2004b) and maintained in a wild-type genetic background by crossing T at each generation to NT coho salmon collected from nature. NT salmon were produced by crossing wild-type males to the same females used to produce TR salmon. NT and TR fish were produced in January 2012, and TF fish were produced in January 2013. Thus, TF fish were of same size and developmental stage as TR and NT fish, but were one year younger. All groups of fish were reared under the same standard conditions (400 fish/4000 L fibreglass tanks, 1 group of fish per tank, 10 ± 1 °C well water, and simulated daylight set to the natural photoperiod). Fish were fed stage-appropriate commercial salmonid diets (Skretting Ltd., Canada) at fixed times of day (9 AM and 2 PM) for at least 3 months prior to the experiment to standardize physiological responses to feeding. Foraging and schooling behaviour of each group was visually observed prior to and during feeding events.

2.2. Sampling and dissection

Three time points were chosen to represent different stages of feeding: pre-feeding, and two post-prandial stages [1 h post-feeding (1 hpf) for satiation, and 4 h post-feeding (4 hpf) for active digestion]. Fish were sampled over a five-day period as follows: Day 1: TR pre-feeding; Day 2: NT pre-feeding; Day 3: TF pre-feeding, TR 1 hpf, and TR 4 hpf; Day 4: NT 1 hpf and NT 4 hpf; Day 5: TF 1 hpf and TF 4 hpf. This sampling approach provided a two-day recovery period between pre-feeding and post-feeding samplings for each group. No differences in feeding behaviours of a population were noted between pre-experimental and sampling periods. Fish were fed normal feeding levels between pre-feeding and post-feeding sample days, and then fed to satiation at the 9 AM