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Growth and endocrine effects of recombinant bovine growth hormone treatment in non-transgenic and growth hormone transgenic coho salmon

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ABSTRACT

To examine the relative growth, endocrine, and gene expression effects of growth hormone (GH) transgenesis vs. GH protein treatment, wild-type non-transgenic and GH transgenic coho salmon were treated with a sustained-release formulation of recombinant bovine GH (bGH; Posilac™). Fish size, specific growth rate (SGR), and condition factor (CF) were monitored for 14 weeks, after which endocrine parameters were measured. Transgenic fish had much higher growth, SGR and CF than non-transgenic fish, and bGH injection significantly increased weight and SGR in non-transgenic but not transgenic fish. Plasma salmon GH concentrations decreased with bGH treatment in non-transgenic but not in transgenic fish where levels were similar to controls. Higher GH mRNA levels were detected in transgenic muscle and liver but no differences were observed in GH receptor (GHR) mRNA levels. In non-transgenic pituitary, GH and GHR mRNA levels per mg pituitary decreased with bGH dose to levels seen in transgenic salmon. Plasma IGF-I was elevated with bGH dose only in non-transgenic fish, while transgenic fish maintained an elevated level of IGF-I with or without bGH treatment. A similar trend was seen for liver IGF-I mRNA levels. Thus, bGH treatment increased fish growth and influenced feedback on endocrine parameters in non-transgenic but not in transgenic fish. A lack of further growth stimulation of GH transgenic fish suggests that these fish are experiencing maximal growth stimulation via GH pathways.

1. Introduction

Many teleost species show a remarkable capacity for growth enhancement [4,23]. Injections of exogenous growth hormone (GH) have resulted in increased growth in many species, including channel catfish [49,50], tilapia [38,68], striped bass [25], grass pickerel [67], rainbow trout [11,26,29], and coho salmon [14,30–32,41,59]. Other physiological changes can be associated with increased growth rate, including increased feed conversion efficiencies, greater energy and nutrient utilization, altered tissue shape and composition, and enhanced hypoosmoregulatory ability [4]. Thus, GH treatment has the potential to improve production efficiencies for aquaculture, however to date has not been applied in commercial production due to public perception issues and a lack of field trials of sufficient scale to demonstrate efficacy.

An alternative to GH injections for growth acceleration is the creation of transgenic fish that over-produce GH from gene constructs [20,21,24,33,39,40,42,45,52,53]. Transgenic salmon that over express GH show similar physiological changes as are found for GH protein injection, including enhanced growth rates [20,21,24], increased appetite and feeding motivation, enhanced feed conversion efficiencies, increased nutrient and energy utilization, and acceleration of development and onset of maturation [1,10,12,15,16,19,47,54,55,61–63]. Elevated levels of GH in salmon, whether by injection or transgenesis affect the growth hormone – insulin-like growth factor-I (GH-IGF-I) axis, modifying gene expression and plasma hormone levels and hence modifying growth rates [3,7,17,28,35,57]. Normally, GH is secreted from the pituitary gland, circulates in plasma, and binds growth hormone receptors (GHR) to stimulate IGF-I mRNA transcription and subsequent IGF-I protein release from the liver and other tissues [51,56,58,66]. IGF-I mediates many GH growth effects and both hormones can feed back on the pituitary to reduce GH mRNA transcription and GH protein production [2,7]. In addition, autocrine and paracrine stimulation by GH and IGF-I may be functioning in many tissues, particularly in transgenic animals [8,9,55].

Intraperitoneally injected GH circulates in the body and acts via endocrine mechanisms, whereas transgenesis has the potential to act in both an endocrine and paracrine/autocrine fashion (and potentially intracellularly). In both transgenic and injected cases, expression or treatment can be adjusted such that saturating effects on growth stimulation are achieved - that is, further GH administration, or increasing the dosage of the transgene has little

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effect on growth. However, the relative effects of transgenesis vs. GH protein injection have not been directly compared, nor has the effect of supplemental GH administration in GH transgenic salmon. In the present experiments, transgenic and non-transgenic fish were injected with a slow-release formulation of bovine GH (Posilac™; Monsanto Corporation), previously shown to increase growth in salmonids [29,35,38,41,46,49,50,64]. Growth was monitored over a 14 week period, and plasma IGF-I and GH hormone levels, and GH, IGF-I and GHR mRNA levels in several tissues were measured to assess effects on the endocrine system.

2. Methods

2.1. Fish culture and sampling

Experiments were conducted on strains of coho salmon (*Oncorhynchus kisutch*). Non-transgenic (NT) coho salmon were derived from crosses using 10 male and 10 female parents from the Chehalis River in southwestern British Columbia, Canada. GH transgenic salmon (T; M77 strain containing the OnMTGH1 construct, Devlin et al. 1994; 2004) contain GH transgenes at a single locus and was originally developed from the same Chehalis River strain. The T strain is maintained by backcrossing at each generation with 10 females derived from wild Chehalis River population to retain a wild background genotype. On March 1, 2004, 102 NT and 102 T coho salmon were weighed, measured for fork-length, and intraperitoneally injected with passive integrated transponder (PIT) tags. Following a 3-day recovery period without feeding, 6 size-matched groups ($n = 17$; 17.5 ± 0.1 g, mean fish weight ± SE) were selected for each genotype, and groups were intraperitoneally injected (at the ventral midline anterior to the anal fin) with (1) a control (CTRL) injection of 0.01 M phosphate buffered saline, pH 7.4, (2) a low (Low) dose of Posilac™ (Monsanto Corporation, St. Louis, MI) at 2.23 mg recombinant bovine GH/g body weight or (3) a high (High) dose of Posilac™ at 6.69 mg recombinant bovine GH/g body weight. Fish group names are described by genotype followed by dosage, i.e. NT-CTRL, NT-Low, NT-High, T-CTRL, T-Low and T-High. One group of each treatment "*x*" genotype was placed into each of two replicate 3000 L tanks (Tank A and B); thus each tank held each of the six fish groups. Tanks were supplied with well water (10.2 mg/L DO_2, 10 °C ± 1 °C) at 1 L/min/kg body weight and reared for 98 days on a simulated natural photoperiod (Vancouver, Canada, 45°15′N, 123°10′W from March to June). Starting on day 1, fish were fed to satiation twice daily using size-appropriate commercial salmon feed (Skretting Canada). Fish rearing and experimentation was conducted as per Canadian Council for Animal Care guidelines and at Fisheries and Oceans Canada's laboratory in West Vancouver which possesses physical containment measures to prevent escape of transgenic fish to the natural environment.

At 2-week intervals following injection, fish were anesthetized in tricane methane-sulphonate (MS 222; 0.2 g/L supplemented with air and 0.2 g of sodium bicarbonate/L), weighed, and measured for fork-length. At 14 weeks post-injection all fish were again assessed for size and 14 NT-CTRL, 13 NT-Low, 12 NT-High, 13 T-CTRL, 12 T-Low and 11 T-High fish were obtained from Tank A and Tank B and rapidly team sampled for blood, pituitary, muscle and liver tissue, with fish taken from the different groups randomly throughout the sampling period. Blood was centrifuged at 4000*g* for 5 min at 4 °C, and plasma removed and frozen at −80 °C for measurement of growth hormone (GH) and insulin-like growth factor-I (IGF-I) protein levels. Pituitary, muscle and liver were placed in RNAlater (Ambion) at 4 °C overnight and then frozen at −80 °C. White muscle was dissected from above the lateral line on the left side of the fish 1 cm behind the operculum, and a liver sample was taken from the tip of the posterior lobe.

In a second experiment, NT and T coho salmon fry (4 weeks old, average weight 0.5 g) were injected with either 4 mg recombinant bovine GH/g body weight or a sham injection as described above and 60 fish were assigned to each of four groups; NT-CTRL, NT-GH, T-CTRL and T-GH. The fry were held in 6 tanks of 40 fish (10 fish from each treatment) at the conditions above and 10 representative fish per treatment were randomly selected and measured for weight and length at 0, 2 and 4 weeks. Specific growth rates (SGR) were calculated as follows: $SGR = 100[\ln (W_2 \times W_1^{-1})](d_2 - d_1)^{-1}$ where W = weight in grams and d = days.

2.2. Hormone analysis

Plasma growth hormone levels were measured using an established ^{125}I-based radioimmunoassay [65] that measures salmon GH and does not cross react with exogenous bGH. Plasma IGF-I concentrations were determined using an ^{125}I radioimmunoassay IGF-I kit (GroPep IGF-I Fish Kit, Adelaide, Australia) as described [55].

2.3. RNA extraction and quantitative PCR

RNA from frozen muscle and liver samples were extracted, quantified and measured for β-actin, GH and IGF-I mRNA levels using quantitative PCR (Q-PCR) as in Raven et al. [55]. Whole pituitaries were weighed to the nearest μg (PerkinElmer AD6 Autobalance), and RNA was extracted by homogenization using a micropestle and Micro-Aqueous Kit (Ambion). To allow quantitation of RNA recovery per pituitary, column elution volumes were measured and RNA concentrations determined using a PicoGreen kit (Applied Biosystems) on a FLUROStar Omega plate reader. 500 ng of RNA was reverse transcribed using a SuperScript III Reverse Transcription Kit (Invitrogen) and Q-PCR was performed and analyzed on an Applied Biosystems 7500 Real-Time PCR System using a dilution series of pooled cDNA. Inter-plate differences within a group were compensated for by measuring four RNA samples on each plate (plate standards) and correcting RNA quantities on each plate relative to the change in the mean of the four samples (mean standard sample quantities from initial plate/mean standard sample quantities on plate of interest *X* experimental sample on plate of interest). β-actin quantities showed no significant differences between groups for muscle and liver (data not shown) and therefore were used to normalize muscle and liver mRNA levels (GH or IGF-I mRNA levels/β-actin mRNA level). Pituitary β-actin did show significant differences between groups and thus was not used to normalize mRNA levels. Pituitary mRNA levels are reported per 0.01 ng RNA for GH and per 10 ng RNA for GHR, levels per whole pituitary, levels per whole pituitary per g fish, and levels per mg pituitary. We assumed that equal pituitary homogenization and extraction of RNA and reverse transcription of RNA occurred between groups, although these technical variables could have increased variance and made detection of differences among groups more difficult.

2.4. Statistical analyses

For all analyses, a two-way ANOVA using the variables of genotype and bGH dose was used to assess treatment effects, followed by Holm-Sidak multiple comparison tests to identify intergroup differences. In cases where data were not normal or variances were not equal, a transformation was applied followed by two-way ANOVA. If data transformation did not result in normal data or equal variances, a Kruskal–Wallis one-way ANOVA on ranks was performed on the six treatment groups (genotype and dose variables combined) followed by Dunn's multiple comparison tests. Grubb's outlier test was performed on growth hormone data and 2 outlier data points were removed. Correlation analyses were performed using Pearson