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Nutritional status and growth hormone regulate insulin-like growth factor binding protein (*igfbp*) transcripts in Mozambique tilapia

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ABSTRACT

Growth in teleosts is controlled in large part by the activities of the growth hormone (Gh)/insulin-like growth factor (Igf) system. In this study, we initially identified *igf-binding protein (bp)1b*, *-2b*, *-4*, *-5a* and *-6b* transcripts in a tilapia EST library. In Mozambique tilapia (*Oreochromis mossambicus*), tissue expression profiling of *igfbps* revealed that *igfbp1b* and *-2b* had the highest levels of expression in liver while *igfbp4*, *-5a* and *-6b* were expressed at comparable levels in most other tissues. We compared changes in hepatic *igfbp1b*, *-2b* and *-5a* expression during catabolic conditions (28 days of fasting) along with key components of the Gh/Igf system, including plasma Gh and Igf1 and hepatic *gh receptor* (*ghr2*), *igf1* and *igf2* expression. In parallel with elevated plasma Gh and decreased Igf1 levels, we found that hepatic *igfbp1b* increased substantially in fasted animals. We then tested whether systemic Gh could direct the expression of *igfbps* in liver. A single intraperitoneal injection of ovine Gh into hypophysectomized tilapia specifically stimulated liver *igfbp2b* expression along with plasma Igf1 and hepatic *ghr2* levels. Our collective data suggest that hepatic endocrine signaling during fasting may involve post-translational regulation of plasma Igf1 via a shift towards the expression of *igfbp1b*. Thus, Igfbp1b may operate as a molecular switch to restrict Igf1 signaling in tilapia; furthermore, we provide new details regarding isoform-specific regulation of *igfbp* expression by Gh.

1. Introduction

Growth in vertebrates is largely controlled by the coordinated activities of the growth hormone (Gh)/insulin-like growth factor (Igf) system. In teleost fishes, the regulation of growth performance by the Gh/Igf system also seems to be highly conserved (Duan, 1997; Wood et al., 2005). Growth performance is often used as an indicator of the status of individuals and populations in culture and the wild, and therefore, major effort has been applied towards garnering a more comprehensive understanding of how multiple components of the Gh/Igf system interact to control growth and metabolism (Picha et al., 2008; Beckman, 2011).

Gh is a member of the Gh/prolactin/somatolactin family of pituitary hormones that regulate numerous physiological processes that include somatic growth, immune function, osmoregulation, lipid and protein metabolism and feeding behavior (Duan, 1997; Kawauchi and Sower, 2006). The physiological actions of Gh on target tissues are mediated via transmembrane receptors that activate the Jak/Stat signaling pathway (Bole-Feysot et al., 1998). Genes encoding Gh receptors (*ghrs*) have been cloned from several teleost species and display a wide distribution of expression across tissues in accord with the pleiotropic actions of Gh (Reindl and Sheridan, 2012). We have identified two *ghr* sequences in Mozambique tilapia (*Oreochromis mossambicus*), denoted *ghr1* and *ghr2* (Kajimura et al., 2004; Pierce et al., 2007). Phylogenetic analyses, tissue expression patterns, and regulation by Gh of these two *ghrs* suggest that *ghr2* encodes the primary Gh receptor (Kajimura et al., 2004; Pierce et al., 2007, 2012). Further evidence of divergent physiological roles for these two

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receptors comes from observations that *ghr1* and *ghr2* transcript expression is differentially responsive to fasting (Saera-Vila et al., 2005; Uchida et al., 2009; Fox et al., 2010), temperature (Gabillard et al., 2006), stressors (Saera-Vila et al., 2009), metabolic hormones (Reindl et al., 2009; Pierce et al., 2012), xenobiotics (Davis et al., 2009), and salinity (Pierce et al., 2007; Breves et al., 2011). Dynamic *ghr* expression within key metabolic tissues following alterations in nutritional, osmoregulatory and endocrine states occurs across teleosts (Reinecke, 2010; Reindl and Sheridan, 2012). Thus, the capacity to modulate *ghr* expression appears to serve as a fundamental mechanism to regulate the sensitivity of target tissues to Gh.

In liver and muscle, Gh can act directly by stimulating mitosis and differentiation among other cellular behaviors, and acts indirectly by initiating the production and release of Igfs (Wood et al., 2005; Duan et al., 2010). In mammals, Igf1 is regarded as the primary somatomedin during postnatal life. Igf2 exhibits minimal dependence upon endocrine Gh and its actions have been largely associated with fetal growth and development (Daughaday and Rotwein, 1989; Constancia et al., 2002). In a subset of teleosts, however, growing evidence supports the operation of Igf2, in addition to Igf1, as a somatomedin throughout the life cycle. For example, hepatic *igf2* expression and plasma Igf2 levels are stimulated by Gh both *in vivo* and *in vitro* (reviewed by Reindl and Sheridan, 2012) and Igf2 administration stimulates growth in juvenile tilapia (Chen et al., 2000). These findings underscore the importance of considering both Igfs when characterizing the actions of Gh in teleosts.

Igf1 and Igf2 interact with a family of binding proteins, termed Igf binding proteins (Igfbps); the specific character of these interactions determines how the biological actions of Igfs are expressed because Igfbps affect hormone availability, transport and receptor binding (Duan et al., 2010). As in mammals, six Igfbps have been identified in fishes (Daza et al., 2011). Although the mechanisms of action are poorly understood, there is good evidence that Igfbps also exhibit ligand-independent activities (Firth and Baxter, 2002). Gh is an important regulator of *igfbp* expression and protein secretion in mammals (Yamada and Lee, 2009). This link in fishes, however, has only been investigated in a restricted number of salmonid species (Cheng et al., 2002; Pierce et al., 2006), with few studies aimed at characterizing the effects of Gh on *igfbp* expression *in vivo*.

We have previously characterized the responses of the Gh/Igf system to changes in metabolic status in Mozambique tilapia with particular attention to plasma Gh and Igf1 levels and the expression of hepatic *ghr2*, *igf1* and *igf2* transcripts (Uchida et al., 2003; Fox et al., 2006, 2010; Pierce et al., 2007; Peddu et al., 2009). In turn, the Mozambique tilapia is positioned as a key *in vivo* model from which to advance our understanding of how the Gh/Igf system, via the activities of Igfbps, responds to nutritional status. In this study, we identify *igfbp1b*, *-2b* and *-5a* as highly expressed hepatic transcripts and assess their regulation by nutrient restriction and Gh, and therefore contribute new details on the physiology of Igfbps in a widely cultured teleost.

2. Materials and methods

2.1. Animals

Male Mozambique tilapia (*O. mossambicus*) were maintained in re-circulating fresh water (FW) under artificial photoperiod (14 h light, 10 h dark) at the Department of Biological Sciences (University of Arkansas, Fayetteville, AK). Fish were fed Aquamax Starter Fingerling 300 (PMI Nutrition International, Brentwood, MO) and water temperatures were maintained between 20 and 22 °C. The Institutional Animal Care and Use Committee of the University of Arkansas approved all housing and experimental protocols.

2.2. igfbp sequences

Sequences for *igfbp1b* (Acc. No. XM_003438121), *igfbp2b* (Acc. No. XM_005450484), *igfbp4* (Acc. No. XM_003454633), *igfbp5a* (Acc. No. XM_003443250.2) and *igfbp6b* (Acc. No. XM_003441337) were identified in the Nile tilapia (*Oreochromis niloticus*) transcriptome (Lee et al., 2010) by searching with tBLASTn at the NCBI web resource using the relevant sequences known from rainbow trout (*Oncorhynchus mykiss*) (Kamangar et al., 2006). The sequence similarities of rainbow trout and Nile tilapia *igfbp1b*, *-2b*, *-4*, *-5a* and *-6b* were 79%, 75%, 76%, 70% and 84%, respectively. Nucleotide similarities between Nile and Mozambique tilapia *igfbp* sequences were $\geqslant$98%.

2.3. Fasting experiment

Tilapia (40–60 g) were distributed into 6 tanks representing two treatment groups (3 fed and 3 fasted). Fish were allowed to acclimate to the experimental tanks for 4 weeks prior to the beginning of the experiment. Following this initial acclimation period, food was withheld from 3 tanks while the animals contained in the other 3 tanks were fed at ~5% of their body weight twice daily.

Body weight and standard length were measured at each sampling point for calculation of condition factor: (body weight, g)/(standard length, cm)^3 × 100. At sampling, all fish were anesthetized in 2-phenoxyethanol (2-PE; 0.3 ml/L; Sigma, St. Louis, MO) and blood was collected from the caudal vasculature by a needle and syringe treated with ammonium heparin (200 U/ml, Sigma). Plasma was separated by centrifugation at 4 °C and stored at −80 °C until analyses for plasma glucose and Igf1. Liver tissue was collected, snap frozen in liquid nitrogen, and stored at −80 °C until RNA isolation.

2.4. Hypophysectomy and Gh injection

A Gh injection experiment was conducted at the Hawaii Institute of Marine Biology, University of Hawaii. Tilapia (70–150 g) were reared in outdoor tanks with a continuous flow of FW under natural photoperiod at 24–26 °C and fed a commercial diet *ad libitum* (Skretting, Tooele, UT). Hypophysectomy was performed by the transorbital technique (Nishioka, 1994). Briefly, tilapia were anesthetized by immersion in buffered tricaine methanesulfonate (100 mg/L, Argent Chemical Laboratories, Redmond, WA) and 2-PE (0.3 ml/L) in FW. Following removal of the right eye and underlying tissue, a hole was drilled through the neurocranium, and the pituitary was aspirated with a modified Pasteur pipette. The orbit was then packed with microfibrillar collagen hemostat (Ethicon, Somerville, NJ) and fish were allowed to recover in brackish water (12‰) composed of seawater (Kaneohe Bay, Hawaii) diluted with FW. Following recovery, fish were transferred to re-circulating experimental aquaria containing aerated brackish water and treated with kanamycin sulfate (National Fish Pharmaceuticals, Tucson, AZ). Upon transfer to experimental aquaria, food was withheld in all experiments to control for the possibility of confounding effects due to disparate feeding patterns between individuals. Water temperatures were maintained between 24 and 26 °C.

To test the effects of Gh on the Gh/Igf system, hypophysectomized fish maintained in brackish water for 3–4 days following surgery (n = 6–9) were administered ovine Gh (oGh; 5 μg/g body weight) or saline vehicle by a single intraperitoneal (IP) injection. oGh was obtained from the National Hormone and Peptide

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