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Comparative Biochemistry and Physiology, Part B 149 (2008) 209–214
CBP
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Metabolic rate and reactive oxygen species production in different genotypes of GH-transgenic zebrafish

C.E. Rosa [a,b,*], M.A. Figueiredo [a], C.F.C. Lanes [a,c], D.V. Almeida [a,b], J.M. Monserrat [a,b], L.F. Marins [a,b,c]

[a] *Fundação Universidade Federal do Rio Grande, Brazil*
[b] *Programa de Pós-Graduação em Ciências Fisiológicas - Fisiologia Animal Comparada, Brazil*
[c] *Programa de Pós-Graduação em Aqüicultura, Brazil*

Received 8 August 2007; received in revised form 12 September 2007; accepted 12 September 2007
Available online 20 September 2007

Abstract

Growth hormone overexpression increases growth and consequently increases the metabolic rate in fishes. Therefore, the objective of this study was to evaluate the effects of growth hormone overexpression in zebrafish *Danio rerio* in terms of growth, oxygen consumption, reactive oxygen species production, lipid hydroperoxide content, antioxidant enzyme activity and glutamate-cysteine ligase catalytic subunit gene expression. The employed models were wild type and transgenic (hemizygous and homozygous) zebrafish expressing the *Odonthestes argentinensis* growth hormone gene directed by the *Cyprinus carpio* beta-actin promoter. Higher growth parameters were observed in the hemizygous group. The homozygous group possessed higher oxygen consumption and reactive oxygen species production. Growth hormone transgenesis causes a decrease in glutamate-cysteine ligase catalytic subunit expression, an enzyme responsible for glutathione synthesis. Although the lipid hydroperoxide content was similar between groups, we demonstrate that growth hormone overexpression has the potential to generate oxidative stress in fishes.

Keywords: Growth hormone; Transgenic zebrafish; Oxygen consumption; Reactive oxygen species; Glutamate-cysteine ligase catalytic subunit

1. Introduction

Growth hormone (GH) is the most frequently employed gene for fish transgenesis and several GH-transgenic fish have demonstrated increased growth performance (Devlin et al., 2006). In fact, GH overexpression in fish generally causes significant enhancement of growth rates, which can result in large differences in size at a particular age, up to a 35 fold increase in weight gain (Devlin et al., 2004; Nam et al., 2001).

GH is a peptide hormone that has a growth-promoting action in teleosts, and furthermore is involved in osmoregulation, reproductive functions, as well as in carbohydrate, lipid and protein metabolism (Le Bail and Boeuf, 1997). This hormone possesses well-established protein anabolic effects, in mammalian and fish species, with an increase in nitrogen retention due to an increase in protein synthesis and/or a decrease in protein degradation, or both (Mauras and Haymond, 2005).

Because GH affects many processes in addition to growth, transgenic fish overexpressing this hormone can show a broad range of pleiotropic effects on morphology, physiology, metabolism, immunology and behavior (Devlin et al., 2006). As a result of GH overexpression or administration, significant increases in metabolic rate and oxygen consumption in Atlantic salmon (Seddiki et al., 1995, 1996; Herbert et al., 2001; Stevens et al., 1998; Cook et al., 2000) and in tilapia have been reported (McKenzie et al., 2000, 2003).

Aerobic metabolism causes the production of reactive oxygen species, even under basal conditions (Sies, 1993). It has been demonstrated that 0.1% of the total oxygen consumed

* Corresponding author. Fundação Universidade Federal do Rio Grande, Programa de Pós-Graduação em Ciências Fisiológicas - Fisiologia Animal Comparada, Avenida Itália, Km 8, CEP 96201-900, Rio Grande, RS, Brazil. Tel./fax: +55 53 32336850.
E-mail address: ninovski@octopus.furg.br (C.E. Rosa).

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doi:10.1016/j.cbpb.2007.09.010

in the aerobic respiration is converted in the mitochondria into reactive oxygen species (ROS) (Fridovich, 2004), which can oxidize and damage biomolecules (Storey, 1996). Under normal conditions, excessive ROS formation and concomitant damage at cellular and tissue levels is controlled by cellular antioxidant defense systems (ADS) (Sies, 1991). Like other vertebrates, fish possesses well-developed ADS which utilizes the enzymatic triad superoxide dismutase (SOD), catalase (CAT) and glutathione-peroxidase among others, in addition to non-enzymatic compounds, including glutathione (GSH), vitamin E and vitamin C (Wilhelm-Filho, 1996). GSH is considered the cell's first line of defense against ROS, because it serves several functions such as: scavenger of reactive oxygen species, a substrate of antioxidant enzymes (GPx and GST), reactivation of enzymes inhibited under oxidative stress and vitamin E regeneration (Griffith, 1999; Dickinson and Forman, 2002). GSH is the most abundant intracellular non-protein thiol, and is found in the millimolar range in most cells (Storey, 1996). GSH synthesis occurs constitutively, but can also be up-regulated in response to stress (Iles and Liu, 2005).

Several reports demonstrate that GH-transgenic mice have augmented growth rate and enhanced metabolism (Bartke et al., 1994). However, in this species, the ADS were not enhanced to cope with the excess of oxygen utilization and probably ROS generation (Hauck and Bartke, 2000; Brown-Borg and Rakoczy, 2000, 2003; Brown-Borg et al., 2005). It has been demonstrated that GH-transgenic coho salmon had higher glutathione content in liver, muscle and plasma in comparison to wild type strains (Leggatt et al., 2007).

Within this context, the objective of the present study was to evaluate the oxygen consumption rate and the ROS generation in different genotypes of GH-transgenic zebrafish. Additionally, the growth performance of hemizygous and homozygous GH-transgenic zebrafish and some others parameters related to oxidative stress such as the activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), glutamate-cysteine ligase catalytic subunit gene expression and lipid peroxidation were also evaluated.

2. Materials and methods

2.1. GH-transgenic fish and maintenance conditions

The GH-transgenic zebrafish lineage (F0104) used here was produced using two transgenes comprised of carp (*Cyprinus carpio*) β-actin promoter driving the expression of the growth hormone cDNA from the marine silverside fish *Odonthestes argentinensis* (Marins et al., 2002), with the same promoter driving the expression of the green fluorescent protein (GFP) gene as a transgenesis label (Figueiredo et al., 2007). Hemizygous (HE) transgenic and wild type (WT) sibling fish were produced by crossing hemizygous males from the F0104 lineage and wild type females. The offspring was analyzed by epifluorescence microscopy (excitation 485 nm, emission 520 nm) for transgenic fish identification through GFP expression. Homozygous (HO) transgenic fish were produced by crossing homozygous animals from the F0104 lineage.

Transgenic and non-transgenic fish were reared until 4 months of age in a closed circulation water system composed of 25 L tanks at 28 °C, 14 h light/10 h dark photoperiod, fed with high-protein (47.5%) commercial food (5% of body weight/day). The groups were maintained separately in a 1.25 animal/L density. At the end of this period a group of seven animals per genotype were employed in the oxygen consumption measurements. Another group was anesthetized (0.1 mg/mL Tricaine). The weight (W) and total length (L) were measured. In addition, the condition factor (K) was calculated ($K=(W/L^3)\times 10^3$). Some of these animals were employed for ROS generation assay or frozen (−80 °C) for the biochemical analysis.

2.2. Oxygen consumption measurements

Standard oxygen consumption rates of individual specimens of WT, HE and HO zebrafish, with similar weight, around 298.40± 15.03 mg (mean wet mass±S.E.) were measured (Nithart et al., 1999). Briefly, the animals, starved for 24 h, were individually maintained in respiration chambers, with a volume of 100 mL, containing fresh water at 28 °C. The animals were acclimated to this condition for at least 10 min before the beginning of the tests. Oxygen concentrations were measured with an oxymeter (Digimed) after 10 min of enclosure within the chamber. Oxygen consumption was expressed as mg $O_2\times h^{-1}\times g\ ww^{-1}$.

2.3. Assessment of intracellular ROS generation

Generation of reactive oxygen species (ROS) production of caudal fin muscle was analyzed using the ROS-sensitive dye diclorofluorescein-diacetate (DCF-DA) (Myhre and Fonnum, 2001). Animals ($n=5$ per group) were anesthetized (0.1 mg/mL Tricaine) and dissected and the fin muscle homogenized (1:3 w/v) in cold buffer containing 0.32 mM sucrose, 20 mM HEPES, 1 mM $MgCl_2$ and 0.5 mM PMSF (pH=7.4). The homogenate was centrifuged for 20,000 g for 20 min (4 °C) and the obtained supernatant employed in the following steps. The total protein content was measured using a commercial diagnostic kit (LABTEST), based on the Biuret reagent, using bovine serum albumin as standard. The supernatant was diluted in cold homogenization buffer for a final concentration of 1 mg/mL of protein, and this was the sample source for the ROS generation assay. Samples were incubated with the fluorogenic compound at a final concentration of 40 μM in HEPES buffer (30 mM). Fluorescence intensity was monitored for 30 min at 28 °C, using a fluorimeter (Victor 2, Perkin Elmer), with an excitation and emission wavelengths of 485 and 520 nm, respectively. The curve areas of fluorescence intensity were integrated and the total area was used for comparison. The results were expressed as Fluorescence Units × min (FU × min).

2.4. Enzyme assays

For the enzymatic assays, six animals of each class (non-transgenic, hemizygous and homozygous) were selected and cryoanesthetized and the caudal fin muscles were dissected for the analysis. The samples were homogenized (1:3 w/v) in cold