Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part B 145 (2006) 27–34
CBP
www.elsevier.com/locate/cbpb

Expression of growth hormone family and growth hormone receptor during early development in the Japanese eel (*Anguilla japonica*)

Yuichi Ozaki [a,*], Haruhisa Fukada [a,1], Hideki Tanaka [b], Hirohiko Kagawa [b,2], Hiromi Ohta [b,3], Shinji Adachi [a], Akihiko Hara [a], Kohei Yamauchi [a]

[a] *Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan*
[b] *National Research Institute of Aquaculture, Fisheries Research Agency, Nansei, Mie 516-0193, Japan*

Received 20 December 2005; received in revised form 3 May 2006; accepted 27 May 2006
Available online 2 June 2006

Abstract

In a previous study, we identified cDNAs encoding the growth hormone receptor (eGHR1) and eGHR1 homologue (eGHR2) in Japanese eel (*Anguilla japonica*). In the present study, changes in the developmental expression of growth hormone (GH), eGHR1 and eGHR2 were investigated in the Japanese eel eggs and preleptocephali by RT-PCR and immunohistochemical methods in an attempt to examine the involvement of these proteins in larval growth. The GH transcripts and the production of GH protein were not detected in the newly hatched larvae and preleptocephali at day 3 post-hatch, however, these were detected at day 6 post-hatch, and also detected at higher levels at day 10 post-hatch. In contrast, prolactin and somatolactin transcripts could not be detected in all preleptocephalus specimens (newly hatched larvae and preleptocephali at day 3, 6 and 10 post-hatch). eGHR1 and eGHR2 transcripts were detected in all preleptocephalus specimens. Therefore, it is plausible that the actions of GH during the preleptocephalus stage are mediated through the eGHRs. The present data suggest that GHR-mediated actions of GH begin at the same time as the initiation of GH production, and that GH plays important roles in larval growth and survival to the leptocephalus stage. eGHR1 mRNA, which is thought to be of maternal origin, was also detected in ovulated eggs. However, the role of eGHR1 mRNA in eggs is not clear.

Keywords: Growth hormone; Growth hormone receptor; Prolactin; Somatolactin; Japanese eel; Preleptocephalus; Egg; Early development

1. Introduction

Growth hormone (GH) is the principal regulator of somatic growth and metabolism in fish, as in other vertebrates (Björnsson, 1997; Pérez-Sánchez, 2000). In tetrapod vertebrates, embryonic and fetal growth had been considered to be independent of pituitary GH, although it is required for postnatal growth and development. However, recent studies suggested that extra-pituitary GH play important roles on the early embryonic growth and differentiation (Sanders and Harvey, 2004). Growth of larvae immediately after hatching is critical for survival. However, little is known about the regulation of growth in teleosts during early development, although involvement of the endocrine system is obvious. The presence of GH in the embryo and early larval stages has been confirmed in several fishes (Ayson et al., 1994; Yang et al., 1999; de Jesus et al., 2002). The presence of GH in the early developmental stages suggests that it plays important roles in larval growth and survival. This has been further supported by the growth-promoting effects of exogenous GH, and the transfer and over-expression of the GH gene demonstrated in the teleost larvae (Chen et al., 1993; Martinez et al., 1996; Ben-Atia et al., 1999).

* Corresponding author. Genetic Strains Research Center, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan. Tel./fax: +81 55 981 5849.
E-mail address: yozaki@lab.nig.ac.jp (Y. Ozaki).
[1] Present address: Faculty of Agriculture, Kochi University, Nankoku, Kochi 783-8502, Japan.
[2] Present address: Department of Biological Production and Environmental Science, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan.
[3] Present address: Department of Fisheries, Faculty of Agriculture, Kinki University, Nara 631-8505, Japan.

1096-4959/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2006.05.009

The Japanese eel (*Anguilla japonica*) is a catadromic teleostean species with a complex life cycle. It is assumed that after completing growth in freshwater habitats, adult Japanese eels migrate thousands of kilometers into the ocean to spawn (Tsukamoto, 1992). After hatching, preleptocephalus larvae develop into leptocephali, which drift with the current toward the coast where the leptocephali metamorphose into glass eels (Tsukamoto et al., 1992; Sakakura et al., 1996). On entering freshwater, the glass eels pigment and then live as immature yellow eels before migrating back to the sea. In eels, it was assumed that higher levels of GH transcripts in females caused higher growth rates in females than in males (Degani et al., 2003). The growth-promoting effect of GH was demonstrated also in elvers (Degani and Gallagher, 1985); however, there is no information about GH during the preleptocephalus stage.

It is believed that the actions of GH are initiated by binding to the GH receptor (GHR), which is localized on the cell membrane of target tissues. This in turn, induces a phosphorylation cascade resulting in cell signaling and gene expression (Herrington and Carter-Su, 2001; Zhu et al., 2001). In order to understand the precise actions of GH, it is necessary to examine the presence and changes in GHR expression. In mammals, the expression and functionality of GHR during early embryonic stages have been demonstrated (Sanders and Harvey, 2004). However, the fish GHR has not been well characterized and the involvement of GHR in the larval growth remains unclear, largely because the GHR gene has only recently been identified. cDNAs for the piscine GHR were first isolated from goldfish and turbot (Lee et al., 2001; Calduch-Giner et al., 2001). In vertebrates, it was generally accepted that only one gene encoding GHR existed. However, a recent study has demonstrated the presence of two different GHR genes in fish (Saera-Vila et al., 2005). In our previous study, we described the isolation of two cDNAs encoding GHR-like genes (eGHR1 and eGHR2) from the Japanese eel. Furthermore, binding assays showed that recombinant eGHR1 specifically binds to GH, although specific binding of recombinant eGHR2 to GH could not been confirmed (Ozaki et al., 2006a).

Because of their considerable structural similarity, GH, prolactin (PRL) and somatolactin (SL) are considered to have evolved from a common ancestral molecule by gene duplication, and thus categorized into the GH/PRL family. Several reports have revealed that the PRL and SL are present during embryonic and larval stages of some fish species, leading to the suggestion that these pituitary hormones may play important roles in growth and development during early larval stages (Ayson et al., 1994; Yang et al., 1999; Pandolifi et al., 2001).

The early development of the Japanese eel has received very little in the way of research attention, largely because of the very limited availability of eggs and larvae. It is very difficult to obtain live specimens of eel larvae from the sea that are in early developmental stages, although it is possible to produce the larvae artificially (Tanaka et al., 2001). In the present study, we studied changes in expressions of GH and eGHRs during early development in the larvae obtained from artificially matured Japanese eel to confirm whether GH mediated via the GHR is involved in the larval growth. In addition, we report associated changes in expressions of PRL and SL.

2. Materials and methods

2.1. Animals

This study utilized preleptocephali of Japanese eel cultured at the National Research Institute of Aquaculture, Mie, Japan. These larvae were acquired by the artificial fertilization of ovulated eggs from cultured female eels previously subjected to repeated injections of salmon pituitary extract (20 mg per fish per week). Ovulation was induced by injection of 17α, 20β-dihydroxy-4-pregnen-3-one (2 mg per kg body weight) (Ohta et al., 1997). Semen was hand stripped from cultivated male eels injected with human chorionic gonadotropin (1 IU per g body weight per week) in order to induce spermiation, and then incubated in artificial seminal plasma containing 15.2 mM KCl for acquisition of motility and fertility (Ohta et al., 1997). Fertilized eggs were incubated in floating nets placed in a natural, filtered flow-through seawater aquarium at 23 °C. Hatching larvae were moved to a five liter acrylic bowl tank with 23 °C natural, filtered flow-through seawater, and cultured until 10 days after hatching without feeding. Ovulated eggs, fertilized eggs, newly hatched larvae, and preleptocephali at day 3, 6 and 10 post-hatch were collected as specimens. Glass eels of Japanese eel (56–61 mm) were obtained from a commercial dealer.

2.2. Production of a polyclonal antibody against eel GH

Recombinant eel GH (reGH) was prepared to use as antigen. cDNA fragments encoding mature peptides of eel GH were amplified by RT-PCR. The primers were designed from the sequence of GH as reported previously (Saito et al., 1988) but including *Bam*HI restriction sites. The amplified fragments were subcloned into the pCR®2.1 plasmid vector using a TA cloning kit (Invitrogen, Carlsbad, CA, USA), and were subsequently digested with *Bam*HI. The digested fragments were subcloned into the pQE-30 expression vector (QIAGEN, Hilden, Germany) with a 6xhistidine-tag upstream of the region encoding eel GH. This expression vector was then used to prepare reGH. After subcloning into the pQE vector, reGH was expressed in *Escherichia coli* following induction by isopropyl β-D-thiogalactopyranoside (IPTG) and purified using The QIAexpressionist (QIAGEN). Briefly, the fusion protein was purified from the bacterial lysate by affinity chromatography using a metal chelate adsorbent nickel–nitrilotriacetic acid agarose. The fusion protein was eluted with elution buffer (8 M urea, 0.1 M sodium phosphate, 10 mM Tris, pH 4.0), and the elutant was dialyzed for 3 days against 20 mM Tris–HCl buffer (pH 8.0).

A rabbit was immunized with 1 mg of the reGH dissolved in 20 mM Tris–HCl emulsified in an equal volume of Freund's complete adjuvant (IATRON, Tokyo, Japan). The antiserum against reGH obtained from the immunized rabbit was mixed with bacterial lysate to absorb the antibodies against the bacterial lysate that might have been generated by contamination during the purification of reGH. Then, the mixed solution was centrifuged at 10,000 rpm for 1 h and the supernatant was collected as antiserum. Moreover, in order to purify rabbit IgG against reGH (anti-reGH) from the antiserum, the antiserum was mixed with saturated