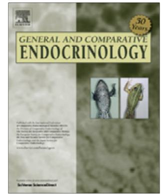
General and Comparative Endocrinology 178 (2012) 427–435

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen



Relationships between gill Na^+,K^+-ATPase activity and endocrine and local insulin-like growth factor-I levels during smoltification of masu salmon (*Oncorhynchus masou*)

Takahiro Shimomura [a], Takuro Nakajima [a], Moeri Horikoshi [a], Anai Iijima [b], Hirokazu Urabe [b], Shinya Mizuno [b], Naoshi Hiramatsu [a], Akihiko Hara [a], Munetaka Shimizu [a,*]

[a] Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan
[b] Salmon and Freshwater Fisheries Research Institute, Hokkaido Research Organization, Kitakashiwagi 3-373, Eniwa, Hokkaido 061-1433, Japan

ARTICLE INFO

Article history:
Received 1 December 2011
Revised 17 April 2012
Accepted 12 June 2012
Available online 28 June 2012

Keywords:
Insulin-like growth factor-I
Salmon
Smoltification
Gill
Na^+,K^+-ATPase
Serum

ABSTRACT

We established profiles of insulin-like growth factor (IGF)-I mRNA in the liver, gill and white muscle and circulating IGF-I during smoltification of hatchery-reared masu salmon, and compared with that of gill Na^+,K^+-ATPase (NKA) activity. Gill NKA activity peaked in May and dropped in June. Liver *igf1* mRNA was high in March and decreased to low levels thereafter. Gill *igf1* increased from March, maintained its high levels during April and May and decreased in June. Muscle *igf1* mRNA levels were relatively high during January and April when water temperature was low. Serum IGF-I continuously increased from March through June. Serum IGF-I during March and May showed a positive correlation with NKA activity, although both were also related to fish size. These parameters were standardized with fork length and re-analyzed. As a result, serum IGF-I and gill *igf1* were correlated with NKA activity. On the other hand, samples from desmoltification period (June) that had high serum IGF-I levels and low NKA activity disrupted the relationship. Expression of two IGF-I receptor (*igf1r*) subtypes in the gill decreased in June, which could account for the disruption by preventing circulating IGF-I from acting on the gill and retaining it in the blood. The present study suggests that the increase in gill NKA activity in the course of smoltification of masu salmon was supported by both endocrine and local IGF-I, and the decrease during desmoltification in freshwater was due at least in part to the down-regulation of gill IGF-I receptors.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

All anadromous salmonids are hatched in freshwater, stay in the river/lake for certain period and migrate to the ocean. With a few exceptions, juvenile salmon are intolerant to full seawater and need to acquire seawater adaptability as well as changes adaptive to ocean life prior to the downstream migration. Such transition is called smoltification (parr-smolt transformation) that involves development of seawater adaptability, body silvering, darkening of fin margins, decrease in condition factor, change in rheotaxis and formation of school [19,55]. These changes are sometimes independent one another but occur in spring through synchronization by photoperiod [8,19,55,56]. Several endocrine systems are involved in smoltification and often act synergistically to induce a change. For instance, the acquisition of seawater adaptability is under control by cortisol and the growth hormone (GH)-insulin-like growth factor (IGF)-I system [26,27]. On the other hand, some changes may be coordinated by a single endocrine system. The GH-IGF-I system controls animal growth and also plays a crucial role in development of seawater adaptability in salmonids [11,26,42]. The GH-IGF-I system promotes growth via multiple pathways [10,22,34]. GH acts on target tissues directly or indirectly through IGF-I, which is primarily produced by the liver in stimulation with GH, secreted into bloodstream and mediates GH actions [10,34]. IGF-I is also expressed in virtually all types of tissues and exerts autocrine/paracrine actions [22]. Understanding how these hormones improve seawater adaptability is particularly important for hatchery programs of several salmonid species since degree of seawater adaptation directly affects initial survival of released fish in seawater, growth in following summer and survive as adults [3,7,12].

The gill, along with the kidney and intestine, is a major organ responsible for maintaining ion concentrations of the body. Improvement of seawater adaptability at the gill level is largely achieved by proliferation, differentiation/transformation and specific localization of the chloride cells with enhanced activity of Na^+,K^+-ATPase (NKA) and other ion transporters/channels [17,37,46]. NKA is located in the basolateral membrane of chloride cells and essential for extrusion of sodium ions from the cells.

* Corresponding author. Fax: +81 138 40 8897.
E-mail address: mune@fish.hokudai.ac.jp (M. Shimizu).

0016-6480/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2012.06.011

Cortisol and GH are known to enhance NKA activity by acting on its mRNA and/or protein and by inducing the changes of the chloride cells [23,25–27,40,43]. As is the case for growth regulation, some of the GH actions on NKA activity may be mediated by IGF-I, and local IGF-I (i.e. gill IGF-I) should also play a role [24,29]. However, what source of IGF-I is important is a matter of debate [42]. Accumulating evidence emphasizes importance of gill IGF-I in osmoregulation [42,44,57]. On the other hand, assessing the involvement of endocrine IGF-I in activating NKA has been encountered by the fact that during smoltification, a rapid lean growth also occurs in response to increasing day length, water temperature and food availability. Circulating IGF-I typically shows an increase during smoltification and may be important for both promoting growth and NKA activity [4,11]. However, what percentages of circulating IGF-I are partitioned to promote growth and osmoregulation, respectively, is not known. In order to analyze the IGF-I roles in the regulation of osmoregulation, a comprehensive data set on circulating IGF-I levels and tissue *igf1* mRNA during smoltification is necessary, which is somewhat incomplete to date. Indeed, there is no study measuring circulating IGF-I and liver *igf1* mRNA levels simultaneously during smoltification. The first goal of this study is to establish profiles of circulating IGF-I and *igf1* mRNA in tissues responsible for growth and osmoregulation (i.e. liver, gill and white muscle) in masu salmon (*Oncorhynchus masou*). We then performed correlation analyses to assess the involvement of endocrine and local IGF-I in increasing gill NKA activity.

2. Materials and methods

2.1. Fish

Under yearling and yearling masu salmon were reared in freshwater at the South Branch of Salmon and Freshwater Fisheries Institute, Hokkaido Research Organization (42°N, 140°E) (Nikai-gun, Hokkaido, Japan). Under yearling masu salmon were sorted by size (>10.5 cm) and visual inspection in November 2009 to remove precociously maturing males and potential non-smolting fish in the following spring. Fish were maintained in the river water in outdoor ponds (24.6 × 3.5 m) and fed twice (November-February) or three times (March-June) a day on a commercial diet (Nippon Formula Feed Mfg, Kanagawa, Japan) with standard rations at 0.4–1.9%/body weight. These fish were for stock enhancement and released to the river in May 2010. Some fish were kept in the same pond and reared until June. From November 2009 to June 2010, seven fish were sampled monthly. Fish were anesthetized by 3.3% 2-phenoxyethanol (Kanto Chemical, Tokyo, Japan) and measured for fork length and body weight. Condition factor was calculated as follows: (body weight) $\times$ 1000/(fork length)^3. Blood was withdrawn by a syringe from the caudal vein, allowed to clot overnight at 4 °C and centrifuged at 8,050*g* for 10 min. Serum was collected and stored at −30 °C until use.

2.2. Cloning of partial cDNAs for IGF-I and elongation factor-1a (EF-1α)

Liver cDNA was prepared from yearling masu salmon reared at Nanae Freshwater Experimental Station, Hokkaido University (Kameda-gun, Hokkaido, Japan) as described in Shimizu et al. [51]. Primer sets designed for Atlantic salmon (*Salmon salar*) IGF-I and EF-1α (Genbank ID: EF432852 and BG933853 [9]) (Table 1) were applied to masu salmon. Reverse transcriptase (RT)-PCR was performed with a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA) and AmpliTaq Gold® 360 Mater Mix (Applied Biosystems, Foster City, CA). PCR cycles consisted of 1 cycle of 95 °C for 10 min; 36 cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 1 min 30 s; 1 cycle of 72 °C for 7 min. PCR products were cloned into the pGEM-T Easy Vector Systems (Promega, Madison, WI) and positive clones were sequenced as described in Shimizu et al. [51].

2.3. RNA extraction and cDNA synthesis

Total RNA was extracted from the tissues as described in Shimizu et al. [51]. One and half microgram RNA was reverse-transcribed using SuperScript VILO cDNA Synthesis kit (Invitrogen, Carlsbad, CA) in a 10 μl reaction according to the manufacturer's instruction. cDNA was stored at −30 °C until use.

2.4. Real-time quantitative PCR (qPCR)

Primer sets for qPCR of IGF-I and EF-1α were designed based on the cloned masu salmon cDNA sequences using MacVector Ver 9 (MacVector Inc., Cary, NC). One primer in each assay was placed across an exon/exon boundary predicted from the gene structure of zebrafish from Ensembl data base (http://asia.ensembl.org/index.html). The primers for IGF-I target the signal peptide region.

RT-PCRs using these primers were performed to prepare assay standards. PCR products run on 1.5% agarose gel were excised and purified using QIAEX II Gel Extraction Kit (Qiagen, Valencia, CA). Copy numbers of the purified amplicon were calculated from the molecular weight of the amplion and concentration. The standard cDNA were serially diluted from 1×10^7 to 3×10^2 copies. qPCR was set up using Power SYBR Green PCR Master Mix (Applied Biosystems) in a reaction volume of 20 μl with primer concentration of 100 nM. The reaction mixture contained 0.1–2 μl cDNA template. qPCR was run on a 7300 Sequence Detector (Applied Biosystems) using the manufacturer's recommended cycling conditions: 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Measured values were normalized by those of *ef1a* and expressed as relative values.

Primers specific to type I IGF receptor subtypes (IGF-IRa and IGF-IRb) were designed based on the sequences of rainbow trout (*Oncorhynchus mykiss*) (Genbank ID: AF062499 and AF062500 [16]) (Table 1). qPCR was performed as described above and measured values were normalized by those of *ef1α*.

2.5. Time-resolved fluoroimuunoassay (TR-FIA) for IGF-I

Prior to the assay, serum IGF-I was extracted with an acid–ethanol as described in Shimizu et al. [52]. IGF-I was quantified by TR-FIA based on the method described in Small and Peterson [53] using recombinant salmon/trout IGF-I (GroPep) as a standard.

Table 1
Primer sequences used for real-time PCR (qPCR) analysis.

Gene	Forward primer 5′-3′	Reverse primer 5′-3′	Product size (bp)
IGF-I	TCTCCAAAACGAGCCTGCG	CACAGCACATCGCACTCTTGA	207 bp
EF-1α	GAATCGGCCATGCCCGGTGAC	GGATGATGACCTGAGCGGTG	142 bp
IGF-IRa	AAGAGAACACATCCAGCCAGGT	TTGTTGGCCGTTGAGGTATGC	97 bp
IGF-IRb	CTGCCAGCATGAGAGAGAGAATA	TAGGACTGGGACGGATCTTTAG	196 bp

Download English Version:

https://daneshyari.com/en/article/2800502

Download Persian Version:

https://daneshyari.com/article/2800502

Daneshyari.com