Comparative Biochemistry and Physiology, Part A 203 (2017) 24–31

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Characterization of the peripheral thyroid system of gilthead seabream acclimated to different ambient salinities

I. Ruiz-Jarabo [a,c,*], P.H.M. Klaren [b], B. Louro [c], J.A. Martos-Sitcha [a,d,1], P.I.S. Pinto [c], L. Vargas-Chacoff [e], G. Flik [b], G. Martínez-Rodríguez [d], D.M. Power [c], J.M. Mancera [a], F.J. Arjona [a,b,2]

[a] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Av. República Saharaui s/n, E11519 Puerto Real, Cádiz, Spain
[b] Department of Animal Ecology & Physiology, Institute for Water and Wetland Research, Faculty of Science, Radboud University, Heyendaalseweg 135, Box 30, 6525 AJ Nijmegen, The Netherlands
[c] Comparative Endocrinology and Integrative Biology Group, Centre of Marine Sciences (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[d] Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Spanish National Research Council, Av. República Saharaui, 2, E11519 Puerto Real, Cádiz, Spain
[e] Instituto de Ciencias Marinas y Limnológicas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

ARTICLE INFO

Article history:
Received 7 June 2016
Received in revised form 2 August 2016
Accepted 16 August 2016
Available online 21 August 2016

Keywords:
Deiodinases
Osmoregulation
Outer ring deiodination
Sparus aurata
Thyroid hormones

ABSTRACT

Thyroid hormones are involved in many developmental and physiological processes, including osmoregulation. The regulation of the thyroid system by environmental salinity in the euryhaline gilthead seabream (*Sparus aurata*) is still poorly characterized. To this end seabreams were exposed to four different environmental salinities (5, 15, 40 and 55 ppt) for 14 days, and plasma free thyroid hormones (fT3, fT4), outer ring deiodination and Na^+/K^+-ATPase activities in gills and kidney, as well as other osmoregulatory and metabolic parameters were measured. Low salinity conditions (5 ppt) elicited a significant increase in fT3 (29%) and fT4 (184%) plasma concentrations compared to control animals (acclimated to 40 ppt, natural salinity conditions in the Bay of Cádiz, Spain), while the amount of pituitary thyroid stimulating hormone subunit β (*tshb*) transcript abundance remained unchanged. In addition, plasma fT4 levels were positively correlated to renal and branchial *deiodinase type 2* (*dio2*) mRNA expression. Gill and kidney T4-outer ring deiodination activities correlated positively with *dio2* mRNA expression and the highest values were observed in fish acclimated to low salinities (5 and 15 ppt). The high salinity (55 ppt) exposure caused a significant increase in *tshb* expression (65%), but *deiodinase* gene expression (*dio1* and *dio2*) and activity did not change and were similar to controls (40 ppt). In conclusion, acclimation to different salinities led to changes in the peripheral regulation of thyroid hormone metabolism in seabream. Therefore, thyroid hormones are involved in the regulation of ion transport and osmoregulatory physiology in this species. The conclusions derived from this study may also allow aquaculturists to modulate thyroid metabolism in seabream by adjusting culture salinity.

1. Introduction

Thyroid hormones (THs) are truly pleiotropic in fish, affecting metabolism, reproduction, growth and osmoregulation, relevant physiological processes for aquaculture (Blanton and Specker, 2007). Thus, understanding how this system is regulated by the environment in cultured species, is key for the optimization of their culture. In the aquaculture ponds of the South of Spain, where culture of gilthead seabream (*Sparus aurata*) is carried out, salinity is highly variable and may well influence the thyroid system. In general, the fish thyroid system responds to stimuli by regulating the release of thyroid stimulating hormone (Tsh) that in turn stimulates the thyroid follicle to secrete thyroxine (T4) into the blood stream (Eales and Brown, 1993). Within the plethora of stimuli regulating the release of Tsh in fish, different salinity concentrations are postulated (Leatherland and Farbridge, 1992). Pituitary *thyroid stimulating hormone subunit β* (*tshb*) gene expression is under negative feedback control by plasma (free) thyroid hormones (Cohn et al., 2010; Manchado et al., 2008).

The pro-hormone T4 is deiodinated into bioactive triiodothyronine (T3) in the peripheral tissues (Bernier et al., 2009; Klaren et al., 2008). The regulation of deiodination in peripheral tissues is therefore a determining factor for the physiological effects of thyroid hormones.

Two iodothyronine deiodinases (Dio1 and Dio2) have outer ring deiodination (ORD) activities and in peripheral organs such as the gills and the kidney produce T3 from T4 that are directly involved in ion

* Corresponding author at: Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Av. República Saharaui s/n, E11519 Puerto Real, Cádiz, Spain.
E-mail address: ignacio.ruizjarabo@uca.es (I. Ruiz-Jarabo).
[1] Current address: Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal, Consejo Superior de Investigaciones Científicas (IATS-CSIC), Ribera de Cabanes, E-12,595 Castellón, Spain.
[2] Current address: Department of Physiology, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, The Netherlands.

http://dx.doi.org/10.1016/j.cbpa.2016.08.013
1095-6433/

transport and osmoregulation (Arjona et al., 2008). The inactivation pathways of THs are catalysed also by Dio1 and by a third iodothyronine deiodinase, Dio3. Both Dio1 and Dio2 ORD activities have distinct substrate and co-substrate preferences (Klaren et al., 2012; Orozco et al., 2000). Reverse T3 (rT3) is usually the preferred substrate for Dio1 in mammals (Orozco et al., 1997) while T4 is the preferred substrate of Dio2 (Garcia-G et al., 2004).

One consequence of increased TH activity is the stimulation of the basal metabolic rate, which seems to result, at least in part, in increased oxygen consumption and ATP hydrolysis. Several studies have reported species-specific changes in plasma TH levels, ORD activity (Arjona et al., 2008) or deiodinase gene expression (Lorgen et al., 2015) when fish are submitted to an osmotic challenge. Osmotic acclimation in fish is also associated with variations in plasma THs and in gilthead seabream plasma free T4 and gill ORD activity respond to a change in environmental salinity from 35 ppt to 1 ppt (Klaren et al., 2007).

Other authors have studied the thyroid system in *S. aurata* in hypo-saline conditions (Klaren et al., 2007; Power et al., 2001). To our knowledge, there are no previous studies characterizing the effects of acclimation to iso- or hypersaline conditions on the thyroid system in this species. We therefore set out to compare the effects of environmental hypo- and hyper-salinity on the thyroid system of the euryhaline gilthead seabream, an important aquaculture species.

2. Materials and methods

2.1. Animal maintenance prior to experimentation

Immature juvenile gilthead seabream juveniles ($N = 32$; 200 ± 44 g body mass, mean ± SD) were provided by *Servicios Centrales de Investigación en Cultivos Marinos* (SCI-CM, CASEM, University of Cádiz, Spain; Operational Code REGA ES11028000312), and maintained in the fish husbandry facility of the Faculty of Marine and Environmental Sciences (Puerto Real, Cadiz, Spain). Fish were acclimated for 35 days in 400-L tanks to seawater (40 ppt, natural salinity condition in the Bay of Cadiz, Spain) in a flow-through system under natural photoperiod (month of May in Cadiz, 14 h light:10 h dark) and temperature (environmental temperature of approximately 19.5 °C). Fish were fed commercial pellets (1% body mass) once a day (9:00) (Dibaq-Diproteg, Segovia, Spain). The experimental procedures complied with the guidelines of the University of Cadiz (Spain) and the European Union (86/609/EU) for the use of animals in research.

2.2. Acclimation to different environmental salinities

Fish were lightly anaesthetized in 0.05% (*v*/v) 2-phenoxyethanol, netted and randomly allocated to 400-L cubic tanks with different salinities (5, 15, 40 and 55 ppt with 140, 364, 1090 and 1546 mOsm kg^{-1} osmolality, respectively) ($N = 8$ per group). During transfer to the experimental tanks, the mass and length of the animals were recorded. Experimental salinities were achieved by mixing full-strength seawater with dechlorinated tap water (Puerto Real, Spain) or by mixing seawater with natural marine salt (Salina La Tapa, Puerto de Santa María, Cádiz, Spain). Each tank had a water recirculation system, which consisted of an external filter (Hydor Prime 30, Sacramento, CA, USA) to ensure optimal water conditions. Water conditions during experimentation were: temperature, ranging between 19.1 and 19.8 °C; 5, 15, 40 and 55 ppt salinity (variations <1 ppt for each tank); pH, ranging between 7.82 and 7.88; dissolved oxygen, >5 mg O_2 L^{-1}; nitrites, between 0.05 and 1.69 mg L^{-1}; nitrates, between 4.13 and 36.41 mg L^{-1}; and ammonium, 0.0–0.2 mg L^{-1}. These parameters were checked daily and did not vary significantly for the duration of the experiment. 20% of the water in circuits was replaced every other day. Fish were maintained in these conditions for 14 days and were fasted for 24 h before sampling. No mortality was observed during the acclimation period.

2.3. Sampling

Fish were netted, anaesthetized in 0.1% (*v*/v) 2-phenoxyethanol, weighed and sampled. Blood was collected with ammonium-heparinized syringes from the caudal vessels and placed into heparinized tubes. Plasma was separated from cells by centrifugation of whole blood (3 min, 10,000 × *g*, 4 °C). Fish were then euthanized by spinal transection and the pituitary gland was collected from each fish. The first gill arch on the left side of fish was excised. Adherent blood was removed by blotting with absorbent paper and a smaller subsample consisting of a few branchial filaments was collected using fine-point scissors. A small portion of the caudal part of the kidney was also collected. Gill filaments and kidney were placed in 100 μL of ice-cold sucrose-EDTA-imidazole (SEI) buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) for the analysis of Na^+/K^+-ATPase activity. The remaining gill tissue and kidney were snap frozen in liquid nitrogen and stored at −80 °C until measurement of outer ring deiodination activities or mRNA extraction. Liver was also collected and weighed to determine the hepatosomatic index (HSI).

2.4. Water chemistry

Water samples were filtered (0.22 μm pore size) prior to analysis. Na^+, K^+ and Mg^{2+} levels were measured using a flame atomic absorption spectrophotometer (UNICAM 939, Servicios Centrales, University of Cadiz). Cl^- and Ca^{2+} levels were measured with commercially available kits following the manufacturers protocol (Spinreact S.A, Sant Esteve d'en Bas, Girona, Spain). Osmolality was measured using a vapour pressure osmometer (Fiske One-Ten osmometer, Fiske, Massachusetts, USA) and expressed as mOsm kg^{-1} H_2O. Water chemistry data are shown in Supplementary File 1.

2.5. Cloning of tshb

The sequence of the beta subunit of *tsh* was originated using a cDNA cloned from a seabream pituitary cDNA library (Louro et al., 2005). Plasmid DNA was extracted using the alkaline lysis procedure (Birnboim and Doly, 1979) and sequenced using the Sanger sequencing method. Sequence identity was determined using the tblastx and blastn algorithms (Altschul et al., 1994) against the non-redundant nucleotide (nr db) and GenBank EST databases. Homologues were defined as those with an E-value <$1e^{-5}$ and a score of >40. Several cDNA clones corresponding to *tshb* were identified; one cDNA clone (281 EP10C7 Sa) was selected as reference and fully sequenced in order to obtain 3-fold coverage.

2.6. Phylogenetic analyses

Clustal Omega (SeaView v4 software, Gouy et al., 2010) with default parameters was used to generate a multiple sequence alignment of *tshb* sequences from representatives of the main vertebrate taxa.

Model Generator v0.85 (Keane et al., 2006) was used to test which substitution model best fitted the amino acid (aa) sequence alignment data. The Maximum Likelihood (ML) method, based on the selected optimal matrix-based model (JTT) (Jones et al., 1992), was used for the evolutionary analyses conducted in MEGA6 (Tamura et al., 2013). The bootstrap consensus tree was inferred from 1000 replicates (Felsenstein, 1985), and only branches corresponding to partitions reproduced in >50% bootstrap replicates were presented. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with a superior log likelihood value. A discrete gamma distribution was used to model evolutionary rate differences among sites [5 categories (+*G*, parameter = 1.7029)]. All positions containing gaps and missing data were eliminated.