General and Comparative Endocrinology 179 (2012) 369–375

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

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

The goitrogenic efficiency of thioamides in a marine teleost, sea bream (*Sparus auratus*)

M.A. Campinho [1,*], I. Morgado [1], P.I.S. Pinto, N. Silva, D.M. Power

CCMAR, CIMAR, Laboratório Associado, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

ARTICLE INFO

Article history:
Received 11 June 2012
Revised 20 September 2012
Accepted 21 September 2012
Available online 29 September 2012

Keywords:
Thioamides
Deiodinases
Goitrogens
Sea bream
Thyroid hormones
Thyroid receptors

ABSTRACT

Studies on the role of thyroid hormones (THs) in teleost fish physiology have deployed the synthetic goitrogens, methimazol (MMI), propilthiouracil (PTU) and thiourea (TU) that are used to treat human hyperthyroidism. However, the action of the goitrogens, MMI, PTU and TU at different levels of the hypothalamic–pituitary–thyroid (HPT) axis in teleosts is largely unknown. The central importance of the hypothalamus and pituitary in a number of endocrine regulated systems and the cross-talk that occurs between different endocrine axes makes it pertinent to characterize the effects of MMI, PTU and TU, on several endpoints of the thyroid system. The marine teleost, sea bream (*Sparus auratus*) was exposed to MMI, PTU and TU (1 mg/kg wet weight per day), via the diet for 21 days. Radioimmunoassays (RIA) of plasma THs and ELISA of the TH carrier transthyretin (TTR) revealed that MMI was the only chemical that significantly reduced plasma TH levels ($p < 0.05$), although both MMI and PTU significantly ($p < 0.05$) reduced plasma levels of circulating TTR ($p < 0.05$). Histological analysis of the thyroid tissue revealed modifications in thyrocyte activity that explain the reduced circulating levels of THs. MMI also significantly ($p < 0.05$) up-regulated transcript abundance of liver deiodinase 1 and 2 while significantly ($p < 0.05$) decreasing TRβ expression in the pituitary, all hallmarks of HPT axis action of goitrogens in vertebrates. The results indicate that in the sea bream MMI is the most effective goitrogen followed by PTU and that TU (1 mg/kg wet weight for 21 days) failed to have a goitrogenic effect. The study highlights the non-uniform effect of goitrogens on the thyroid axis of sea bream and provides the basis for future studies of thyroid disrupting pollutants.

1. Introduction

Thyroid hormones (THs) constitute a major signaling system in vertebrates, participate in a huge variety of cellular and physiological processes in juveniles and adults and are also essential for normal development [2,11,37,38]. Thyroid hormones (TH) are produced in the thyroid gland and exported to the serum in the form of the pro-hormone thyroxine (T4). The active form, triiodothyronine (T3), is mainly produced by the deiodinase enzymes (D1 and D2) and deiodinases (D1 and D3) are also responsible for TH inactivation [28,36,43,44]. THs are synthesized and liberated by the thyroid gland, carried in the blood by thyroid hormone-binding proteins and bind to nuclear TH receptors (TR) in target tissues to mediate their cellular responses.

Systemic central TH homeostasis is tightly regulated by a negative feedback mechanism involving the hypothalamus-pituitary-thyroid gland (HPT) axis and any imbalance in the thyroid axis and TH synthesis or degradation can seriously compromise normal TH actions. Abnormally high or low serum levels of THs are indicators of hyper- and hypothyroidism, respectively, and these are usually the standard parameters used to evaluate thyroid status in vertebrates [16]. In humans, hyperthyroidism is clinically treated with synthetic anti-thyroid drugs such as the goitrogens that suppress the function of the thyroid gland and inhibit the production of THs. This results in stimulation of the pituitary to synthesize and release thyroid stimulating hormone (TSH) which in turn stimulates thyroid follicles and ultimately leads to goitre formation. The action of anti-thyroid drugs has been explored in relation to thyroid gland pathologies [20,39,45]. In addition, manipulation of thyroid balance with anti-thyroid drugs has also been used to investigate the function of the thyroid axis and the consequences of altered thyroid states in several vertebrates [1,17,19,27,45,49–51] and is based on the assumption that the thyroid system is conserved across all taxa and the mechanism of action of anti-thyroid drugs in vertebrates is similar to what occurs in humans.

A number of researchers have employed anti-thyroid drugs (methimazol (MMI), propylthiourea (PTU) and thiourea (TU)) to induce disruption of the thyroid axis and investigate metabolism and the role of THs in fish reproduction, embryogenesis, larval

* Corresponding author. Address: Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. Fax: +351 289818419.
E-mail address: macampinho@ualg.pt (M.A. Campinho).
[1] These authors contributed equally to this work.

0016-6480/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2012.09.022

development and growth [6,15,29,30,47,51,52]. However, the results of such studies are inconsistent as the effect of anti-thyroid drugs do not appear to be limited to TH synthesis but in common with endocrine disrupting chemicals also affect other endpoints of the thyroid system [18,51]. Better characterization of the effect of known synthetic goitrogens (MMI, PTU and TU) on the thyroid axis of teleost fish would be desirable, particularly in light of the increasing concern about the impact of aquatic pollutants on the thyroid system in fish [3,7,13,32,33].

The present study compares the action of commonly used synthetic anti-thyroid drugs, MMI, PTU and TU on the thyroid axis of a marine teleost, the sea bream (*Sparus auratus*). The chemicals used are thiourylene drugs that in mammals block TH synthesis by interfering with iodide incorporation in thyroglobulin in the thyroid gland. Sea bream were exposed via the diet to the chemicals and their impact on different thyroid system parameters, plasma thyroid hormones, plasma transthyretin, thyroid follicle morphology and the TH-regulatory genes TSHβ, TTR, TRβ and deiodinases 1 and 2, evaluated.

2. Materials and methods

2.1. Animals and experiment conditions

Maintenance and manipulation of animals used in the study was carried out under a Group-1 license from the Direcção-Geral de Veterinária, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas, Portugal in accordance with the Guidelines of the European Union Council (86/609/EU). Immature sea bream (approx. 50 g body weight) were obtained from TIMAR (Tavira, Portugal). Fish were maintained in open sea water circuits under normal ambient temperature (20–21 °C) and salinity (36 parts per thousand, ppt) for October in Algarve (Portugal) using a 12 h light/dark photoperiod with daybreak set at 07.00 h. Fish were fed twice daily (1.5% total fish weight per tank per day) with a commercial sea bream diet (Dourasoja size 2 mm). After two weeks of acclimatization the fish were weighed and measured and those of a similar size were distributed between four tanks (65 l), which corresponded to the control (average wet weight fish (W) = 57.86 ± 7.38 g), thiourea (TU, W = 60.29 ± 10.27 g), propylthiouracil (PTU, W = 58 ± 6.76 g) and Methimazole (MMI, W = 59.14 ± 11.82 g), at a density of seven fish per tank. No mortality was observed during the experiment.

2.2. Administration of goitrogenic compounds

All the goitrogenic compounds were administered to fish via the diet and the dose corresponded to the daily medium range value for the goitrogens in humans, approximately 1 mg goitrogen/kg wet fish weight. The cumulative dose at the end of the experiment (21 days) in a 50–60 g fish was approximately 1 mg. Approximately 6 g of feed/tank/day was provided and gave a food intake of 1.5% of the body mass. Feed was prepared weekly using freshly prepared drug solutions to avoid oxidation and was stored in an airtight bag at 4 °C in the dark. Food was prepared by spraying it with an appropriate concentration of chemical dissolved in 5 ml of ethanol. For the control group food was sprayed with ethanol alone. Particular care was taken to ensure food was evenly coated with the chemical solution by turning the food pellets frequently. The procedure was carried out in a fume cupboard and the food left overnight to ensure all the ethanol had evaporated. Just before feeding the food was lightly sprayed with cod liver oil to increase its palatability. Fish were observed during feeding to ensure all the food provided was consumed and to verify all fish were feeding.

2.3. Sampling

After 21 days of treatment, fish were anesthetized in phenoxyethanol (0.02% (v/v) phenoxyethanol, Sigma–Aldrich), and blood samples collected from the caudal region using heparinized syringes and centrifuged at 1000*g* for 10 min at 4 °C. The plasma was frozen in liquid nitrogen and stored at −20 °C until analysis. Fish were subsequently killed by decapitation and pituitaries, liver and kidney were collected, frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Tissue from the insertion of the gill arch into the lower mandible was fixed in Bouin-Holland for thyroid histology.

2.4. TH Radioimmunoassays (RIA)

Thyroid hormone (T3 and T4) plasma concentrations were measured by radioimmunoassay (RIA) as previously described [32,35]. Plasma samples were diluted (1/10) in phosphate buffer (0.01 M, pH 7.6), with the exception of plasma from MMI treated fish that was not diluted. Samples were heat extracted as described in Morgado, et al. (2007b). Plasma from six fish was analyzed in the control, MMI and PTU groups and 5 samples were analyzed in the TU group. The total assay volume was 300 μl and comprised 100 μl of standard (1–2500 pg of T_3 or T_4, Sigma–Aldrich, Madrid) or diluted, heat treated plasma (20 μl of the 1/10 dilution further diluted in phosphate buffer to 100 μl final volume), 100 μl of anti-T_3 sera (1:15000, Sigma–Aldrich) or anti-T_4 sera (1:8000, Sigma–Aldrich) and 100 μl of tracer ([^{125}I]-T_3 or [^{125}I]-T_4, Amersham Biosciences). Separation of the free and bound hormone was performed using the second antibody method [35].

2.5. Total plasma protein quantification

The total protein concentration in plasma was determined using the Lowry method as previously described [26,32] with bovine serum albumin as the standard. Six individual animals were analyzed per treatment group. Absorbance was measured at 745 nm in a spectrophotometer.

2.6. Elisa

Plasma TTR levels were quantified using an indirect competitive ELISA as previously described [32,35] using recombinant sea bream TTR (sbTTR) and specific antisera. The assay was carried out in triplicate for the standard curve points (sea bream recombinant TTR) and in duplicate for plasma samples (n = 7 for control group, n = 5 for thiourea, n = 6 for methimazol and PTU) in 96-well plates (Nunc, Apogent, Denmark). Plasma samples were diluted (1:5 to 1:40 in accordance with preliminary optimization studies) and anti-sbrTTR was used at a 1:10000 dilution. A standard curve, non-specific binding and maximal binding were determined in all assays.

2.7. Histology

For analysis of thyroid tissue the entire pharyngeal region was fixed in Bouin–Holland solution (n = 3/treatment group) for 4 days at room temperature with occasional agitation. Excess fixative was removed by rinsing in distilled water and the tissue was subsequently decalcified in a solution of EDTA, pH 8 (GE) at room temperature with occasional agitation over 7 days. Paraffin blocks of the fixed tissue were prepared for sectioning by dehydration through a graded ethanol series from 70% to 100%, followed by xylene (100%) and finally embedded in paraffin (Histosec, Merk, Germany) using an automated tissue processor (Leica, TP1020). Sagittal serial sections of embedded tissue (8 μm) were cut using