REVIEW

The Role of Thyroid Hormone in Fish Development with Reference to Aquaculture

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Abstract

Recent progress in an understanding of the role of thyroid hormone (TH) on fish development was reviewed with particular reference to fish farming. TH is a small liposoluble hormone produced in thyroid follicles and has two bioactive forms, tetraiodothyronine (thyroxine, T4) and triiodothyronine (T3). Mother fish deposits a considerable amount of TH in developing oocytes. The amount of TH and ratio of T3 to T4 in eggs vary among species and stocks of the same species. The role of TH in eggs is not yet clear, though enhanced survival and growth of embryos and larvae by the enrichment of the eggs with TH have been reported in some cases. On the other hand, the role of TH that promotes larva-juvenile transition or metamorphosis has been established in teleost fish. The level of TH elevates at the onset of metamorphosis, and the treatment with TH can induce precocious metamorphosis. Considering the TH role in fish development, the disorder of its proper function could lead to deformity in juveniles, which is frequently observed in hatchery. In addition, many environmental chemicals have potential disrupting TH function in developing embryos and juveniles of fish.

Discipline: Aquaculture

Additional key words: egg, environmental disruptor, deformity, metamorphosis

Introduction

In higher vertebrates, thyroid hormone (TH) is known to control a wide range of physiology including development, basal metabolism, homeostasis, etc. In lower vertebrates, the role as a metamorphosis-inducing hormone in amphibians is probably the best known feature of TH. The lack of TH or disorder of its function causes serious problems for life such as cretin disease in humans. This is a hypothyroidism characterized by undergrowth and mental retardation. Since TH in higher vertebrates exerts its biological effects from early stages of life, the hormone is also expected to play an indispensable role during embryonic and larval periods of fish development. Although the role of TH in fish development had long been obscure, a wide body of knowledge about TH in fish development, mostly in teleosts, has been accumulated in the last two decades. The most important findings are the presence of a high level of TH in eggs and the role of TH for promoting larva-juvenile transition. In this review, TH function in fish development is discussed.

Overview of the production and action of thyroid hormone

A basic understanding of the production, metabolism and action of TH, particularly of fish TH, is summarized in this section. The details have been described in previous reviews.7,25,51 Fig. 1 shows the regulation and metabolism of TH. TH is produced in thyroid follicles, which consist of a single layer of epithelial cells. Unlike mammals, the thyroid follicles of most fish species do not form a single organ, but are dispersed along the afferent artery. The production of TH is regulated by thyroid-stimulating hormone (TSH) from the pituitary, while a hypothalamic factor controlling TSH secretion is unknown in fish; thyrotropin-releasing hormone (TRH), which stimulates TSH releasing in mammals and birds, is not effective in fish. TH is a small liposoluble molecule with non-species specificity, and has two bioactive forms, tetraiodo-L-thyronine (thyroxine, T4) and triiodo-L-thyronine (T3), thus containing four or three iodine atoms in...
Unlike higher vertebrates, the shortage of iodine in the diet is not a problem for fish because iodine is taken predominantly from ambient water through the gills. The major hormone synthesized in the thyroid gland is likely to be T₄ in fish. T₄ is metabolized to more biologically potent T₃ by outer-ring deiodination or, alternatively, to inactive reverse T₃ (rT₃) by inner-ring deiodination, mostly in peripheral tissues, particularly in the liver. T₃ and rT₃ can be metabolized further to diiodothyronine (T₂). The inactivation of TH is also achieved by

![Diagram of thyroid hormone production]

**Fig. 1. The cascade of thyroid hormone production**

A: Scheme of the pituitary-thyroid axis. B: Deiodination pathways of iodothyronines. TSH: Thyroid-stimulating hormone, TH: Thyroid hormone, T₄: Tetraiodothyronine (thyroxine), T₃: Triiodothyronine, rT₃: Reverse triiodothyronine, T₂: Diiodothyronine.

**Table 1. The contents of thyroid hormones in eggs**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>TH levels (ng/g)*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coho salmon</td>
<td><em>Oncorhynchus kisutch</em></td>
<td>T₄: 28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND: 19</td>
</tr>
<tr>
<td>Coho salmon</td>
<td><em>Oncorhynchus kisutch</em></td>
<td>16–20</td>
<td>5: 10</td>
</tr>
<tr>
<td>Chinook salmon</td>
<td><em>Oncorhynchus tschawytscha</em></td>
<td>8–11</td>
<td>20: 10</td>
</tr>
<tr>
<td>Chum salmon</td>
<td><em>Oncorhynchus keta</em></td>
<td>13–15</td>
<td>8–10: 41</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td><em>Oncorhynchus mykiss</em></td>
<td>1.4</td>
<td>4.7: 42</td>
</tr>
<tr>
<td>Amago salmon</td>
<td><em>Oncorhynchus masou macrostomus</em></td>
<td>3.8</td>
<td>3.5: 42</td>
</tr>
<tr>
<td>Biwa salmon</td>
<td><em>Oncorhynchus masou rhodous</em></td>
<td>3.4</td>
<td>6.1: 42</td>
</tr>
<tr>
<td>Chum salmon</td>
<td><em>Oncorhynchus keta</em></td>
<td>15</td>
<td>9.4: 42</td>
</tr>
<tr>
<td>Red sea bream</td>
<td><em>Pagrus major</em></td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;: 18</td>
</tr>
<tr>
<td>Black sea bream</td>
<td><em>Acanthopagrus schlegeli</em></td>
<td>0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;c&lt;/sup&gt;: 42</td>
</tr>
<tr>
<td>Sea bass</td>
<td><em>Lateolaborax japonicus</em></td>
<td>0.1</td>
<td>3.5: 42</td>
</tr>
<tr>
<td>Striped bass</td>
<td><em>Morone saxatilis</em></td>
<td>5.2</td>
<td>4.5: 2</td>
</tr>
<tr>
<td>Japanese flounder</td>
<td><em>Paralichthys olivaceus</em></td>
<td>0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.6&lt;sup&gt;c&lt;/sup&gt;: 42</td>
</tr>
<tr>
<td>Puffer</td>
<td><em>Takifuga niphobles</em></td>
<td>0.5</td>
<td>3.1: 42</td>
</tr>
<tr>
<td>Rabbitfish</td>
<td><em>Siganus guttatus</em></td>
<td>10–16</td>
<td>1–2: 1</td>
</tr>
<tr>
<td>Goldstriped amberjack</td>
<td><em>Seriola lalandi</em></td>
<td>ND</td>
<td>1.1: 40</td>
</tr>
<tr>
<td>Greater amberjack</td>
<td><em>Seriola dumerili</em></td>
<td>ND</td>
<td>2.1: 8</td>
</tr>
<tr>
<td>Red spotted grouper</td>
<td><em>Epinephelus aakara</em></td>
<td>ND</td>
<td>4.9: 8</td>
</tr>
<tr>
<td>Goldfish</td>
<td><em>Carassius auratus</em></td>
<td>0.1</td>
<td>0.1: 42</td>
</tr>
<tr>
<td>Ayu</td>
<td><em>Plecoglossus altivelis</em></td>
<td>4.3</td>
<td>0.5: 42</td>
</tr>
</tbody>
</table>

* TH concentrations in unfertilized eggs are indicated if available.
  a): 10 days before hatching,  b): Fertilized eggs,  c): 5–20 h after fertilization,  ND: Not determined.
glucuronide or sulfate conjugation, deamination or decarboxylation. A large part of circulating TH (approximately 99% in salmonids) is bound to plasma binding proteins like prealbumin. The difference in the bioactivity of iodothyronines appears due to the difference in the binding affinity to a TH receptor (TR): TR has about ten times greater affinity for T₃ than for T₄, and little for rT₃ and T₂. TR belongs to the nuclear hormone receptor family, which includes receptors for steroids, retinoids and vitamin D. In mammals, it is well demonstrated that the ligand-TR complex forms a homodimer or heterodimer with the retinoid receptor and then modulates gene expression through binding to a specific regulatory nucleotide sequence of a target gene.

Maternal TH in eggs

1. TH content in eggs

Yolk of unfertilized eggs of fish examined to date contained a substantial amount of TH. The levels of T₁ and T₄ in eggs of cultured species are listed in Table 1. Those levels and the proportion of T₁ to T₄ in eggs vary among species and even between different stocks of the same species. Generally, the eggs of fresh water fish contain higher concentration of T₁ than T₄, whereas the eggs of seawater species show an opposite tendency. The levels of T₁ and T₄ in eggs appear to be similar or slightly higher levels than those in the blood of adult fish.

2. TH function during embryogenesis

The presence of a considerable amount of TH in unfertilized eggs together with the evidence that the levels of TH in eggs decrease during embryonic development suggests its significant roles in fish embryogenesis. In addition, the expression of TR in embryos before hatching was demonstrated in zebrafish and sea bream. Experiments of TH administration to improve embryonic or larval survival and growth are summarized in Table 2. In some cases, TH enhanced survival and growth of the fish. These results support the meaningful role of TH during embryonic development. On the other hand the cases without any positive effects are also present despite the successful enrichment of TH in eggs. Furthermore, in medaka fish, although the treatment of mother fish with goitrogen, an anti-thyroidal drug, largely decreased the TH level in eggs, this did not alter the survival or growth of the offsprings. However, these results do not necessarily mean that TH is unessential in fish embryogenesis and larval development, since only a small amount of TH stored in eggs may be sufficient to exert hormonal effects and the amount of required TH may differ among species. Thus, many pieces of evidence like the expression of TR in embryos and enhanced survival and growth of larvae by TH treatment in some fish species suggest a significant role of TH in the early development of fish, while the mechanism of how TH exerts its effects has not been understood yet.

3. Transport of TH into eggs

The origin of TH in eggs is apparently maternal. Since the artificial TH enrichment in eggs can be achieved easily by the treatment to mother fish, it is a useful method to improve the egg quality if TH in eggs is

| Table 2. Effects of thyroid hormone administration on embryonic and larval development of fish |
|---|---|---|---|---|---|
| Common name | Scientific name | Treatment | Administered hormone | TH delivery to eggs | Effects | Reference |
| Tilapia | Sarotherodon mossambicus | DL | T₁ | 0.1 ppm | E | E | 20 |
| Tilapia | Tilapia nilotica | DL | T₁ | 0.1, 0.3 ppm | E* | 32 |
| Carp | Cyprinus carpio | DE, DL | T₁ | 0.01–0.05 ppm | E | E | 21 |
| Goldfish | Carassius auratus | DL | T₁ | 0.01–0.05 ppm | E | E | 35 |
| Striped bass | Morone saxatilis | IM | T₁ | 20 mg / kg BW | E | E | 3, 4 |
| Goldstriped amberjack | Seriola lalandi | IMF | T₁ | 20 mg / kg BW | E | I | I | E | I | 40 |
| Japanese parrot fish | Oplegnathus fasciatus | IM | T₁ | 20 mg / kg BW | E | I | I | E | I | 8 |
| Red sea bream | Pagrus major | IM | T₁ | 20 mg / kg BW | E | I | I | I | I | 8 |
| Japanese whiting | Sillago japonica | IMF | T₁ | 20 mg / kg BW | E | I | I | I | I | 8 |
| Rabbitfish | Signus guttatus | IM | T₁ | 1–100 mg / kg BW | E | I | I | I | I | 1 |

effective for the development of embryo and larvae. TH is considered to enter into eggs through the blood circulation, although the detailed mechanism is still uncertain. Three possible mechanisms have been proposed; passive diffusion, entry with vitellogenin, and involvement of other transporter proteins.

**TH function in larva-juvenile transition**

1. **Activation of the thyroid gland**

   The thyroid gland of salmonids becomes active before hatching, whereas the thyroid gland is unlikely to have differentiated at hatching in fish that reach hatching within several days after spawning. For those fish, a few small follicles appear first at the early stages of the larval period. Then the follicle increases gradually in number and size. The small follicles are capable of producing TH, since the thyroid follicles show immunoreaction with antiserum against TH and TH is detectable in larval fish though the concentration is relatively low.

   The larvae of most fish species greatly differ in their external appearances from the adults and undergo dramatic morphological transformation when they develop into juveniles, which are basically miniatures of adult fish. This transformation is often termed as metamorphosis on the analogy with amphibian metamorphosis. Fish metamorphosis is typically seen in certain taxonomical groups such as flatfishes and eels. Early in the 20th century, distinct morphological activation of the thyroid gland, which is characterized by the thickening of follicular epithelium and endocytosis of thyroidal colloid by the cells, has been described in the flatfish and eel. Later, the assay of TH during flounder metamorphosis demonstrated a clear elevation of TH at the metamorphic climax (Fig. 2). Similarly, the concomitant increase in TH levels with metamorphosis or larva-juvenile development was remarked in conger eel, black sea bream, red sea bream, grouper, summer flounder, etc. The fish undergoing distinct transformation in a shorter period seems to show clearer elevation of TH.

   Besides teleost fish, lampreys undergo apparent metamorphosis, but show a surprising developmental profile of TH, in which the TH level reaches a peak during the larval period and declines sharply at the onset of metamorphosis. The decline of the TH level seems to be a cue for the initiation of metamorphosis, although the detailed mechanism is unknown.

2. **Roles of TH**

   The action of TH on the metamorphosis was further verified experimentally by the treatment of larva with the hormone or anti-TH drugs: TH administration to larvae induced precocious metamorphosis or larva-juvenile transition, whereas the goitrogen caused developmental retardation in various fish species. In addition to morphological changes in their external appearances, the development is accompanied by behavioral, functional and biochemical alterations. Exogenous TH stimu-
lated the transition of muscle proteins\textsuperscript{15}, replacement of red blood cells from larval to adult type\textsuperscript{15}, skin pigmentation\textsuperscript{13,16}, development of the gastric glands\textsuperscript{13,15} (Fig. 3), scale formation\textsuperscript{35}, and fin formation\textsuperscript{5,35}. Simultaneous action on various tissues and organs is characteristic of TH during the metamorphic period. Concomitantly with these changes, the demands for nutrition and habitat also shift greatly.

These changes are believed to be triggered by the modification of gene expression by TR. Fish TR genes have been cloned from several species so far. Two types of TR, TR\textsubscript{\(\alpha\)} and TR\textsubscript{\(\beta\)}, were found in most fish species as in higher vertebrates\textsuperscript{9,17,24,33,48,49}. TR\textsubscript{\(\alpha\)} of a given species shows higher similarities to TR\textsubscript{\(\alpha\)} of different species than its own TR\textsubscript{\(\beta\)}, even between fish and mammals, and so with TR\textsubscript{\(\beta\)}. This could imply a distinct and indispensable function of each TR type throughout vertebrate animals. The differential localization and timing of gene expression of TR\textsubscript{\(\alpha\)} and TR\textsubscript{\(\beta\)} during metamorphosis and early development also suggest their functional differentiation\textsuperscript{24,50}. Thus, TH is capable of modulating TH function at the receptor level, which may partly explain why TH can influence on various parts of the body simultaneously. It is well established in higher vertebrates that TR regulates its responsive genes by binding to a cis-acting element of the gene. Such direct target genes have not been determined in fish, however.

### Hormonal disorder

**1. Morphological abnormality in aquaculture**

In hatchery, deformed fish often appear and sometime occupy the majority. The deformity is predominantly observed in bones of various parts. The curvature of the backbone occurs in severe cases, while malformation in cranial bones and tailbones is frequent. Abnormal pigmentation of the skin often occurs in flatfishes (Fig. 4). Since the occurrence of deformed fish results in economic loss, a preventive countermeasure is of particular interest. However, the cause of the deformity is not well understood in many cases. Because sensitive tissues for deformity are target sites for TH, involvement of TH in the deformity is worth being examined. In fact, precocious metamorphosis in flounder by exogenous TH induced a high percentage of albinism on the ocular side, which should be black-pigmented. Furthermore, the treatment to flounder larva with retinoic acids, which interact with TH via their nuclear receptors, induced pigmentation of the bottom side\textsuperscript{29} and the deformity of the backbone\textsuperscript{11}. Thus the experimental modification of TH function could induce morphological abnormalities in juveniles. The disorder of proper function of TH may be one of the causes of the deformity of juveniles reared in hatcheries.

**2. Environmental chemicals**

As recently reviewed by Rolland\textsuperscript{36} and Brawn et al.\textsuperscript{6}, many toxic chemicals such as polychlorinated biphenyl
(PCB), planar halogenated aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH), heavy metals, steroids, etc., have potentials for affecting thyroidal states. Administration of these chemicals to fish has influences on morphology of the thyroid gland and on levels of circulating TH positively or negatively according to cases. Although thyroidal disorder due to those endocrine disruptors has been rarely reported in wild fish, it was actually observed in wild salmon in the Great Lakes where the salmon had the enlarged thyroid gland\textsuperscript{23}. Moreover, the rodents fed the Great Lakes salmon caused hypothyroidism\textsuperscript{39}, suggesting the involvement of some chemical endocrine disruptors that can be stored in the body of the salmon. It should be noted that most of the studies on thyroidal disorder have evaluated the effects of chemicals by measuring plasma TH levels of adult fish probably due to the difficulty in examining larval fish. As mentioned earlier, TH exerts its prominent biological effects in early life stages. Therefore, future studies on thyroidal disorder should be focused on the young stages of fish.

**References**


