Changes in Intestinal and Hepatic Thyroid Hormone Deiodination During Spontaneous Metamorphosis of the Sea Lamprey, *Petromyzon marinus*

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ABSTRACT We measured microsomal low- K_m outer-ring deiodination (ORD) and inner-ring deiodination (IRD) activities for thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) in intestine and liver in nonmetamorphosing (undersized) larvae, immediately premetamorphic larvae, animals in stages 1–7 of metamorphosis, and immediately postmetamorphic sea lampreys (*Petromyzon marinus*). For *intestine*: T_4 ORD activity was relatively low in nonmetamorphosing larvae, increased in premetamorphic individuals, was highest in stages 1 and 2 and was very low during stages 3–7; T_4 IRD activity was negligible until stage 3 but increased 4.7-fold through stages 3 to 7 such that T_4 IRD activity was 14 times T_4 ORD activity at stage 6; T_3 ORD activity was undetectable; T_3 IRD activity was not measured through stages 3–7 but correlated with T_4 IRD activity at other stages. For *liver*: deiodination was only measured up to stage 2 and in postmetamorphic animals; in contrast to intestine, T_4 ORD activity fell to low levels at stage 2 and was low during postmetamorphosis; T_4 IRD and T_3 IRD activities were very low and uninfluenced by developmental stage; T_3 ORD activity was undetectable. We conclude that (1) deiodination activity is usually much higher in intestine than in liver, (2) intestinal ORD and IRD activities change reciprocally so that ORD predominates in early metamorphosis but IRD predominates in mid and late metamorphosis, and (3) changes in intestinal deiodination may contribute to the characteristic depression of plasma T_4 and T_3 levels during spontaneous metamorphosis. *J. Exp. Zool.* 286:305–312, 2000. © 2000 Wiley-Liss, Inc.

Plasma levels of thyroid hormones (TH) undergo characteristic changes during lamprey metamorphosis. Plasma levels of both thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) peak in larval life and then decrease sharply as metamorphosis progresses (Wright and Youson, '77; Lintlop and Youson, '83a; Leatherland et al., '90; Youson et al., '94). This decrease may be necessary for metamorphosis (Youson, '97), since T_3 has some effect in blocking the initiation of the event (Youson et al., '97). In at least some lamprey species, metamorphosis can be induced by potassium perchlorate which presumably depresses endostylar iodide uptake resulting in a decline in circulating TH levels (Hoheisel and Sterba, '63; Suzuki, '86; Holmes and Youson, '93; Youson et al., '95). This perchlorate-induced metamorphosis can also be blocked by maintaining high blood levels of TH (Manzon and Youson, '97; Manzon et al., '98). The cause of the natural fluctuations in blood TH levels during metamorphosis is unknown. It may involve changes in TH production by the metamorphosing larva, asso-

ciated with the reorganization of the endostyle into the thyroid follicles (Wright and Youson, '76; Wright et al., '80), or it may involve changes in TH peripheral metabolism (Leatherland et al., '90).

In teleost fish, extrathyroidal deiodination is a major mechanism for regulating plasma TH levels (reviewed by Eales and Brown, '93; Mol et al., '97, '98). Changes in extrathyroidal deiodination could contribute to the changing profile of blood TH during lamprey metamorphosis. Induction of metamorphosis in the sea lamprey (*Petromyzon marinus*) by potassium perchlorate is not accompanied by a change in deiodination activity responsible for conversion of T_4 to T_3 (Manzon et al., '98). However, to date there has been no thorough in-

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vestigation of deiodination during spontaneous metamorphosis of any lamprey species.

We therefore investigated the possibility that during spontaneous metamorphosis of laboratory-maintained sea lampreys, *P. marinus*, there are changes in the peripheral deiodination of TH that might explain the changes in blood TH levels. We examined the low- K_m T_4 outer-ring deiodination (ORD) to produce T_3 and, where material was available, we also examined the T_4 inner-ring deiodination (IRD) to produce inactive 3,3',5'-triiodothyronine (rT_3) and T_3 IRD to produce 3,3'-diiodothyronine (3,3'- T_2). In contrast to teleost fish, the intestine and not the liver is the main deiodination site in larvae and parasitic-stage lampreys (Eales et al., '97). We therefore focused on the intestine but we also examined liver when samples were available. The overall developmental profile was derived from three studies. In study I we analyzed metamorphic stages 3 to 7; in study II we analyzed postmetamorphic lampreys; in study III we analyzed nonmetamorphosing (undersized) larvae, immediately premetamorphic larvae and metamorphic stages 1 and 2.

MATERIALS AND METHODS

Animal collection and maintenance

Study I

Larval sea lampreys were collected on May 9–12, 1995 from Beaverdam Brook, a tributary of the Salmon River which drains into Lake Ontario, near Pulaski, New York. Larvae of 120 mm or longer and weighing at least 3.0 g were held in glass aquaria containing about 7 cm of sand and receiving a continuous supply of running dechlorinated freshwater. Water temperature ranged from 15°C in early May to 21°C in mid-June to 15°C in mid-November. Lighting was fluorescent and the photoperiod was 15L:9D. Animals were fed baker's yeast once weekly at a ration of 1 g yeast/animal/week. Animals were not fed during non-trophic metamorphosis. Metamorphosing animals ($n = 18$ –30) corresponding to stages 3 to 7 of development (Youson and Potter, '79) were sampled periodically between August 15 and November 15, 1995. Each animal was anesthetized in 0.05% MS-222, weighed, measured and organs removed. The intestine was frozen at -70°C for deiodination analysis.

Study II

Larvae were collected on May 31 and June 1, 1996 from Oshawa Creek, a tributary of Lake

Ontario, near Toronto, and used in an experiment to determine extreme and optimal temperatures for metamorphosis (Holmes and Youson, '98). The animals that entered metamorphosis were pooled on August 15, 1996, and held without food under the conditions described for study I. By December 20–25, 1996, young fully metamorphosed parasitic juveniles (hereafter referred to as post-metamorphic animals) began emerging from the sand. They were held without food until they were sampled ($n = 20$) on January 28, 1997.

Study III

Larvae were collected on May 13–15, 1998, from Fish Creek, New York, and held in the laboratory under the conditions described in study I at 14–16°C. Beginning on June 1, water temperature was raised by 1°C/day to 21°C and then held at this level. On June 8 we sampled 18 premetamorphic larvae (>120 mm, >3.0 g, condition factor (CF) > 1.50) which were predicted, on the basis of size and CF, to initiate metamorphosis in about 1 month (Youson et al., '93). On June 23 we sampled 20 larvae (<120 mm, <3.0 g) that were predicted, on the basis of size, would not metamorphose; hereafter this undersized group is referred to as nonmetamorphosing larvae. On July 14, approximately 1–1.5 weeks after the start of metamorphosis, we sampled 10 animals in each of stages 1 and 2.

Study IV

This study represents previously published data (Eales et al., '97) on the adult feeding period. We included it here to provide a complete view of the life cycle from larva to feeding adult.

Deiodination analyses

Microsomal fractions were prepared (Shields and Eales, '86) as pools from four (study I) or two (Studies II and III) individuals at the same development stage and stored at -76°C . Deiodination assays were run on the microsomal fractions using 0.11–0.18 (study I), 0.25–0.4 (study II) or 0.1–0.4 (study III) mg of microsomal protein/ml incubate. The assay method followed that of Johnston and Eales ('95) and was conducted at 12°C, at pH 7.2, with 10 mM dithiothreitol (DTT) for an incubation of 2 hr, using high-specific activity [^{125}I] T_4 or [^{125}I] T_3 (New England Nuclear) as substrate. The end-products were analyzed by HPLC (Sweeting and Eales, '92). No unlabelled substrate was added to the labelled stock substrate provided by the manufacturer. This proce-

ture was followed to maximize conversion of labelled substrate for the purpose of HPLC detection. Changes in specific activity of the stock material resulted in small variations in substrate level between studies. In order to compare between studies, all deiodinase activities were corrected for a common substrate level of 0.15 nM, assuming that over the narrow substrate range used in the different studies, deiodination activity would be proportional to substrate concentration. T₄ORD activity was assessed from formation of labelled T₃, T₄IRD activity from formation of 3,3',5'-triiodothyronine (rT₃), T₃ORD activity from formation of radioiodide and T₃IRD activity from formation of 3,3'-T₂. Deiodination activity was expressed as fmols T₄ or T₃ deiodinated/hr/mg microsomal protein.

Statistical analyses

Within each of studies I and III the effect of stage of development was determined by one-way ANOVA. If a significant ($P < 0.05$) difference was found, individual means were then compared by Student-Neuman-Keuls test ($P < 0.05$).

RESULTS

Table 1 gives sampling dates and mean values for body length, body mass and condition factor for studies I, II, and III.

Intestine

T₄ORD activity (Fig. 1A) was significantly higher in stages 1 and 2 (≈ 40 fmols/hr/mg) than

in nonmetamorphosing larvae (7 fmols/hr/mg). However, T₄ORD activity was low at stage 3 (3 fmols/hr/mg) and did not change significantly to stage 7 or during postmetamorphosis. T₄IRD activity (Fig. 1B) was negligible until stage 3 (15 fmols/hr/mg) but increased during metamorphosis to 70 fmols/hr/mg at stage 7, and was still appreciable (19 fmols/hr/mg) during postmetamorphosis. The ratio of T₄IRD/T₄ORD activities (Fig. 1C) rose to a peak ($\approx 14:1$) during stages 6 and 7. Representative HPLC radiochromatograms (Fig. 2) show through stages 3 to 7, the low T₃ formation by T₄ORD but the considerable rT₃ formation by T₄IRD.

T₃ORD activity was undetectable (Fig. 3B). T₃IRD activity (Fig. 1D) was not measured from stages 3–7 due to insufficient material. However, T₃IRD activity tracked T₄IRD activity in that it was negligible in nonmetamorphosing larvae, premetamorphic larvae and stages 1 and 2, but was appreciable in postmetamorphic lampreys (21 fmols/hr/mg) (Fig. 1D).

Liver

Liver T₄ORD activity (10 fmols/hr/mg) (Fig. 4A) was comparable to intestinal T₄ORD activity in nonmetamorphosing larvae (7 fmols/hr/mg) but fell significantly to 1.5 fmols/hr/mg by stage 2. T₄ORD activity was not measured for stages 3–7, but was low (1 fmol/hr/mg) at postmetamorphosis. T₄IRD and T₃IRD activities were very low (≤ 2 fmols/hr/mg) for all developmental stages examined (Figs. 3B, C and 4B, C). T₃ORD activity was undetectable (Fig. 3C).

TABLE 1. Metamorphic stages, sampling dates, lengths, weights, and condition factors of the larvae or postmetamorphic (PM) lampreys used in studies I, II and III¹

Stage	Date	Length (mm)	Weight (g)	Condition factor ²	n
Study I					
3	8/15/95	130.8 $\pm$ 1.85	4.16 $\pm$ 0.18	1.84 $\pm$ 0.05	18
4	8/16/95	126.8 $\pm$ 1.84	3.76 $\pm$ 0.16	1.82 $\pm$ 0.02	18
5	8/16/95	129.6 $\pm$ 1.93	3.85 $\pm$ 0.16	1.74 $\pm$ 0.02	18
6	9/12/95	130.0 $\pm$ 1.94	3.52 $\pm$ 0.16	1.62 $\pm$ 0.02	20
7	11/14/95	135.5 $\pm$ 2.36	3.45 $\pm$ 0.17	1.36 $\pm$ 0.02	30
Study II					
PM ³	1/28/97	143.7 $\pm$ 2.29	3.64 $\pm$ 0.15	1.22 $\pm$ 0.02	20
Study III					
L ⁴	6/23/98	104.9 $\pm$ 1.89	1.95 $\pm$ 0.09	1.68 $\pm$ 0.02	20
PR ⁵	6/08/98	132.7 $\pm$ 1.59	3.64 $\pm$ 0.15	1.54 $\pm$ 0.03	18
1	7/14/98	140.7 $\pm$ 4.21	4.61 $\pm$ 0.38	1.63 $\pm$ 0.03	10
2	7/14/98	135.2 $\pm$ 2.40	4.27 $\pm$ 0.31	1.70 $\pm$ 0.03	10

¹Means $\pm$ SEM.

²Condition factor = [M (g)/liter (mm)³] $\times 10^6$.

³Postmetamorphic lampreys.

⁴Nonmetamorphosing larvae.

⁵Premetamorphic larvae.

Fig. 1. Intestinal deiodination activities during lamprey development (means $\pm$ SEM; $n = 5-10$). (A) T₄ORD; (B) T₄IRD; (C) Ratio of T₄IRD to T₄ORD; (D) T₃IRD. The profiles were derived from three studies in 1995 (I), 1997 (II), and 1998 (III) and also from Eales et al. ('97) (IV). L = nonmetamorphosing larvae; PR = premetamorphic larvae; 1-7 = stages of metamorphosis; PM = postmetamorphic lampreys; P = parasitic lampreys. There was a significant effect of stage of development on T₄ORD (study III) and T₄IRD activity (study I). Within a given study, means with similar letters do not differ significantly ($P < 0.05$).

DISCUSSION

We showed previously in nonmetamorphosing sea lamprey larvae that T₄ORD activity was higher in intestine than in liver, kidney or muscle and that T₄IRD, T₃ORD and T₃IRD activities were undetectable in any of these tissues (Eales et al., '97). We also found T₄ORD, T₄IRD and T₃IRD ac-

Fig. 2. HPLC radioactivity profiles for extracts of incubates of [¹²⁵I]T₄ and microsomes from larval lamprey intestine. Retention times of UV-detected authentic T₄, T₃ and rT₃ are shown; I⁻, radioiodide. HPLC conditions: C-18 150 $\times$ 4.6-mm Econosphere column at 30°C; isocratic elution with 38% acetonitrile with 0.1% trifluoroacetic acid (TFA)/62% water with 0.1% TFA. (A) Control (no microsomes); (B) stage 3; (C) stage 7.

Fig. 3. HPLC radioactivity profiles for extracts of incubates of [125 I]T₃ and microsomes from postmetamorphic lamprey tissues. Retention times of UV-detected authentic T₃ and 3,3'-T₂ are shown; I⁻, radioiodide. HPLC conditions: C-18 150 $\times$ 4.6-mm Econosphere column at 30°C; acetonitrile (0.1% TFA)/water (0.1% TFA) isocratic from 0–10 min, increasing linearly to 47% acetonitrile at 14 min and then decreasing to 42% acetonitrile at 16 min. (A) Control (no microsomes); (B) intestine; (C) liver.

tivities in intestine of metamorphosed parasitic-stage lampreys, but low or negligible deiodinating activities in other tissues. We therefore focused here on intestinal deiodination, but we also examined liver when it was available. Our present data confirm that the intestine usually has more deiodinating activity than the liver. Only in

Fig. 4. Liver deiodination activities during lamprey development (means $\pm$ SEM; $n = 5$ –10). (A) T₄ORD; (B) T₄IRD; (C) T₃IRD. The profiles were derived from three studies in 1995 (I), 1997 (II), and 1998 (III) and also from Eales et al. ('97) (IV). L = nonmetamorphosing larvae; PR = premetamorphic larvae; 1–7 = stages of metamorphosis; PM = post-metamorphic lampreys; P = parasitic lampreys. There was a significant effect of stage of development on T₄ORD (study III). Within study III, means with similar letters do not differ significantly ($P < 0.05$).

nonmetamorphosing larvae was liver T₄ORD activity comparable to that of intestine. At other developmental stages deiodination activity was considerably greater for intestine than liver.

In order to compare liver and intestine at different developmental stages, all our *in vitro* estimates of deiodination activity were based on a TH substrate level of 0.15M. The activity values we report therefore represent the deiodinating potential of the tissues at this standardized TH concentration. The actual amount of TH deiodinated by these tissues *in vivo* will of course depend on the intracellular concentrations of the appropriate TH substrates, which were not measured in this study.

Our survey of deiodinating activity during development from the nonmetamorphosing larva to the parasitic-stage adult indicates a reciprocal relationship between intestinal T₄ORD and T₄IRD

activities. Intestinal T_4 ORD activity is low in nonmetamorphosing larvae, reaches a peak during premetamorphosis and early metamorphosis, and then drops dramatically during metamorphosis to eventually rise again in parasitic-stage lampreys. Indeed the level of T_4 ORD activity during premetamorphosis is likely higher than that described here since it is only possible to select immediately premetamorphic larvae with 60–80% accuracy (Youson et al., '93; Holmes et al., '94). In contrast, T_4 IRD activity is negligible until stage 3. It then climbs to a peak value at stage 7 but falls to modest levels in parasitic-stage lampreys. T_3 IRD activity was not measured during stages 3–7 due to insufficient material, but at all other stages T_3 IRD activity closely tracked T_4 IRD activity. This is expected if there is a common enzyme, comparable to the mammalian type III, that deiodinates both T_4 and T_3 from the inner ring. We conclude that during the initiation of metamorphosis (including before external signs are apparent) there is a major increase in intestinal T_3 production, followed by a shutdown of T_4 conversion to T_3 and a major induction of IRD of both T_4 and T_3 during mid metamorphosis. The biological significance of these changes in deiodination activity cannot be determined from this study. They could contribute to tissue-specific local regulation of thyroidal status as suggested for metamorphosing amphibia by Becker et al., ('97). They could also contribute to both the high plasma levels of T_4 and T_3 immediately prior to metamorphosis and the dramatic fall in these levels during metamorphosis (Wright and Youson, '77; Lintlop and Youson, '83a; Leatherland et al., '90; Youson et al., '94, '97).

Liver T_4 ORD activity was significantly lower in stage-2 larvae than in nonmetamorphosing larvae, suggesting that during the early stages of metamorphosis there is a decrease in hepatic T_3 production by T_4 ORD. In contrast intestinal T_4 ORD activity increased from nonmetamorphosing larvae to stage-2 larvae so that T_4 ORD activity of the intestine at stage 2 was 20 times that of liver. Although there could be changes in deiodinating activity in tissues that we did not examine, our data suggest that as larvae enter metamorphosis the intestine is the predominant peripheral deiodination site.

Metamorphosis can be induced prematurely by $KClO_4$ treatment that also depresses plasma T_4 and T_3 levels (Youson et al., '95; Manzon and Youson, '97), but does not alter intestinal T_4 ORD activity (Manzon et al., '98). Thus there is a dif-

ference in the intestinal T_4 ORD activity during $KClO_4$ -induced and spontaneous metamorphosis. However, during $KClO_4$ treatment the decline in plasma T_4 is likely due to decreased T_4 production by the endostyle, and the decline in T_3 is likely due to deficiency of T_4 substrate to form T_3 . Furthermore, IRD pathways were not measured in our previous treatment with $KClO_4$. These pathways could have contributed to the decline of both plasma T_4 and T_3 .

Our present correlative study shows that changes in intestinal deiodination could explain the metamorphic decline in plasma TH levels. However, other factors may contribute to changes in plasma TH levels (Youson, '97). There could be decreased production of TH from the larval endostyle, which during metamorphosis undergoes structural reorganization to form the follicular thyroid characteristic of the parasitic stage (Wright and Youson, '76; Wright et al., '80). However, the transforming endostylar tissue continues to be capable of incorporation of radioiodine (Wright and Youson, '76). There is also some suggestion for increased binding capacity of thyroid hormone receptors for T_3 in at least one tissue (Lintlop and Youson, '83b). It is possible, therefore, that changes in intestinal deiodination work in concert with other mechanisms and result in a lowering of plasma TH levels during lamprey metamorphosis.

The high deiodinating activity in the intestine of lamprey larvae may reflect the route whereby TH normally enter the larval circulation. Endostylar secretions containing thyroid hormones may be discharged to the gastrointestinal tract and absorbed from there into the blood (reviewed by Eales, '97). The intestine is therefore strategically placed to regulate the levels of TH delivered to the blood. However, during metamorphosis this mechanism could be greatly impaired as the entire alimentary canal undergoes a reorganization (Youson and Connelly, '78). This restructuring also involves the intestinal epithelium (Youson and Horbert, '82). Despite these changes, the intestine seems to be efficient in deiodination.

It is interesting to compare larval lamprey deiodination profiles with those of amphibians in which high levels of TH are required for the tadpole-to-frog metamorphosis (Galton, '88). At the start of lamprey metamorphosis T_4 ORD activity is high and both plasma T_4 and T_3 levels are high so that TH could be involved in the initiation of metamorphosis. However, in contrast to amphibian metamorphosis, morphological and physiological changes in lamprey metamorphosis correspond

with a decrease and not an increase in plasma TH levels. Nevertheless, in both lampreys and amphibians, changes in ORD and IRD pathways feature prominently in the regulation of thyroidal status and the intestine is a major deiodination site (Galton and Hiebert, '87, '88; Becker et al., '97). Deiodination also occurs in amphibian skin (Galton and Hiebert, '88; Becker et al., '97) but has not yet been studied in lamprey skin. T₄IRD has been reported for trout skin (Fenton et al., '97).

Significant intestinal T₄ and T₃ IRD, but undetectable levels of hepatic T₄ and T₃ ORD and IRD activities, have been reported in lampreys in the parasitic period (stage IV; Eales et al., '97). Therefore the immediately postmetamorphic state may represent the condition that persists into the feeding phase of adult life. During the parasitic period, when the sanguivorous lamprey may be ingesting significant amounts of TH from its host, the intestinal inner-ring pathways that degrade TH may be important in regulating entry of TH to the circulation.

Negligible T₃ORD activity was observed in intestine or liver at any stage of development. This is consistent with previous observations on the sea lamprey (Eales et al., '97), teleost fish (Eales and Brown, '93; Mol et al., '97, '98) and higher vertebrates (McNabb, '92).

We conclude that IRD is induced during sea lamprey metamorphosis, and that this event in conjunction with depressed T₄ORD activity may contribute to the decrease in plasma TH levels which is so characteristic of early metamorphosis and which may be required for the event to proceed.

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