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Study of the relationship between thyroid hormones and lipid metabolism during KClO_4 -induced metamorphosis of landlocked lamprey, *Petromyzon marinus*[☆]

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Abstract

This study examines the role of thyroid hormones (TH) (thyroxine and triiodothyronine) in regulating lipid metabolism of landlocked larval sea lampreys, *Petromyzon marinus*. Larvae were treated with either thyroxine (0.5 or 1 mg l⁻¹ water) or triiodothyronine (0.25 or 1 mg l⁻¹ water) in the presence or absence of the goitrogen, potassium perchlorate (KClO_4) (0.05% w/v), for 4, 8, and 16 weeks. Treatment with KClO_4 alone, which induced metamorphosis after 8 weeks and lowered plasma TH levels, reduced hepatic and renal total lipid content after 8 weeks of treatment. KClO_4 -induced lipid depletion after the 8-week treatment was supported by an increased rate of hepatic lipolysis, as indicated by increased triacylglycerol lipase activity. Furthermore, reduced lipogenesis in the liver was indicated by decreased hepatic acetyl-CoA carboxylase and diacylglycerol acyltransferase (DGAT) activities, and by decreased renal DGAT activity following 8 weeks of KClO_4 treatment. Treatment of larvae for 4 weeks with TH alone resulted in either no change or a slight increase of lipid in the liver and kidney. TH treatments in combination with KClO_4 failed to induce metamorphosis and, after up to 8 weeks, several TH treatments blocked changes in lipid content and enzyme activity associated with KClO_4 -induced metamorphosis. These experimental results suggest that TH deficiency during metamorphosis may promote lipid catabolism, while the presence of TH tends to protect/promote lipid reserves, perhaps favoring the larval condition. The actions of TH and KClO_4 on metamorphosis-associated lipid metabolism in sea lampreys may be direct, permissive, and/or indirect via other factors. © 1999 Elsevier Science Inc. All rights reserved.

Keywords: Thyroid hormones; Potassium perchlorate; Lipid metabolism lipolysis; Lipogenesis; Sea lamprey; *Petromyzon marinus*; Metamorphosis

1. Introduction

The metamorphosis of sea lampreys (*Petromyzon marinus*) involves a marked depletion of body lipid [26] for energy use during this non-trophic period when there also is extensive development and reorganization

of tissues [32,33]. Depletion of stored lipid during sea lamprey metamorphosis primarily results from the specific catabolism of stored triacylglycerol [11]. The biochemical basis of these changes involves increased lipid breakdown, as indicated by enhanced activity of triacylglycerol lipase [12] in the liver and kidney, and decreased lipid synthesis, as indicated by reduced activities of malic enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and isocitrate dehydrogenase in the liver and muscle [20]. Decreased lipogenesis is also indicated by reduced activities of acetyl-CoA carboxylase (ACC) and diacyl-

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glycerol acyltransferase in the liver and kidney [12]. The mechanisms that serve to control metamorphosis-associated alterations in lipid metabolism of lampreys are not known.

The endocrine system of lampreys, like other poikilothermic vertebrates which possess a metamorphic episode as part of their life history, is inextricably linked to the metamorphic process, and may provide the essential chemical link between the organism and environmental and metabolic cues [2,7,25,34,35]. Although many endocrine factors may be involved with regulating lamprey metamorphosis, thyroid hormones (TH) (thyroxine (T4) and triiodothyronine (T3)) have been studied most extensively and results suggest their involvement in some capacity [35]. For example, numerous studies have reported high levels of TH in premetamorphic larvae and that these levels decline sharply at the time external changes are first evident in spontaneous metamorphosis of lampreys [15–17,27,29,30,34]. In addition, spontaneous metamorphosis in sea lampreys was inhibited by T3 treatment [40]. Moreover, potassium perchlorate (KClO_4) induced the metamorphosis of three Northern Hemisphere lamprey species, *Lampetra planer* [9], *Lampetra reissneri* [28], and *Petromyzon marinus*; in *P. marinus*, KClO_4 -induced metamorphosis was accompanied by reduced T4 and T3 concentrations [10,18,39].

The declining concentrations of plasma TH observed during spontaneous metamorphosis of lamprey are coincident with the depletion of lipids occurring during the same period [11,38]. A role for TH in modulating lipid metabolism in lamprey has been suggested by studies in adult animals. Administration of T3 in vivo lowered the plasma concentration of fatty acids (FA) initially, but resulted in elevated plasma FA 48 h after injection; whereas propylthiouracil treatment decreased plasma FA [22]. Whether TH regulates metamorphosis-associated alterations in lipid metabolism of lampreys has not been determined.

In this study, the landlocked form of sea lamprey, *P. marinus*, was used to evaluate the role of TH in regulating development-associated alterations in lipid metabolism. Our hypothesis was that TH and/or altered thyroid status affect the metabolism of lipids in the liver and kidney of larval and metamorphosing lamprey. Liver and kidney were chosen because we had previously identified these tissues as lipid depot sites in metamorphosing sea lampreys [11].

2. Materials and methods

2.1. Experimental animals

Sea lamprey larvae (ammocoetes) were collected and sampled as described by Manzon et al. [19]. The larvae

were collected from the Salmon River near Keeseville, NY, in mid-September and were transported to the University of Toronto at Scarborough, Ont., Canada. The larvae were maintained in a 21 l glass aquaria with a sandy substrate that was supplied with aerated, dechlorinated water (12 l) at an ambient temperature of 15–18°C under a photoperiod of 15 h light and 9 h dark, and fed with baker's yeast once per week (1 g per animal).

2.2. In vivo experiment

The experimental design is described by Manzon et al. [19]. Briefly, a total of 300 larvae with a body length greater than 120 mm were randomly assigned to 30 aquaria (10 larvae per aquarium). Larvae were acclimated to laboratory conditions for 9 days prior to the start of the experiment, which consisted of 10 treatment groups and three sampling times. The 10 treatment groups were as follows: (1) no treatment, (2) low dose of T4 (0.5 mg l^{-1}), (3) high dose of T4 (1 mg l^{-1}), (4) low dose of T3 (0.25 mg l^{-1}), (5) high dose of T3 (1 mg l^{-1}), (6) 0.05% KClO_4 , (7) 0.05% KClO_4 + low dose of T4, (8) 0.05% KClO_4 + high dose of T4, (9) 0.05% KClO_4 + low dose of T3, (10) 0.05% KClO_4 + high dose of T3. The untreated group of larvae were exposed only to dechlorinated water. Treatments were made by delivering the appropriate quantity of T3 dissolved in NaOH (20 ml); the appropriate quantity of T4 in a suspension of dechlorinated water (150 ml); and the appropriate volume of a 1% stock solution to aquaria to achieve the final desired concentrations. During the experiment, water was changed and fresh treatments were added every 14 days. All treatments began on 6 December (time of the year when spontaneous metamorphosis does not occur) and the animals were sampled 4, 8, and 16 weeks afterwards. At each sampling period, larvae were anesthetized with 0.05% tricaine methanesulfonate, evaluated for their stage of metamorphosis, and sacrificed by decapitation. Tissues (liver and kidney) were removed, frozen in liquid N_2 , and stored at -70°C for later determination of total lipid and enzyme activity. The kidney sample consisted of the entire paired nephric tissue extending from the heart region to the cloaca. Measures of organ weights and indices at the time of biochemical analysis are shown in Table 1.

2.3. Biochemical analysis

Liver and kidney lipids were extracted and analyzed as described by Kao et al. [11]. One-third of each tissue was homogenized in 20 volumes of chloroform/methanol (2:1 v/v) [3]. Total lipids were determined on aliquots of the lipid extracts after evaporation of the solvent by a sulphophospho-vanillin colorimetric

Table 1
Effects of thyroid hormones and the goitrogen, potassium perchlorate (KClO₄), on weights and somatic indices of kidney and liver in larval lamprey, *Petromyzon marinus*[†]

Parameter	Without 0.05% KClO ₄					With 0.05% KClO ₄				
	No hormone	Low T4	High T4	Low T3	High T3	No hormone	Low T4	High T4	Low T3	High T3
<i>Liver (mg)</i>										
4 weeks	24.2 ± 1.5	24.3 ± 1.3	27.1 ± 3.3	26.0 ± 0.9	23.6 ± 1.4	22.5 ± 1.5	23.1 ± 1.8	25.9 ± 1.1	19.8 ± 0.8	22.5 ± 1.3
8 weeks	25.9 ± 1.3	25.2 ± 2.1	23.3 ± 1.2	24.0 ± 1.5	27.3 ± 1.7	16.1 ± 0.8 ^{ab}	20.3 ± 1.2 ^b	25.1 ± 1.5 ^c	20.4 ± 1.4 ^b	19.4 ± 0.7 ^b
16 weeks	22.2 ± 1.3 ^a	23.3 ± 0.9	22.5 ± 1.2	27.2 ± 0.9 ^b	26.6 ± 1.7 ^b	19.5 ± 1.4	22.8 ± 1.6	21.2 ± 1.1	19.2 ± 1.5	22.3 ± 1.6
<i>HSI (g g⁻¹ × 100%)</i>										
4 weeks	0.73 ± 0.02	0.74 ± 0.03	0.81 ± 0.05	0.79 ± 0.04	0.71 ± 0.03	0.66 ± 0.03	0.66 ± 0.02	0.75 ± 0.04	0.69 ± 0.03	0.73 ± 0.02
8 weeks	0.69 ± 0.04	0.73 ± 0.07	0.71 ± 0.07	0.73 ± 0.09	0.76 ± 0.05	0.61 ± 0.04 ^a	0.67 ± 0.05 ^{ab}	0.81 ± 0.07 ^b	0.64 ± 0.06 ^a	0.66 ± 0.05 ^a
16 weeks	0.71 ± 0.02	0.71 ± 0.03	0.70 ± 0.02	0.78 ± 0.03	0.71 ± 0.02	0.68 ± 0.03 ^{ab}	0.73 ± 0.02 ^b	0.62 ± 0.02 ^a	0.69 ± 0.03 ^a	0.69 ± 0.02 ^a
<i>Kidney weight (mg)</i>										
4 weeks	116 ± 13	107 ± 11	120 ± 28	126 ± 13	117 ± 15	121 ± 11	126 ± 17	112 ± 17	91 ± 12	104 ± 13
8 weeks	137 ± 19	98 ± 11	119 ± 15	117 ± 23	108 ± 20	88 ± 13 ^{**}	100 ± 15	102 ± 16	120 ± 15	90 ± 10
16 weeks	73 ± 64	98 ± 12	104 ± 17	101 ± 144	104 ± 14	96 ± 15 [*]	92 ± 12	123 ± 16	77 ± 154	108 ± 16
<i>KSI (g g⁻¹ × 100%)</i>										
4 weeks	3.45 ± 0.26	3.21 ± 0.24	3.26 ± 0.48	3.68 ± 0.23	3.37 ± 0.31	3.60 ± 0.34	3.51 ± 0.26	3.19 ± 0.43	3.04 ± 0.27	3.26 ± 0.26
8 weeks	3.54 ± 0.42	2.72 ± 0.27	3.58 ± 0.57	3.75 ± 0.88	3.00 ± 0.47	3.23 ± 0.47	3.39 ± 0.60	3.45 ± 0.71	3.78 ± 0.44	3.06 ± 0.36
16 weeks	2.33 ± 0.14	2.90 ± 0.28	3.01 ± 0.28	2.78 ± 0.24	2.70 ± 0.27	3.25 ± 0.37 [*]	2.86 ± 0.21	3.38 ± 0.23	2.55 ± 0.34	3.19 ± 0.32

[†] Data presented as means ± SEM (*n* = 10). Student's *t*-test (**, *P* < 0.10; *, *P* < 0.05) was used to compare the difference between the no hormone without KClO₄ (untreated) group and the no hormone with KClO₄ group. ANOVA was used to compare the difference between the no hormone group and a particular hormone treatment in either the presence or absence of KClO₄. Groups with different letters are significantly different from each other (*P* < 0.05); groups with no letter designation are not different.

method [4] using a gravimetrically quantified lipid extract of trout liver as standard. The remaining tissue was used to determine the rates of lipolysis, lipogenesis, and oxidative metabolism [12]. Pooled tissue samples (two animals per pool) were homogenized in five volumes of 25 mM HEPES buffer (containing 100 mM KF, 5 mM EDTA, 5 mM EGTA, 0.1 mM phenylmethylsulphonyl fluoride, pH 7.5), and the homogenate was centrifuged at 16 000 × *g* and 4°C. Aliquots of the resulting supernatant were used for enzyme assay after protein determination (see Bio-Rad Technical Bulletin 1177). The rate of lipid breakdown was evaluated by assessing triacylglycerol lipase (TGL) (EC 3.1.1.2) activity. Units of enzyme activity are expressed as picamoles ³H-oleic acid released per hour per milligram protein from the ³H-triolein (tri-[9,10(*n*)-³H]-oleate glycerol). The rate of lipid synthesis was evaluated by assessing separately de novo fatty acid synthesis, as determined by ACC (EC 6.4.1.2) activity, and triacylglycerol synthesis, as determined by diacylglycerol acyltransferase (DGAT) (EC 2.3.1.20) activity. Units of ACC activity and DGAT activity are expressed as nanomoles ¹⁴C-malonyl-CoA formed per hour per milligram protein and picamoles ¹⁴C-TG formed per minute per milligram protein, respectively. The rate of

oxidative metabolism was evaluated by assessing citrate synthase (CS) (EC 4.1.3.7) activity. Units of CS activity are expressed as nanomoles CoASH formed per minute per milligram protein.

2.4. Statistical analysis

Data are expressed as means ± SEM. Analysis of variance (ANOVA) and Duncan's multiple range test were used to examine differences among the various means. The Student's *t*-test analysis determined differences between the untreated group and the group treated with KClO₄ alone. A probability level of 0.05 was used to indicate significance. All statistics were performed using SIGMASTAT (Jandel Scientific, Palo Alto, CA).

3. Results

The metamorphosis of lamprey from larvae to stage 1, 2, 3, or 4 was induced after 8 and 16 weeks of treatment with KClO₄ alone [19]. However, larvae treated with KClO₄ in combination with T3 or T4, irrespective of hormone dose, failed to initiate meta-

morphosis. Similarly, untreated larvae or those treated with TH alone did not metamorphose. KClO_4 -treated animals displayed reduced levels of TH compared with the untreated control group or to the groups receiving KClO_4 in combination with TH, while the TH-treated animals exhibited higher plasma levels of TH than their untreated counterparts [19].

3.1. Organ weights and indices

There was no difference in animal size (length and weight) among groups at the commencement of the study [19]. However, a number of body characteristics of lamprey were affected by T4, T3, and KClO_4 after 4, 8, and 16 weeks of these treatments (Table 1). Eight weeks of exposure to KClO_4 alone tended to decrease the liver weight, hepatosomatic index, and kidney weight, whereas 4 and 16 weeks of exposure generally had no effect on these parameters. Compared with the sea lampreys treated with KClO_4 alone, TH tended to prevent KClO_4 -induced alterations in the liver weight, and kidney weight after 8 weeks of treatment. Compared with untreated animals, treatment of larvae with TH alone generally did not change liver and kidney weight after 4, 8, and 16 weeks; although T3 treatment significantly increased liver weight after 16 weeks.

3.2. Effects of KClO_4

Substantial alteration in lipid metabolism accompanied KClO_4 -induced metamorphosis of sea lampreys. KClO_4 -treated larvae displayed decreased total lipid content compared with untreated animals in liver and kidney samples after 8 weeks of treatment (Fig. 1A). KClO_4 -induced hepatic and renal lipid depletion was supported by changes in lipolysis as well as lipogenesis. Enhanced hepatic TGL activity was observed following 8 and 16 weeks of KClO_4 treatment (Fig. 1B). De novo fatty acid synthesis in the liver and triacylglycerol synthesis in both the liver and kidney, on the other hand, were reduced in the presence of KClO_4 . The activity of ACC in liver removed from KClO_4 -treated animals was significantly lower than that in control animals at each sampling period (Fig. 1C). DGAT and CS activity in both the liver and the kidney was reduced following 8 weeks of KClO_4 treatment (Fig. 1D,E).

3.3. Combined effects of thyroid hormones and KClO_4

In general, treatment of larvae with TH tended to block KClO_4 -induced changes in lipid metabolism. However, the extent of the lipid preserving effect of T4 and T3 on KClO_4 -induced changes in lipid content depended on hormone dose and duration of treatment. Four weeks of exposure to the high dose of T4 in the

presence of KClO_4 resulted in significantly higher hepatic total lipid content than in the livers of animals treated with KClO_4 alone (Fig. 2A). Treatment with the low dose of T3 for 8 weeks in the presence of KClO_4 also resulted in significantly higher hepatic total lipid content than was observed in livers of animals treated with KClO_4 alone (Fig. 3A).

The pattern of TH to preserve tissue lipid content in the presence of KClO_4 was reflected by the activities of lipolytic and lipogenic enzymes. Hepatic and renal TGL activity was reduced in larvae treated with a combination of TH (particularly T3) and KClO_4 compared with animals treated with KClO_4 alone (Fig. 2B and Fig. 3B). Lipogenesis also tended to be stimulated by the presence of TH in combination with KClO_4 in comparison with KClO_4 alone. The presence of T4 for 4–8 weeks in combination with KClO_4 resulted in higher hepatic and renal ACC activity than observed in larvae treated with KClO_4 alone (Fig. 2C). A combination of the high dose of T4 or either dose of T3 with KClO_4 , however, resulted in reduced renal ACC activity after 16 weeks (Fig. 2C and Fig. 3C). The presence of T3 for 8 weeks in combination with KClO_4 resulted in significantly higher hepatic ACC and renal DGAT activities compared with those observed in animals treated with KClO_4 alone (Fig. 3C,D). In addition, the presence of TH for 8 weeks in combination with KClO_4 tended to result in higher hepatic and renal rates of oxidative metabolism, as indicated by higher hepatic and renal CS activity (Fig. 2E and Fig. 3E), compared with those observed in animals treated with KClO_4 alone.

3.4. Effects of thyroid hormones alone

The effects of TH alone on lipid metabolism varied somewhat and depended on the dose and the duration of the treatment (Figs. 4 and 5). T4 at the high dose increased liver total lipid content of larvae after 4 and 16 weeks of treatment (Fig. 4A). After 8 weeks of high-T4 treatment, there was enhanced TGL activity (Fig. 4B). The rate of de novo fatty acid synthesis, as measured by ACC activity, was significantly decreased in the liver removed from animals treated for 4 weeks with the low-T4 dose; whereas in the kidney, ACC activity was significantly elevated after 8 weeks of treatment with the low dose only (Fig. 4C). DGAT and CS activities were not changed in the liver and kidney after 8 weeks of T4 treatment (Fig. 4D,E).

Treatment with T3 alone showed some alteration in lipid metabolism in the liver and kidney of larvae (Fig. 5). The low dose of T3 significantly increased hepatic total lipid content after 4 and 16 weeks of treatment. High doses of T3 resulted in significantly higher renal lipid content after 16 weeks than in tissue from

Fig. 1. Effects of potassium perchlorate (KClO₄) on (A) total lipid content, (B) rate of lipolysis, (C) rate of de novo fatty acid synthesis, (D) rate of triacylglycerol synthesis, and (E) rate of oxidative metabolism in the liver and kidney of landlocked lamprey, *Petromyzon marinus*, after 4, 8, and 16 weeks of treatment. Values are expressed as the means $\pm$ SEM ($n = 5$ pools, two animals per pool). An asterisk represents a significant ($P < 0.05$) difference between the untreated group and the KClO₄-treated group.

untreated control animals (Fig. 5A). The tendency toward reduced hepatic lipid content observed after 8 weeks of T3 treatment (Fig. 5A) was accompanied by enhanced triacylglycerol lipase activity (Fig. 5B). There was a trend for the rate of fatty acid synthesis in the liver and kidney to decrease after 4 weeks of the high dose T3 treatment (Fig. 5C), but the differences from the control were not significant. The rates of triacylglycerol synthesis and oxidative metabolism for liver and kidney were not significantly changed after 8 weeks of either low or high dose T3 treatment compared with the control group (Fig. 5D,E).

4. Discussion

A clear pattern of the role of thyroid hormones in regulating lipid metabolism of vertebrates has not emerged. In adult lampreys, *Entosphenus tridentatus*, and hagfish, *Eptatretus stouti*, in vivo administration of T3 results in lowered plasma fatty acid levels (consistent with a lipogenic role) 24 h after injection; but by 48 h, the pattern is reversed [22]. In teleosts, TH generally promote lipid depletion from depot sites [22,25] and favor the smoltification-associated lipid depletion pattern of coho salmon [24]. In anuran amphibians, TH

Fig. 2. Combined effects of T₄ and KClO₄ on (A) total lipid content, (B) rate of lipolysis, (C) rate of de novo fatty acid synthesis, (D) rate of triacylglycerol synthesis, and (E) rate of oxidative metabolism in the liver and kidney of landlocked lamprey, *Petromyzon marinus*, after 4, 8, and 16 weeks of treatment. Values are expressed as the means $\pm$ SEM ($n = 5$ pools, two animals per pool). An asterisk represents a significant ($P < 0.05$) difference between the KClO₄-treated group and the KClO₄ + hormone groups.

tend to favor lipogenesis during their metamorphosis, particularly during premetamorphosis [8,14]. In birds, TH stimulate fatty acid synthesis in chick hepatocytes in vitro by enhancing activities of ACC, fatty acid synthase, and malic enzymes [6]. In mammals, TH treatment or hyperthyroidism can stimulate fatty acid synthesis and triacylglycerol synthesis in a tissue-specific manner [1,5,23,31]. The results of the present study on larval lampreys provide support for our initial hypothesis and suggests that TH play a role in regulating lipid metabolism during the metamorphosis of sea

lampreys.

This conclusion is based primarily on the fact that we showed that the catabolism of lipids accompanies TH deficiency brought about by KClO₄-induced metamorphosis of sea lampreys and that TH supplementation with this goitrogen tends to reverse these effects. KClO₄ treatment of 8 weeks resulted in lipid depletion from the primary depot sites, the liver and kidney, and this was accompanied by enhanced TG lipase activity, and reduced lipogenesis as assessed by ACC and DGAT activities. The treated animals at this time were showing

Fig. 3. Combined effects of T_3 and potassium perchlorate (KClO_4) on (A) total lipid content, (B) rate of lipolysis, (C) rate of de novo fatty acid synthesis, (D) rate of triacylglycerol synthesis, and (E) rate of oxidative metabolism in the liver and kidney of landlocked lamprey, *Petromyzon marinus*, after of 4, 8, and 16 weeks treatment. Values are expressed as the means $\pm$ SEM ($n=5$ pools, two animals per pool). An asterisk represents a significant ($P < 0.05$) difference between the KClO_4 -treated group and the KClO_4 + hormone groups.

external signs that they were undergoing metamorphosis, and their liver and kidney weights and their hepatosomatic index had declined. Such patterns of lipid metabolism are reminiscent of those observed during spontaneous metamorphosis in which plasma T_4 and T_3 levels decline [38] concomitant with depletion of lipids from the whole body and these lipid depot organs [26]. The KClO_4 treatment of larvae in the present study resulted in reduced serum levels of T_4 and T_3 and 80% of these larvae underwent metamorphosis [19]. Although it is possible that KClO_4 may have effects on

lipid metabolism independent of its action on the thyroid axis, the present study provided some data to support the view that elevated serum TH not only favor the larval phenotype, but also favor lipid accumulation during this phase of the lifecycle. This contention also is supported by a number of observations. Namely, TH had a tendency to block KClO_4 -induced alterations in lipid metabolism. For example, T_4 (4 weeks) and T_3 (8 weeks) treatment in the presence of KClO_4 reversed hepatic total lipid depletion that had been promoted following treatment with KClO_4 alone. This result was

Fig. 4. Effects of T₄ alone on (A) total lipid content, (B) rate of lipolysis, (C) rate of de novo fatty acid synthesis, (D) rate of triacylglycerol synthesis, and (E) rate of oxidative metabolism in the liver and kidney of landlocked lamprey, *Petromyzon marinus*, after 4, 8, and 16 weeks of treatment. Values are expressed as the means $\pm$ SEM ($n = 5$ pools, two animals per pool). An asterisk represents a significant ($P < 0.05$) difference between the untreated group and the hormone-treated groups.

supported by reduced activity of lipolytic enzymes and enhanced fatty acid synthesis by T₄ and T₃ with KClO₄ compared with KClO₄ alone. Furthermore, the trend for T₄ and T₃ treatment alone was to slightly increase or maintain total lipid content in liver and kidney. Larval sea lampreys, which possess relatively high endogenous levels of TH [38], are in a phase of lipid accumulation [26]. However, the unusually high serum levels of TH in TH-treated animals of the present study [19] may have had a marked effect on the general pattern of lipid metabolism in larvae. Thus, lipolytic

effects of TH on sea lampreys after 8 weeks of treatment may be pharmacological since concentrations of serum TH in this group following exogenous administration [18,19] are beyond the physiological levels of larvae [38].

The results of this study provide insight into the potential role of TH in coordinating lipid metabolism prior to and during spontaneous metamorphosis of sea lampreys. For instance, in the present study, TH administration tended to support a lipogenic rather than a lipolytic pathway. The gradual increase in levels of

Fig. 5. Effects of T₃ alone on (A) total lipid content, (B) rate of lipolysis, (C) rate of de novo fatty acid synthesis, (D) rate of triacylglycerol synthesis, and (E) rate of oxidative metabolism in the liver and kidney of landlocked lamprey, *Petromyzon marinus*, after 4, 8, and 16 weeks of treatment. Values are expressed as the means $\pm$ SEM ($n = 5$ pools, two animals per pool). An asterisk represents a significant difference ($P < 0.05$) between the untreated group and the hormone-treated groups.

serum TH observed during the growth of larval sea lampreys to reach a peak concentration in the oldest larvae [38] is concomitant with the high lipid content observed in immediately premetamorphic larvae [36]. A decline in serum levels of TH by KClO₄ treatment is consistent with lipid depletion. As metamorphosis proceeds, declining levels of TH [38] support a shift from lipid anabolism to lipid catabolism, resulting in the depletion of stored lipid [11,12].

Despite the presented evidence for a possible direct TH–fat depot interaction, we do not know the mode of action of KClO₄ on the thyroid axis or whether, in fact,

it acts directly upon any component of the thyroid axis. Perhaps this goitrogen acts directly on the fat stores [35]. There also is evidence that TH may interact with other tissues which yield other factors, such as insulin (INS) and somatostatin (SS), known to be involved in lipid metabolism. Preliminary studies have shown that serum TH levels decline after acute immunoneutralization with anti-lamprey insulin and increase following anti-lamprey somatostatin treatment [37]. In addition, insulin stimulates lipid deposition in storage sites [13,21], while somatostatin promotes lipid depletion from storage sites [13]. The actions of TH on the lipid

depots in sea lampreys may also be direct through a TH nuclear receptor pathway, as reported in other vertebrate systems [8]. T3 hepatocyte nuclear receptors were reported to have slightly altered binding capacity when those from larvae and metamorphosing *P. marinus* were compared [17,29]. Further studies on the in vitro effects of TH and on the associated signal transduction system are needed to elucidate the hormonal regulation of development-associated alterations in lipid metabolism in larval sea lampreys.

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