Temperature and KClO₄-induced metamorphosis in the sea lamprey (Petromyzon marinus)

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Abstract

Larval sea lampreys (Petromyzon marinus) were exposed to either a warm (18°C) or a cold (3°C) water temperature and either with (treated) or without (untreated) the presence of potassium perchlorate (KClO₄). After 23 weeks, larvae were examined for signs of metamorphosis and serum samples were collected to assay thyroxine (T₄) and 3,5,3'-triiodothyronine (T₃) concentrations. Water temperature did not significantly affect serum T₄ or T₃ concentrations in untreated larvae and no metamorphosis occurred in these groups. Serum T₄ concentrations were not significantly different between the two temperature groups treated with KClO₄. However, serum T₃ concentrations were significantly higher in the cold water, KClO₄-treated larvae (5.4 nmol/l) than in the warm water, KClO₄-treated larvae (1.2 nmol/l). KClO₄ treatment at a warm water temperature induced metamorphosis in all larvae and resulted in serum T₄ and T₃ concentrations which were 66 and 95% lower, respectively, than untreated larvae in warm water. Despite having significantly lower serum T₄ and T₃ concentrations (73 and 80%, respectively) than untreated cold water larvae, metamorphosis was not observed in cold water, KClO₄-treated larvae. The results of this study indicate that warm water is a requirement for the successful induction of metamorphosis with KClO₄, and provide further evidence of water temperature as an important factor in the metamorphosis of lampreys. © 1999 Elsevier Science Inc. All rights reserved.

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1. Introduction

The life cycle of the sea lamprey (Petromyzon marinus) includes a sedentary, filter-feeding, larval period lasting 3–7 years and a true metamorphosis in which larvae undergo many morphological and physiological changes in preparation for a free-swimming, parasitic, juvenile phase [21,22]. Numerous studies (see for example Refs. [23,24]) have investigated the environmental and physiological factors which potentially influence the onset, rate, and incidence of metamorphosis in lampreys. Included among these factors are temperature, lipid reserves, and thyroid hormones (TH).

Temperature is the predominant environmental factor influencing metamorphosis in lampreys. Studies have indicated that temperature can affect the rate, the time of onset, and the incidence of spontaneous metamorphosis in larval sea lampreys [13,16,17,26]. In the aforementioned studies, warm water temperatures (21°C) were more favourable for metamorphosis than cool water temperatures (13°C).

Throughout the larval growth phase in sea lampreys, serum thyroxine (T₄) and 3,5,3'-triiodothyronine (T₃) concentrations gradually rise and reach their peak just prior to the onset of metamorphosis [27]. Subsequent to a peak in serum TH concentrations and concomitant with early metamorphic change, serum TH concentrations decline rapidly [11,20,27]. The significance of this peak and decline is not fully understood, but several experiments suggest that they are keys to metamorphosis. Treatment of larval sea lampreys (including larvae smaller than the minimum size required for spontaneous metamorphosis) with potassium perchlorate (KClO₄) depressed serum TH concentrations and induced metamorphosis at a time of year when sponta-
neous metamorphosis does not occur [6,28]. Furthermore, KClO₄-induced metamorphosis can be blocked with exogenous T₄ or T₃ treatments [14,15], and T₃ retards spontaneous metamorphosis [29]. These KClO₄ treatment studies were conducted at nonseasonal, warm, water temperatures (16–20°C) in the winter months when spontaneous metamorphosis does not occur. The effects of temperature on the induction process have not been investigated.

The primary objective of the current study was to determine if warm water is required for KClO₄ treatment to induce metamorphosis. Another goal was to investigate the effects of warm and cold water conditions in the presence and absence of KClO₄ on serum TH concentrations.

2. Materials and methods

Larval sea lampreys (Petromyzon marinus) greater than 120 mm in length were collected from Putnam Creek, New York in June 1995 and housed at a temperature of approximately 11°C at the University of Toronto at Scarborough [15]. On October 10, larval sea lampreys were removed in groups of five from a holding tank (without substrate) containing 150 individuals. The lengths and masses of these larvae were recorded, and larvae were assigned to one of four experimental aquaria (21 l; 40 x 20 x 25 cm) until each aquarium contained 10 larvae. Aquaria were maintained static (not on a flow-through system), and contained 12 l of dechlorinated tap water and 6–7 cm of industrial sand for substrate. Water in the experimental aquaria was aerated and all aquaria were maintained on a 15-h light, 9-h dark photoperiod throughout the study. Each aquarium was assigned one of four experimental groups: untreated cold water (untreated cold); untreated warm water (untreated warm); potassium perchlorate (KClO₄)-treated cold water (cold KClO₄); or KClO₄-treated warm water (warm KClO₄). Animals and experimental groups were randomly assigned to aquaria by lottery. Larvae were left undisturbed in their new environment for 1 day prior to any change in temperature and 8 days prior to the addition of KClO₄. Water temperature in the cold water groups was gradually lowered by approximately 2°C per day until a temperature of 3°C was attained [typical winter temperatures for Putnam Creek (1–4°C); J.E. Gersmehl, pers. commun.]. Cold water temperatures were achieved using a circulating water chiller (Frigid Units) to regulate the water temperature in a large insulated tank which was used as a bath to maintain the temperature of the experimental aquaria. Water temperature in the warm water groups was maintained at ambient room temperature (16–20°C). The experiment was initiated on October 17, when 500 ml of a KClO₄ stock solution was added to the warm and cold KClO₄ aquaria to achieve a final concentration of 3.6 mM (0.05%).

The instantaneous water temperature in each of the untreated aquaria was recorded twice daily, larvae were fed 100 ml of a Fleischmann’s baker’s yeast solution equal to 1 g of yeast/animal per week [12], and water was added to the aquaria as needed to keep the total volume at 12 l. Aquaria were cleaned, the water was changed, and fresh treatments were added every 2 weeks without disturbing the burrowed larvae. The accumulation of waste products, e.g. ammonia, was not determined throughout the course of the experiment. Spent larvae were replaced using animals marked with a latex dye injected into the caudal sinus; marked animals were excluded from all analyses. Eight weeks from the onset of the study all sea lampreys were anesthetized in a solution of 0.05% tricaine methanesulfonate and examined for external signs of the seven stages (1 to 7) of metamorphosis [25]. The experiment was terminated 23 weeks from its onset at which time sea lampreys were anesthetized, their lengths, masses, and metamorphic stages were recorded and serum was collected [14].

Serum T₄ and T₃ concentrations were measured using the Amersham Amerlex TT4 and TT3 radioimmunoassay (RIA) kits, respectively. The kits were modified for sea lamprey serum TH concentrations and small working volumes [9]. All serum samples were assayed in duplicate; assay sensitivities were 7 and 0.4 nmol/l for T₄ and T₃, respectively, and intra- and inter-assay variances were 9.4 and 11.8% for T₄, and 7.2 and 12.9% for T₃, respectively.

Differences in animal length and mass, and serum T₄ and T₃ concentrations between aquaria (groups) were tested for statistical significance using analysis of variance (ANOVA) and Tukey’s HSD multiple comparison test. Prior to ANOVA analyses, all data were tested for heteroscedasticity of variances using Cochran’s Q-test. Data which did not meet the assumption of homoscedasticity were transformed (log_{10}); subsequent to transformation all data satisfied this assumption [19]. All statistical calculations were performed using Statistica for Microsoft DOS, data are presented as mean ± 2 S.E., and all differences were considered statistically significant if P < 0.05.

3. Results

Mean water temperatures in warm and cold water aquaria were 18 ± 0.17 and 3 ± 0.07°C, respectively. The mean length and mass of all larval sea lampreys at the onset of the study were 138 ± 4 mm and 3.89 ± 0.38 g, respectively, and these size parameters did not differ significantly among groups. Additionally, significant differences in animal length and mass were not ob-
served among the groups at the termination of the experiment when the overall mean animal size was $137 \pm 4$ mm and $3.70 \pm 0.37$ g.

Eight weeks from the onset of the study, metamorphosis was not observed in larvae from the untreated warm, untreated cold, or cold KClO$_4$ experimental groups. However, at this time 80% of the warm KClO$_4$ larvae had commenced metamorphosis. Five animals were determined to be in stage 1 of metamorphosis, three in stage 2 of metamorphosis, and the remaining two individuals were larvae. At the end of the study (following 23 weeks of treatment), metamorphosis was still not observed in any untreated or cold KClO$_4$ larvae. There had been some mortality of unknown causes in the warm KClO$_4$ experimental group but all surviving (8/8) animals had commenced metamorphosis (Fig. 1). Of these metamorphosing individuals, one was described to be at stage 1, two between stages 3 and 4, one at stage 4, three at stage 5, and one at stage 6 of metamorphosis. Warm KClO$_4$ larvae were clearly undergoing metamorphosis, but the timing and morphology of KClO$_4$-induced development was asynchronous. In several individuals the eyes, teeth, and/or body coloration had undergone changes in advance of structures such as the oral disc and branchiopores. In spontaneous metamorphosis the changes in the morphology of these structures are highly synchronized within and among individuals [25].

Serum T$_4$ and T$_3$ concentrations of untreated larvae were not significantly affected by temperature. Mean serum concentrations were 92 and 100 nmol/l for T$_4$, and 23.7 and 27.6 nmol/l for T$_3$ in the untreated warm and cold larvae, respectively (Fig. 2). Similarly, serum T$_4$ was not affected by temperature in the presence of KClO$_4$; mean concentrations were 31 and 26 nmol/l for the warm and cold KClO$_4$ groups, respectively (Fig. 2A). However, KClO$_4$ treatment significantly depressed serum T$_4$ concentrations in both warm and cold KClO$_4$ larvae relative to untreated warm and cold larvae. Warm and cold KClO$_4$ larvae had serum T$_4$ concentrations 66 and 73% lower than their respective untreated warm and cold larvae. KClO$_4$ treatment also significantly depressed serum T$_3$ concentrations in both warm and cold KClO$_4$ larvae relative to untreated warm and cold larvae. Serum T$_3$ concentrations in warm and cold KClO$_4$ larvae were 95 and 80% lower than untreated warm and cold larvae, respectively. Furthermore, mean serum T$_3$ concentrations in warm KClO$_4$ larvae (1.2 nmol/l) were significantly lower than in cold KClO$_4$ larvae (5.4 nmol/l; Fig. 2B).

4. Discussion

The goitrogen KClO$_4$ induces precocious metamorphosis and a concomitant decline in serum TH concentrations in larval sea lampreys [6,14,15,28].
aforementioned studies controlled for temperature and time of year; they were conducted during the winter months at warm (summer) water temperatures (16–20°C). In the current study, larval sea lampreys were exposed to either a warm or cold water temperature (equivalent to summer and winter conditions, respectively) in the presence or absence of KClO₄; the experiment was conducted at a time of year when spontaneous metamorphosis is not expected. Exposure of larvae to KClO₄ at warm water temperatures significantly depressed serum TH concentrations and induced metamorphosis in 100% of all surviving individuals; mortality was due to causes seemingly unrelated to the treatment. These results with KClO₄ treatment at a warm water temperature are similar to those of earlier studies [6,14,15,28]. However, KClO₄ treatment at a cold water temperature did not induce metamorphosis in any larvae despite significant declines in both serum T₄ and T₃ concentrations. The absence of metamorphosis in cold KClO₄ larvae further emphasizes the importance of water temperature in the initiation of lamprey metamorphosis and indicates that a decrease in serum TH concentrations is not sufficient to induce metamorphosis. T₃-induced metamorphosis in amphibians is also temperature-dependent. Frieden et al. [5] and Ashley et al. [1] found no change in frog tail length or urea production, respectively, following T₃ treatment at 5°C but responses were detected at temperatures ≥ 7.5°C.

Serum T₃ concentrations in warm KClO₄ larvae were significantly lower than serum T₃ values in cold KClO₄ larvae; furthermore, the magnitude of the declines in serum T₃ concentrations from values in respective untreated larvae were greater for warm KClO₄ larvae (95%) than cold KClO₄ larvae (80%). Despite the differences in serum T₃ concentrations between warm and cold KClO₄ larvae, it is not likely that these differences contributed to the absence of metamorphosis in cold KClO₄ larvae. In two previous studies, metamorphosis was observed in larval sea lampreys treated with KClO₄ at a warm water temperature in which serum T₃ concentrations were only 72–77% lower than values for untreated control larvae [14,15]; these declines in serum T₃ levels are similar to those observed in cold KClO₄ larvae of the current study. Thus, the absence of metamorphosis in cold KClO₄ larvae may have been a result of the effects of the cold water temperature on regulatory processes of metamorphosis other than serum TH concentrations.

Many biological processes essential to life in poikilothersms are dependent either directly or indirectly on environmental temperature which has been shown to alter both basal metabolic rate and the rate at which developmental processes proceed [2]. In lampreys, it has been established that metabolic rate (measured as oxygen consumption) decreases with decreasing temperature [10,18]. Cold water temperatures may reduce the metabolic rate in lampreys below a minimum required for metamorphosis or may simply reduce the rate of metamorphic development.

We have previously shown that KClO₄ treatment at warm water temperatures does not affect intestinal T₄ outer-ring (5’) deiodination to T₃ (T₄,ORD), the primary site and deiodination pathway, in larval sea lampreys [15]. However, it has been shown that changes in deiodinase activity coincide with changing developmental and/or physiological state in lampreys [3,4]. Although KClO₄ treatment at warm water temperatures did not alter intestinal T₄,ORD activity [15], perhaps the activities of this or other deiodinase pathways were sufficiently altered in cold KClO₄ larvae of the current study to prevent the induction of metamorphosis. Temperature has been shown to affect TH deiodinase activity in rainbow trout [7]. Furthermore, Kaltenbach [8] posed the question: Does temperature alter the number of TH receptors in anurans? Could this question also apply to lampreys? Future research should be directed towards elucidating the function of the TH deiodinase pathways and TH receptors in metamorphosis and their responses to temperature change.

The initiation of lamprey metamorphosis occurs when a number of physiological and environmental requirements are met [23]. KClO₄ depresses serum TH concentrations and can override some of these requirements such as size, condition factor, essential fat stores, and thyroid status resulting in the induction of metamorphosis [24]. However, water temperature is a very important cue in lamprey metamorphosis which cannot be eliminated from the sequence of events involved in the initiation of this developmental process.

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References


