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Effect of Alloxan and Insulin Immunoneutralization on Circulating Thyroid Hormone Levels in Larval Landlocked Sea Lampreys, *Petromyzon Marinus*

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ABSTRACT—The effects of alloxan, an insulin (INS)-secreting cell toxin, and INS immunoneutralization on circulating levels of thyroid hormones (thyroxine, T₄; triiodothyronine, T₃) were examined in larval landlocked sea lampreys, *Petromyzon marinus*. Animals were injected intraperitoneally with either (Experiment 1) saline (0.6%) or alloxan (20 or 200 µg/g body weight), or with (Experiment 2) normal rabbit serum or anti-lamprey INS. Alloxan (200 µg/g) decreased plasma T₃, but not T₄, in larvae by about 45–80%. Three, six, or nine hr after acute immunoneutralization of lamprey INS with anti-lamprey INS, plasma T₃ levels decreased by 13–30%, relative to controls. These data indicate that INS deficiency can regulate the thyroid system of larval lampreys. There is some suggestion that INS may mediate the metamorphic processes by modulating thyroid hormone concentrations.

Key words: insulin, thyroxine, triiodothyronine, alloxan, lamprey

INTRODUCTION

The endocrine system of lampreys, like other poikilothermic vertebrates that possess a metamorphic episode as part of their life history (Dickhoff, 1993), is inextricably linked to the metamorphic process (Youson, 1997). It has been suggested that thyroid hormones (TH; thyroxine, T₄, and triiodothyronine, T₃) are important factors in the metamorphic development of lampreys (Youson, 1997). This notion is supported by the observation that TH deficiency, caused by treatment with goitrogens, induces precocious metamorpho-

sis of four Northern Hemisphere lamprey species, *Lampetra planeri* (Hoheisel and Sterba, 1963), *L. reissneri* (Suzuki, 1986), *P. marinus* (Youson *et al.*, 1995), and *L. appendix* (Holmes *et al.*, 1999). In addition, TH treatment completely blocked KClO₄-induced metamorphosis (Manzon *et al.*, 1998) and inhibited KClO₄-induced changes in lipid metabolism that are similar to those observed during spontaneous metamorphosis (Kao *et al.*, 1999a). A recent report indicated that, with the exception of propylthiouracil, several different goitrogens (i.e., potassium thiocyanate, sodium perchlorate and methimazole) induced metamorphosis in *P. marinus* larvae (Manzon *et al.*, 2001).

Numerous reports, consistent with the aforementioned goitrogen experiments, have shown that premetamorphic larval lampreys maintain relatively high plasma TH titers which decline rapidly at the onset of spontaneous metamorphosis (Lintlop and Youson, 1983; Suzuki, 1986; Leather-

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land *et al.*, 1990; Youson *et al.*, 1994). The falling plasma TH levels observed during the metamorphosis of lamprey were coincident with the rising concentrations of insulin (INS) in the serum (Youson *et al.*, 1994). In the hagfish (another agnathan), *Eptatretus stouti*, INS insufficiency, achieved 24 hr following the administration of guinea pig anti-human INS serum, lowered plasma T₃, but had a tendency to increase plasma T₄ levels (Plisetskaya *et al.*, 1983). To date little is known about the regulatory influences which INS has on TH titers in lampreys nor the ultimate role this plays in lamprey metamorphosis.

The objective of this study was to examine the effects of alloxan and INS immunoneutralization TH levels in larval sea lamprey in order to gain further insight into the evolution of TH regulatory systems and the endocrine control of metamorphosis.

MATERIALS AND METHODS

Sea lamprey, *P. marinus*, larvae (body weight, 2.7–2.9 g; body length, 116–130 mm) were housed and injected intraperitoneally (10 µl/g body weight) as previously described by Kao *et al.* (2001). Briefly, larvae were administered 0.6% of saline (control) or alloxan (5, 6-dioxyuracil monohydrate; dissolved in 0.6% saline; Sigma) at a low dose of 20 µg or high dose of 200 µg per gram body weight once per day for 2 days. Alloxan is a β-cell cytotoxin (Lukens, 1948; Morris and Islam, 1969). Twelve to 14 hr after the last injection, individual animals were anesthetized with 0.05% tricaine methanesulfonate. Acute neutralization of pancreatic hormones was induced essentially as previously described by Plisetskaya *et al.* (1989). Groups of 10 larvae received an intraperitoneal injection of 10 µl/g body weight of 1:1 mixture of 0.6% saline and either anti-lamprey INS or normal rabbit serum (control). The antisera were preabsorbed with excess thyroglobulin before injection. The validation of the antisera was described by Plisetskaya *et al.* (1988), and Youson *et al.* (1992). Blood was collected into heparinized-capillary tubes from the severed caudal vasculature 3, 6, or 9 hr after the injection. Plasma was collected and stored at –70°C for later determination of TH concentrations. Plasma TH was measured by the radioimmunoassay (RIA) method described by Manzon *et al.* (2001). Inter- and intra-assay variance was about 10% for both assays, and assay sensitivities were 8.0 and 0.24 nM for T₄ and T₃, respectively. The T₄ antiserum used in the RIAs has a cross-reactivity of 12% with T₃, and the T₃ antiserum a cross-reactivity of 0.3% with T₄. Data are expressed as mean±SEM. Unpaired Student *t*-test was used to examine differences between two groups. Analysis of variance (ANOVA) and Duncan's multiple range test were used to examine differences among the various groups in the dose-dependent experiment. A probability level of 0.05 was used to indicate significance. All statistics were performed using SigmaStat (Jandel Scientific, Palo Alto, CA).

RESULTS AND DISCUSSIONS

Plasma concentrations of T₄ and T₃ differed in larva; T₄ levels were 3–5 times greater than T₃ levels (Fig. 1A). The high dose (200 µg/g body weight) of alloxan, a β-cell toxin and oxidant, decreased plasma T₃ concentrations in larvae, but had no effect on plasma T₄ levels in larval animals (Fig. 1A). Acute neutralization of lamprey INS into larvae treated with anti-lamprey INS for 3, 6 or 9 hr tended to reduce the

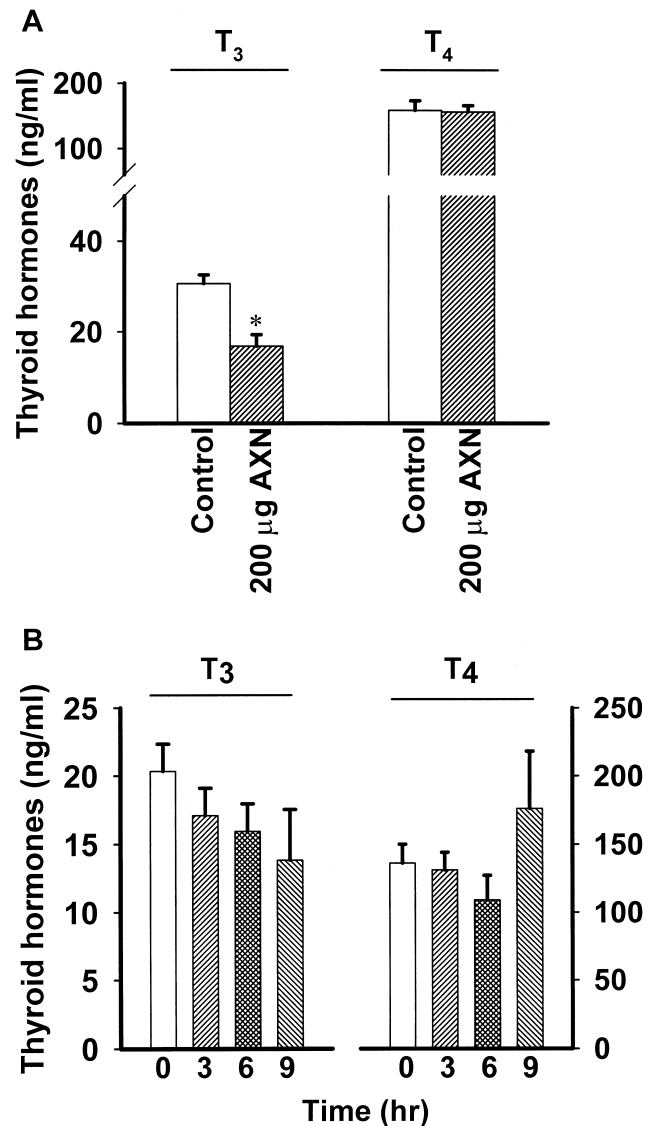


FIG. 1. Effect of alloxan (AXN; **A**) and acute immunoneutralization with lamprey INS antiserum (**B**) on plasma thyroid hormone (T₃, triiodothyronine; T₄, thyroxine) levels of larval lampreys, *Petromyzon marinus*. Data presented as mean±SEM. (n=5–6 animals in **A** and n=10 animals in **B**). In (**B**), the Arabic numbers, 3, 6 and 9 indicate hours after injection of anti-lamprey INS serum. An asterisk symbol indicates a significant (a critical probability value<0.05) difference from the control group.

concentrations of plasma T₃ and T₄; however, the elevation of plasma T₄, above control levels, was observed 9 hr after the administration (Fig. 1B).

The present study provides the observations that acute INS deficiency, brought about by anti-lamprey INS serum or by alloxan treatments, reduced plasma T₃, but not T₄, concentrations in larval sea lampreys. These data are consistent with those for hagfish, another cyclostome, in which INS insufficiency 24 hr after guinea pig anti-human INS serum administration resulted in a decline of plasma T₃ levels (Plisetskaya *et al.*, 1983). In contrast, salmon had a higher concentration of plasma T₃ 12 hr after the administration of

anti-salmon INS serum (Plisetskaya *et al.*, 1989). This suggests that the mechanism of TH regulation by INS in teleosts and cyclostomes is fundamentally different.

The mechanism by which INS deficiency modulates thyroid system in lamprey is not known. However, the lamprey INS antiserum-induced decrease in larval plasma TH titers is clearly a response to the neutralizing endogenous INS because plasma INS levels were reduced by 100% after injection with lamprey INS antiserum (Youson *et al.*, 1992). Whether the low concentrations of plasma TH in larval lamprey caused by INS deficiency due to the increase in the deiodinase activity (Eales *et al.*, 2000) needs further investigations.

The observed alloxan-induced decrease of plasma TH in lamprey is consistent with that reported in mammals (Kumaresan and Turner, 1966). We used alloxan in this study to provide additional insight into INS action and assumed that alloxan would alter thyroid system of sea lamprey by ablating INS. This assumption was supported by previous observations that alloxan damaged β cells and increased plasma glucose in larval *L. planeri* (Morris and Islam, 1969) and adult *L. fluviatilis* (Bentley and Follett, 1965). Many of our results, indeed, including those on increased plasma FA and plasma glucose (Kao *et al.*, 1999b and 2001) were consistent with INS disruption. Whether oxidative stress generated by alloxan oxidant (Dulin *et al.*, 1983) and diabetic glucose (Evans *et al.*, 2002) affects lamprey β cells is unknown. On the other hand, our immunohistological findings (Kao *et al.*, 1999b), as reported in lamprey (Morris and Islam, 1969; Biuw, 1970) and in mammals (Dulin *et al.*, 1983), suggest that in the time frame of our experiment (2 days, 0.2 mg/g body weight), alloxan toxicity in larval sea lampreys is primarily on extra-islet tissues and/or enzymes. This effect may also result in alterations in β -cell functions and/or thyroid functions as well as explain why alloxan injection in this study did not oppose all INS-induced alterations in TH concentrations of larvae and stage 6 transformers (unpublished data). Marked species differences to alloxan treatment in terms of the β cell toxicity have been observed (Lukens, 1948).

It appears that INS plays a role in lamprey metamorphosis. This conclusion is supported by the following observations. First, INS deficiency as reported here modulates TH concentrations. Second, TH deficiency induces precocious metamorphosis of *P. marinus* (Youson *et al.*, 1995; Manzon *et al.*, 2001); whereas, TH treatment blocks KClO₄-induced metamorphosis (Manzon *et al.*, 1998). Finally, alterations in plasma levels of INS and TH are spontaneously associated with lamprey metamorphosis (Youson *et al.*, 1994) and both hormones play an anabolic role in the metamorphosis-associated changes in lamprey lipid metabolism (Kao *et al.*, 1999a and 1999b).

Given the ancient lineage of lamprey, these findings suggest that the thyroidoregulatory roles of INS emerged early during the evolution of vertebrates. Furthermore, the findings suggest a role for INS in lamprey metamorphosis

and support the view that T₃ is the most important TH at this phase of the life cycle (Youson, 1997).

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REFERENCES

- Bentley PJ, Follett BK (1965) The effects of hormones on the carbohydrate metabolism of the lamprey *Lampetra fluviatilis*. *J Endocrinol* 31: 127–137
- Biuw LW (1970) Alloxan effects on blood glucose level and pancreatic islet tissue in *Lampetra fluviatilis*. *Gen Comp Endocrinol* 15: 43–51
- Dickhoff WW (1993) Hormones, metamorphosis, and smolting. In "The Endocrinology of Growth, Development, and Metabolism in Vertebrates" Ed by MP Schreibman, CG Scanes and PKT Pang, Academic Press, New York, pp 519–540
- Dulin WE, Gerritsen GC, Chang AY (1983) Experimental and spontaneous diabetes in animals. In "Diabetes Mellitus: Theory and Practice" Ed by M Ellenberg and H Rifkin, Elsevier/North Holland, New York, pp 361–407
- Eales JG, Mcleese JM, Holmes JA, Youson JH (2000) Changes in intestinal and hepatic thyroid hormone deiodination during spontaneous metamorphosis of the sea lamprey, *Petromyzon marinus*. *J Exp Zool* 286: 305–312
- Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2002) Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of Type 2 diabetes. *Endocr Rev* 23: 599–622
- Hoheisel G, Sterba G (1963) Über die Wirkung von Kaliumperchlorat (KClO₄) auf ammocoeten von *Lampetra planeri* block. *Z Mikrask Anat Forsch* 70: 490–516
- Holmes JA, Chu H, Khanam S, Manzon RG, Youson JH (1999) Spontaneous and induced metamorphosis in the American brook lamprey, *Lampetra appendix*. *Can J Zool* 77: 959–971
- Kao YH, Manzon RG, Sheridan MA, Youson JH (1999a) Study of the relationship between thyroid hormones and lipid metabolism during KClO₄-induced metamorphosis of landlocked lamprey, *Petromyzon marinus*. *Comp Biochem Physiol* 122C: 363–373
- Kao YH, Youson JH, Holmes JA, Sheridan MA (1999b) Effects of insulin on lipid metabolism of larvae and metamorphosing landlocked sea lamprey, *Petromyzon marinus*. *Gen Comp Endocrinol* 114: 405–414
- Kao YH, Youson JH, Holmes JA, Sheridan MA (2001) Effect of somatostatins and insulin on blood glucose levels of larvae and metamorphosing landlocked sea lamprey, *Petromyzon marinus*. *Zool Sci* 18: 1113–1116
- Kumaresan P, Turner CW (1966) Effect of alloxan on thyroid hormone secretion rate and replacement therapy with insulin in rats. *Endocrinology* 79: 828–829
- Leatherland JF, Hilliard RW, Macey DJ, Potter IC (1990) Changes in serum thyroxine and triiodothyronine concentrations during metamorphosis of the Southern Hemisphere lamprey *Geotria australis*, and the effect of propylthiouracil, triiodothyronine and environmental temperature on serum thyroid hormone concen-

- trations of ammocoetes. *Fish Physiol Biochem* 8: 167–177
- Lintlop SP, Youson JH (1983) Concentration of triiodothyronine in the sera of the sea lamprey, *Petromyzon marinus*, and the brook lamprey, *Lampetra lamottenli*, at various phases of the life cycle. *Gen Comp Endocrinol* 49: 187–194
- Lukens FDW (1948) Alloxan diabetes. *Physiol Rev* 28: 304–330
- Manzon RG, Eales JG, Youson JH (1998) Blocking of $KClO_4$ -induced metamorphosis in premetamorphic sea lampreys by exogenous thyroid hormones (TH); effect of $KClO_4$ and TH on serum TH concentrations and intestinal thyroxine outer-ring deiodination. *Gen Comp Endocrinol* 112: 54–62
- Manzon RG, Holmes JA, Youson JH (2001) Variable effects of goitrogens in inducing precocious metamorphosis in sea lampreys (*Petromyzon marinus*). *J Exp Zool* 289: 290–303
- Morris R, Islam DS (1969) The effect of hormones and hormone inhibitors on blood sugar regulation and the follicles of Langerhans in ammocoete larvae. *Gen Comp Endocrinol* 12: 81–90
- Plisetskaya EM, Sower SA, Gorbman A (1983) The effect of insulin insufficiency on plasma thyroid hormones and some metabolic constituents in Pacific hagfish, *Eptatretus stouti*. *Gen Comp Endocrinol* 49: 315–319
- Plisetskaya EM, Pollock HG, Elliott WM, Youson JH, Andrews PC (1988) Isolation and structure of lamprey (*Petromyzon marinus*) insulin. *Gen Comp Endocrinol* 69: 46–55
- Plisetskaya EM, Sheridan MA, Mommsen TP (1989) Metabolic changes in coho and chinook salmon resulting from acute insufficiency in pancreatic hormones. *J Exp Zool* 249: 158–164
- Suzuki S (1986) Induction of metamorphosis and thyroid function in the larval lamprey. In "Frontiers in Thyroidology, Vol. 1" Ed by G Mederises-Neto and E Gaitan, Plenum Press, New York, pp 667–670
- Youson JH (1997) Is lamprey metamorphosis regulated by thyroid hormones? *Am Zool* 37: 441–460
- Youson JH, Leatherland JF, Plisetskaya EM, Sheridan MA (1992) Metabolic and hormonal changes in larval lampreys (*Petromyzon marinus*) following acute neutralization with anti-insulin and anti-somatostatin. Proceedings of the Second International Symposium on Fish Endocrinology. Saint-Malo, France
- Youson JH, Plisetskaya EM, Leatherland JF (1994) Concentrations of insulin and thyroid hormones in the serum of landlocked sea lampreys (*Petromyzon marinus*) of three larval year classes, in larva exposed to two temperature regimes, and in individuals during and after metamorphosis. *Gen Comp Endocrinol* 94: 294–304
- Youson JH, Holmes JA, Leatherland JF (1995) Serum concentrations of thyroid hormones in $KClO_4$ -treated larval sea lampreys (*Petromyzon marinus* L.). *Comp Biochem Physiol* 111C: 265–270

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