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Effect of Somatostatins and Insulin on Blood Glucose Levels of Larvae and Metamorphosing Landlocked Sea Lamprey, *Petromyzon marinus*

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ABSTRACT—We examined the effects of insulin (INS) and somatostatin 14 (SS-14) on glycemic regulation in larval and metamorphosing landlocked sea lamprey, *Petromyzon marinus*. Animals were injected intraperitoneally with either (Experiment 1) saline (0.6%), somatostatin-14 (SS-14; 50 or 500 ng/g body weight), insulin (INS; 10 or 100 ng/g body weight), or alloxan (20 or 200 μg/g body weight), or with (Experiment 2) normal rabbit serum, anti-SS-14, anti-lamprey SS-34 or anti-lamprey INS. Somatostatin-14 (500 ng/g) increased plasma glucose levels in larvae. Injection of anti-SS-14 and anti-lamprey SS-34 resulted in hypoglycemia compared to the controls. Insulin (100 ng/g) resulted in hypoglycemia in both larvae and stage 6 metamorphosing lamrpey. Acute insufficiency of lamprey INS in larvae treated with anti-lamprey INS elevated plasma glucose levels. Similarly, alloxan (200 μg/g, a cytotoxin of insulin-secreting cells) resulted in hyperglycemia in larvae. These data indicate that SS-14 is hyperglycemic in sea lamprey, whereas INS is hypoglycemic, and suggest that the glucoregulatory roles of SS-14 and INS emerged early during the evolution of vertebrates.

Key words: Somatostatin, insulin, lamprey, metamorphosis, glucose homeostasis

INTRODUCTION

Pancreatic hormones such as insulin (INS) and somatostatins (SSs) are known to play an important role in regulating the metabolism of carbohydrates in teleost fish (Epple and Brinn, 1987; Plisetskaya and Duguay, 1993). In lampreys, which are an evolutionarily ancient vertebrate and have a unique metamorphic episode that transforms filter-feeding larvae to either nonparasitic or pelagic parasitic juveniles (Hardisty, 1979), much less is known. Insulin induction of hypoglycemia has been reported in larval and adult lampreys, including *Lampetra planeri* and *L. fluviatilis* (Plisetskaya, 1965; Bentley and Follett, 1965; Leibson and Plisetskaya, 1968; Morris and Islam, 1969). However, there have been no reports of INS action in sea lamprey, *P. marinus*, particularly in the period of preadult life including larval growth and metamorphosis. In addition, nothing is known about the effects of SSs on carbohydrate metabolism of lampreys. The objective of this study was to examine how INS and SSs affect glycemic levels in larval and metamorphosing sea lamprey in order to gain further insight into the evolution of carbohydrate regulating systems.

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MATERIALS AND METHODS

Sea lamprey, *P. marinus*, larvae (body weight, 2.7–2.9 g; body length, 120–128 mm) and stage 6 (body weight, 2.8–4.4 g; body length, 125–140 mm) metamorphosing lampreys (hence referred to as transformers) were housed and injected intraperitoneally (10 μl/g body weight) as previously described by Kao *et al*. (1998, 1999). Briefly, animals were maintained in 21 L glass aquaria with a sandy substrate and 12 L of dechlorinated City of Toronto water at an ambient temperature of 15–18°C under a photoperiod of 15 hr light and 9 hr dark. Larvae were fed baker's yeast once a week (18 g/25 larvae), whereas the transformers did not feed spontaneously (Hardisty, 1979). Larvae and transformers were administered with 0.6% of saline (control), SS-14 (Sigma Cat# S-9129) at a low dose of 50 ng or high dose of 500 ng SS-14 (dissolved in 0.6% NaCl)/g body weight, bovine INS (Sigma Cat# I-5500) at a low dose of 10 ng or high dose of 100 ng INS (dissolved in 0.6% NaCl at pH 4.0)/g body weight, or alloxan (5,

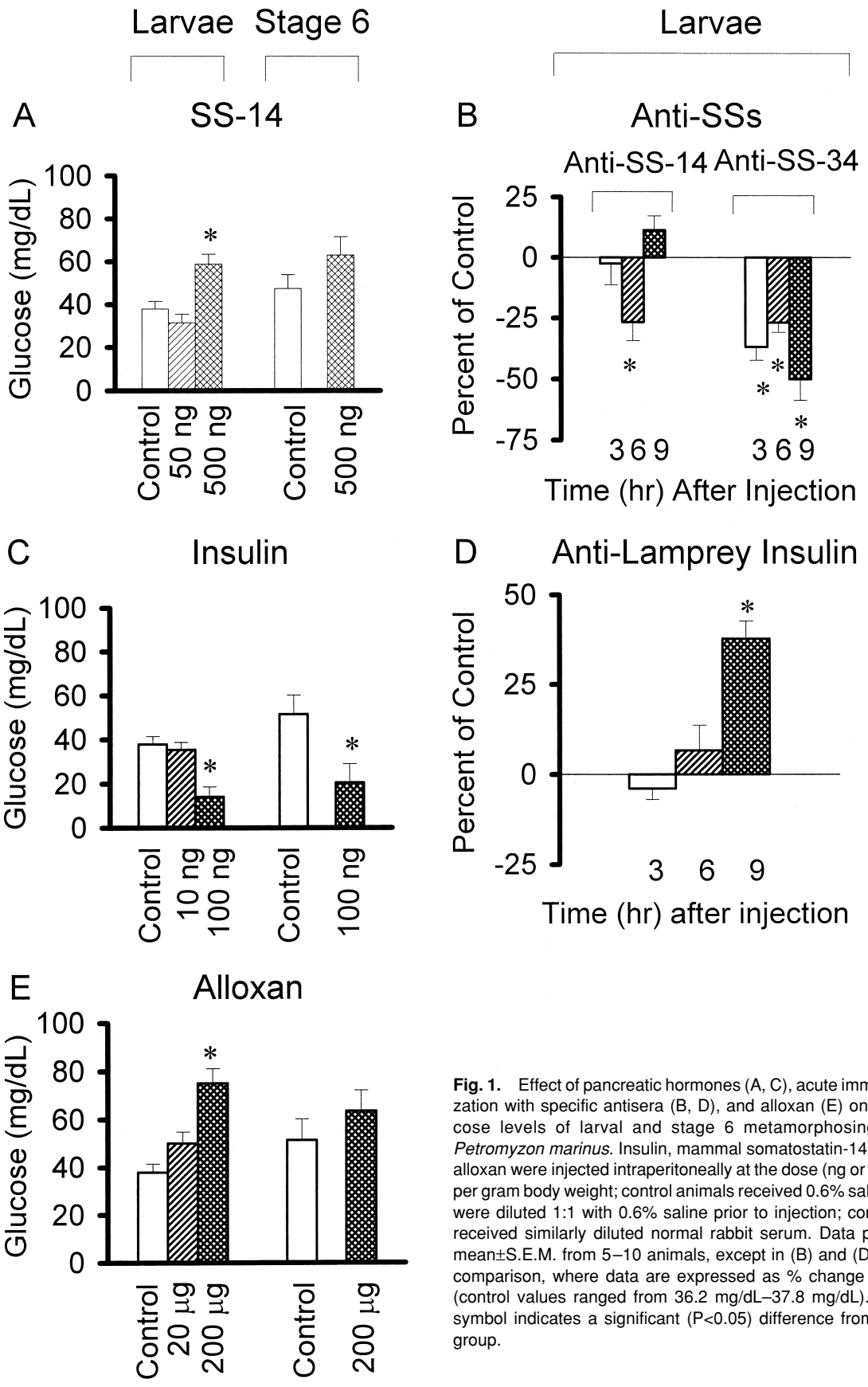

Fig. 1. Effect of pancreatic hormones (A, C), acute immunoneutralization with specific antisera (B, D), and alloxan (E) on plasma glucose levels of larval and stage 6 metamorphosing lampreys, *Petromyzon marinus*. Insulin, mammal somatostatin-14 (SS-14) and alloxan were injected intraperitoneally at the dose (ng or μg) specified per gram body weight; control animals received 0.6% saline. Antisera were diluted 1:1 with 0.6% saline prior to injection; control animals received similarly diluted normal rabbit serum. Data presented as mean±S.E.M. from 5–10 animals, except in (B) and (D) for ease of comparison, where data are expressed as % change from control (control values ranged from 36.2 mg/dL–37.8 mg/dL). An asterisk symbol indicates a significant ($P<0.05$) difference from the control group.

6-dioxyuracil monohydrate; Sigma) at a low dose of 20 μg or high dose of 200 μg alloxan (dissolved in 0.6% saline)/g body weight once per day for a two-day period. Twelve to 14 hr after the last injection, animals were anesthetized individually with 0.05% tricaine methanesulfonate. For the experiment of acute neutralization of pancreatic hormones as previously described by Plisetskaya *et al*. (1989), groups of 10 larvae (excluding the transformers because they were not that easy to obtain) received an intraperitoneal injection of 10 μl/g body weight of 1:1 mixture of 0.6% saline and either anti-mammalian SS-14, anti-lamprey SS-34, anti-lamprey INS or normal rabbit serum (control). The validation of the antisera was described by Andrews *et al*. (1988), Plisetskaya *et al*. (1988), and Youson *et al*. (1992). Blood was collected into heparinized-capillary tubes from the severed caudal vasculature 3, 6 and 9 hr after the injection. Plasma was collected and stored at –70°C for later determination of glucose. Plasma glucose was measured by the *o*-toluidine method described by Huvarinen and Nikkila (1962). 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

There was a trend of development-dependent differences in plasma glucose levels. Plasma glucose levels tended to be higher in transformers than larval lamprey, but these differences were not significant. Injection of SS-14 (500 ng/g) significantly increased plasma glucose in larvae, but did not significantly affect transformers (Fig. 1A). In a separate experiment (Fig. 1B), plasma glucose levels of larvae were significantly depressed 6 h after anti-SS-14 serum. Injection of anti-lamprey SS-34 serum reduced plasma glucose levels over the 9-h course of the experiment.

Insulin injection reduced plasma levels of glucose in both larvae and transformers (Fig. 1C); however, this effect was only significant at 100 ng/g. In a separate experiment, acute insufficiency of INS in larvae injected with anti-lamprey INS serum resulted in significant hyperglycemia 9 hr after injection (Fig. 1D). Alloxan (a cytotoxin of insulin-secreting cells) treatment (200 μg/g body weight) also resulted in hyperglycemia in larvae (Fig. 1E).

DISCUSSION

These results indicate that SSs and INS modulate carbohydrate metabolism when administered *in vivo* to larvae and transformers. The plasma glucose levels in larval *P. marinus* observed in this study tended to be lower than those of transformers. This observation is consistent with that of O'Boyle and Beamish (1977) for the same species that showed higher levels of plasma glucose in transformers than in larvae, in parallel with decreased glycogen content in the liver and muscle as this nontrophic phase of metamorphosis proceeds.

We report here that SSs promote hyperglycemia in lamprey. The hyperglycemic actions of SSs were supported by exogenous injection of SS-14 as well as by acute neutralization of SSs induced by injection of anti-SS-14 and anti-lamprey SS-34 sera. Somatostatin-induced hyperglycemia probably results from increased glycogenolysis. Increased intestinal-pancreatic SS-14 concentration during spontaneous metamorphosis of sea lamprey, *P. marinus*, is consistent with decreased glycogen content in liver and muscle and with increased plasma glucose levels (O'Boyle and Beamish, 1977; Elliott and Youson, 1991). In addition, decreased rates of lipid synthesis in fat depot tissues such as liver, kidney and muscle caused by SS-14 (Kao *et al*., 1998) possibly reflect a decrease in the glucose uptake by these tissues from the blood. Somatostatins have been shown to promote hyperglycemia in association with increased glycogenolysis in numerous other species of vertebrates (Eilertson and Sheridan, 1993).

In this study, SS-14 seems different from the larger form of lamprey SS-34 in modulating the circulating glucose levels since 3- and 9-hr postinjection of anti-lamprey SS-34, but not anti-SS-14, resulted in significantly decreased plasma glucose levels. Such differences imply that SS-14 and SS-34 may have different roles in regulating plasma INS concentration in lamprey and subsequently differentially affect plasma glucose levels. This is evidenced by the finding that 3-hr postinjection of anti-lamprey SS-34 into the larval lamprey resulted in increased plasma INS and such increase was about 2-fold high than that of the anti-SS-14 injection (Youson *et al*., 1992) and that SS-14 immunoreactive cells and SS-34 immunoreactive cells were differentially and development-dependently localized in the brain, intestine, and pancreas (Cheung *et al*., 1991). In rainbow trout, differential effects of SS-14 and SS-25 on carbohydrate and lipid metabolism have been reported; dependently on time and strength and such differences are due to the alterations in INS and glucagon levels circulating in the plasma caused by these peptides (Eilertson and Sheridan, 1993). Three-hour of SS-14 injection caused hyperglycemia, reduced plasma glucagon concentration and had no effect on plasma INS levels; whereas, SS-25 elevated plasma glucose levels in association with reduced glycogen content and reduced plasma glucagon and INS levels (Eilertson and Sheridan, 1993).

The mechanism by which SSs modulate plasma glucose levels in lamprey is not known. It appears likely, however, that SSs act indirectly by interacting with INS. This conclusion is supported by several lines of evidence. First, acute neutralization of lamprey SS-34 resulted in increased plasma INS (Youson *et al*., 1992). Second, acute neutralization of SS-14 resulted in increased plasma INS (Youson *et al*., 1992). These suggest that SSs inhibit plasma INS levels in lampreys and thereby result in the subsequent hyperglycemia. Somatostatin-25 (the larger form of salmon SS) inhibition of pancreatic INS secretion has been reported in rainbow trout (Eilertson and Sheridan, 1993). Whether SSs modify directly the kinetics of lamprey INS receptor (Lappova and Leibush, 1995) is unknown. The possibility also remains that SSs acts directly on lamprey target organs, as is the case in teleosts (Sheridan and Bern, 1986)

Insulin is hypoglycemic in larval and metamorphosing sea lampreys. This conclusion is evidenced by the findings that

(1) exogenous administration of INS resulted in reduced plasma glucose levels in larvae and transformers, (2) anti-lamprey INS administration resulted in hyperglycemia, and (3) alloxan, which is a β-cell cytotoxin (Lukens, 1948; Morris and Islam, 1969), resulted in elevated plasma glucose levels in larvae and transformers. The observation of INS-induced hypoglycemia in *P. marinus* is consistent with that reported by Leibson and Plisetskaya (1968) for larval *L. planeri* and for adult *L. fluviatilis* injected with a dose of 30–60 IU INS/kg body weight. Insulin has been shown to be hypoglycemic in most vertebrates (Epple and Brinn, 1987), including the hagfish (another major group of cyclostomes) (Hardisty, 1979), such as *Eptatretus stouti* (Inui *et al*., 1978). The reduction of plasma glucose levels in larvae treated with INS observed in this study may result from the stimulation of glucose uptake for the subsequent glycogen synthesis and/or from the inhibition of glucose output from glycogenolysis. This supposition is supported by (1) INS suppression of liver glucose-6-phosphatase activity (Leibson and Plisetskaya, 1969), (2) INS stimulation of lipid synthesis (Kao *et al*., 1999), possibly reflecting an increase in the glucose influx from the blood to lipid storage sites, and (3) neutralization of lamprey INS by anti-lamprey INS administration reduced hepatic glycogen content (Youson *et al*., 1992).

In summary, we have shown that SS-14 is hyperglycemic in sea lamprey, whereas INS is hypoglycemic. These findings suggest that the glucoregulatory roles of SS and INS (see Epple and Brinn, 1987; Plisetskaya and Duguay, 1993) (for a discussion of INS actions; see Chan and Steiner, 2000) emerged early during, or perhaps prior to, the evolution of vertebrates.

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