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The Effect of Insulin Insufficiency on Plasma Thyroid Hormones and Some Metabolic Constituents in Pacific Hagfish, *Eptatretus stouti*[1]

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The effect of guinea pig anti-human insulin serum was studied in Pacific hagfish. The metabolic indicators of insulin insufficiency, such as elevation of plasma glucose, free fatty acids, and α-amino nitrogen levels were obtained at 72 and 120 hr after anti-insulin serum injection. Plasma triiodothyronine levels declined significantly 24 hr after anti-insulin serum administration. These findings supplement earlier data with the same species and suggest that the regulatory metabolic action of insulin and thyroid hormones are related in cyclostomes.

The production of an insulin deficiency by administration of antimammalian insulin serum (AIS) has been demonstrated in lampreys and teleosts (Plisetskaya and Leibush, 1972; Plisetskaya and Bondareva, 1974; Ince and Thorpe, 1975). AIS has been shown to cause hyperglycemia and elevation of plasma (free) fatty acids (PFA) in the Baltic lamprey *Lampetra fluviatilis* (Plisetskaya *et al.*, 1976; Plisetskaya, 1980). However, no equivalent successful experiment studies on acute insulin deficiency have yet been reported for a myxinoid cyclostome. The only experiment performed on the administration of the mammalian AIS into Atlantic hagfish *Myxine glutinosa* resulted in no effect on glycemic level (Falkmer and Wilson, 1967).

Aspects of the isolation, molecular structure, biosynthesis, and physiological role of hagfish insulin have been comprehensively investigated by Emdin (1981). It has been shown that the purified hagfish hormone has little crossreactivity with anti-mammalian insulin serum in radioimmunoassays (Emdin and Steiner, 1980). Additionally, and surprisingly, administration of Atlantic hagfish insulin to the fish of the same species had no substantial effect on plasma glucose, α-amino nitrogen, triglycerides, or PFA at least 32 hr after injection. This finding seems to be unlike the situation in most other vertebrates, including cyclostomes, since it has been demonstrated that mammalian insulin causes a long-lasting hypoglycemia in both lamprey and hagfish (Leibson *et al.*, 1963; Bentley and Follett, 1965; Falkmer and Matty, 1966; Falkmer and Wilson, 1967; Leibson and Plisetskaya, 1968, 1969; Inui and Gorbman, 1977), a decrease in PFA concentration in lamprey (Plisetskaya, 1980), as well as a decrease in α-amino nitrogen concentration in hagfish (Inui and Gorbman, 1977).

Previously Murat and Serfaty (1970, 1971) proposed that enhanced insulin secretion in teleost fish may be involved in the metabolic action of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). In continuing our efforts to explore the role of thyroid hormones in regulation of metabolic parameters in cyclostomes we have chosen to test the theses of Murat and Serfaty by determining first whether AIS raised against mammalian hormone can cause an

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insulin deficiency in hagfish; secondly, in the event of an insulin deficiency so produced, we wish to determine the relationship between this phenomenon and plasma thyroid hormone levels.

MATERIALS AND METHODS

Pacific hagfish (*Eptatretus stouti*) were trapped in Bamfield, Vancouver Island, British Columbia, Canada, in January 1981. The hagfish were transported on the same day to the Department of Zoology, University of Washington, Seattle, Washington and maintained at 8–10° in 100-liter dark, plastic, covered tanks containing aerated seawater. The water was changed on a more or less regular weekly basis, and the hagfish were not fed through the period before and during the experiment. Emdin (1981) reported that hagfish could survive in an apparently healthy metabolic responsive state after some months of starvation. The sexually immature animals used were of both sexes, ranging between 45 and 89 g in body weight and between 28 and 45 cm in body length.

Five days before the experiment, in April 1981, the hagfish were transferred to charcoal-filtered seawater tanks in a room in which temperature was controlled at 15°. Before the injection or blood sampling the hagfish were anesthetized by immersion in phenoxyethanol at 0.1 ml/liter in seawater.

Blood was sampled at 0 hr followed by intraperitoneal injection of guinea pig anti-human insulin serum (a gift from the Diabetes Research Center of the University of Washington, Seattle) at 1 ml/100 g bw. The control hagfish were injected with the same volume of normal guinea pig serum (NS) (Miles Laboratories, Inc., Elkhart, Indiana). Blood (0.5 ml) from each hagfish was taken from the caudal subcutaneous sinus into heparinized syringes. Subsequent blood samples were taken at 24, 72, and 120 hr after injection. The plasma samples were frozen and stored at −20° until assayed for glucose, PFA, α-amino nitrogen, and total protein.

Plasma T_4 and T_3 were measured by radioimmunoassays as described by Dickhoff *et al.* (1978) and Plisetskaya *et al.* (1982). Plasma glucose was determined by use of *o*-toluidine reagent, essentially according to Hüvarinen and Nikkilä (1962), the only modification being that of deproteinization of hagfish blood with 8–10% TCA instead of 3–5% TCA. PFA were measured according to Prochorov *et al.* (1977), modified from the method of Noma *et al.* (1973). α-Amino nitrogen was estimated by use of 2,4-dinitrofluorobenzene reagent (Goodwin, 1968). Total plasma protein concentration was measured according to Lowry *et al.* (1951). Hematocrit values were determined after centrifugation of blood in heparinized capillary tubes. The significance of difference between values was calculated using Student's *t*-test. Differences were considered significant when $P < 0.05$.

RESULTS

The results of the determination of plasma constituents are shown in Figs. 1 and 2. Plasma glucose, PFA, and α-amino nitrogen increased significantly as compared with control at 72 hr after AIS injection. The differences of total plasma protein between AIS and NS treated hagfish were statistically insignificant (Fig. 2B).

The changes induced by AIS administration could not be dependent upon hematocrit values which did not significantly differ between AIS and NS injected hagfish ranging from 17 to 20%. The levels of serum constituents in both of the sera injected (AIS and NS) were practically the same, and therefore could not have influenced the results of the experiment (e.g., glucose concentration in NS was 120 mg/100 ml, in AIS 143 mg/100 ml, which means that hag-

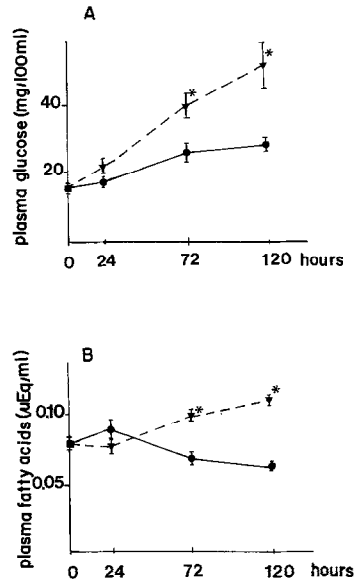

FIG. 1. The effects of intraperitoneal administration of anti-insulin serum (1 ml/100 g bw) on plasma glucose (A) and plasma fatty acids (B) in hagfish. —, After normal guinea pig serum injection; ---, after guinea pig anti-insulin serum injection. Each symbol with a vertical line represents mean ± SE based on 5–7 animals. *$P < 0.05$.

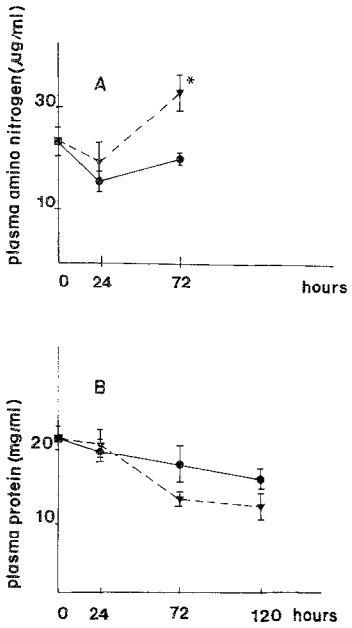

FIG. 2. The effect of intraperitoneal anti-insulin serum injection on plasma α-amino nitrogen (A) and plasma protein (B) levels in hagfish. The designations are the same as in Fig. 1.

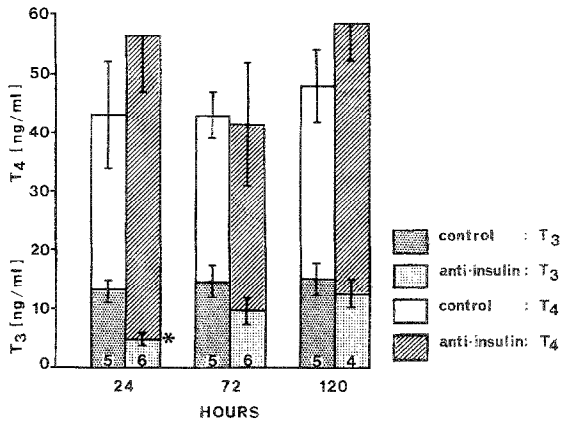

FIG. 3. The effect of intraperitoneal anti-insulin serum injection on plasma thyroxine and triiodothyronine levels in hagfish. The number of plasma samples is indicated at the base of the columns. T_4 values begin at 0.

fish weighing 100 g received either 1.4 mg of glucose contained in 1 ml of AIS or 1.2 mg contained in 1 ml of NS). Falkmer and Matty (1966) previously reported that 50 mg of glucose injected into hagfish of equal weight was fully eliminated during a 24-hr period. The same reasoning would seem to apply in respect to other metabolites not shown here.

Following administration of AIS T_4 concentrations did not significantly differ from those of controls; however, plasma T_3 decreased: 4.8 ± 0.76 ng/ml at 24 hr after treatment in comparison with 10.3 ± 1.89 ng/ml in the control group (Fig. 3).

DISCUSSION

The immunoreactivity of the AIS used in the present study was demonstrated by the production of an immunostaining reaction in histological sections of rat pancreas; it reacted to a far lesser degree with Pacific hagfish islet tissue (Nozaki, personal communication). This agrees with a similar earlier report by Östberg *et al.* (1975) for Atlantic hagfish. In the present experiments, 1 ml of AIS administered to hagfish at a dilution of 1:80,000 binds 6 ng of human insulin, so that even if hagfish insulin has only 0.5% crossreactivity with the AIS (cf. Emdin, 1981), 1 ml of undiluted serum should allow for fairly complete binding of the circulating endogenous hormone in hagfish blood. Acute insulin deficiency so produced in hagfish, at least as manifested by elevation of the levels of some metabolites following AIS administration, was observed in this study. However, the apparent insulin deficiency developed over a longer period of time than was observed in lamprey, some marine teleosts, and pike (Ince and Thorpe, 1975; Plisetskaya *et al.*, 1976). In these other species the changes in carbohydrate metabolism were fully manifested by 6–24 hr after AIS treatment. In the present study, even if insulin deficiency eventually developed earlier than 72 hr after AIS injection, it took more than 24 hr after treatment for it to be first manifested. Reasons offered for this apparent slower effect in hagfish may include differences in the nature of the anti-insulin serums used and the varying

methods of administration of serum. For example, Ince and Thorpe (1975) injecting 10-fold smaller volumes of AIS and NS than that used in our experiment, but directly into the bloodstream (intraarterially), obtained an immediate hypersensitive reaction (coma) in pike. On the contrary, we have never seen any signs of coma either in lamprey and hagfish, or in teleosts, after intraperitoneal administration of insulin antisera (Plisetskaya *et al.,* 1976). The effect of AIS treatment persisted in hagfish at least up until 120 hr after treatment. Aside from other possibilities, such prolongation might be induced by a hypersomatostatinemia in insulin deficient animals (Wasada *et al.,* 1981) which, in turn, might inhibit insulin secretion in hagfish (Stewart *et al.,* 1978).

The present study indicates that the lack of insulin in Pacific hagfish results in hyperglycemia, and in increase of both PFA and α-amino nitrogen levels. It has been previously reported that mammalian insulin in various dosages or crude extracts of hagfish islets administered to hagfish induce a long-lasting hypoglycemic condition (Falkmer and Matty, 1966; Falkmer and Wilson, 1967; Inui and Gorbman, 1977) and a decrease in plasma α-amino nitrogen (Inui and Gorbman, 1977). Such insulin responsiveness is contrary to Emdin's finding (1981) that no effect on metabolic constituents in Atlantic hagfish was manifested after hagfish insulin treatment; however, this treatment elevated serum insulin levels 5- to 7-fold higher than controls during 6–24 hr after injection. The important difference between these experiments is in Emdin's use of purified hagfish insulin (10 μg/100 g bw). It would seem that the effects of hagfish insulin treatment need further investigation before definitive conclusions can be drawn.

Plasma T_3 levels were lower in hagfish treated with AIS compared to those of controls. It was reported recently that T_4 to T_3 conversion in mammals, both in clinical and in experimental diabetes mellitus, is strikingly decreased due to a defect in the peripheral deiodination mechanism (Pittman *et al.,* 1981). In the present study the plasma T_4 and T_3 levels are reported for the first time in hagfish concomitantly insulin deficient. It is unknown, and remains to be studied, whether thyroxine conversion rates were affected.

Recently we observed that both T_4-, and especially T_3-administration, to Pacific hagfish decreased plasma glucose and elevated PFA, together with slight changes in α-amino nitrogen and protein (Plisetskaya and Gorbman, 1982; Plisetskaya *et al.,* 1982). Additionally, it was demonstrated that 6-propylthiouracil, an antithyroid compound administered to hagfish, elevated plasma glucose and α-amino nitrogen, and decreased PFA concentrations in a pattern similar to the effects of these plasma constituents (with the exception of fatty acids) noted in the present study after administration of AIS serum. Accordingly, these observations suggest that an interrelationship exists between insulin and thyroid hormonal levels and their regulatory action on metabolism, even in such primitive vertebrates as cyclostomes.

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