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Insulin and Corticosterone in the Teleost *Anabas testudineus* (Bloch): Effects on Certain Biochemical Parameters of Intermediary Metabolism

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Administration of bovine insulin, (4U/100 g body wt) significantly increased the contents of glycogen, protein and lipid in the liver at 6, 12 and 24 hr after the injection. The activity of glucose-6 phosphatase (G-6-Pase) significantly decreased after insulin treatment, whereas acid phosphatase (Ac. Pase) exhibited an increase in activity on a time-dependent manner. Injection of alloxan (4 mg/100 g body wt), a β-cell cytotoxin, significantly decreased the contents of glycogen, protein and lipid, and Ac. Pase activity. However, the activity of G-6-Pase increased after alloxan treatment. Administration of insulin to alloxanized fish prevented the alloxan-mediated decrease in the biochemical contents and the enzyme activities. Injection of corticosterone (20 μg/100 g body wt) while increasing the glycogen and protein contents, and G-6-Pase activity, significantly decreased lipid content and Ac.Pase activity. The results suggest that insulin and corticosterone exert an anabolic influence on the intermediary metabolism of fish.

Key Words: **Insulin, Alloxan, Corticosterone, Biochemical Indices, Fish**

Introduction

Insulin facilitates utilization of glucose by the cells and prevents the process of glycogenolysis resulting in hypoglycemia. Insulin acts to promote metabolic processes at the level of Krebs cycle dehydrogenases and respiratory metabolism in mammals (Bessman et al. 1986). Recent investigations reveal that insulin promotes mitochondrial metabolism in teleosts (Ignatius & Oommen 1990). Administration of insulin reduces the conversion of amino acids into glucose (Nadkarni & Chitinis 1983) and is reported to enhance protein synthesis in the trout *Salmo gairdneri* (Ablett et al. 1981). Fishes are sensitive to alloxan treatment resulting in beta cell necrosis and insulin deficiency as has been observed in streptozotocin

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treatments (Ablett et al. 1981, Liux et al. 1994, Plisetskaya & Ducass 1994). Alloxan administration decreases the insulin-mediated oxidative metabolism in teleosts (Ignatius & Oommen 1990).

Glucocorticoids cause hyperglycemia and glycogen deposition in a wide variety of vertebrate species (Matty 1985, Norris 1985, Guyton 1991). However the role of glucocorticoids especially corticosterone has received little attention in fishes. Corticosterone has been shown to influence oxidative processes in fish tissues (Ignatius & Oommen 1990). A dose dependent stimulatory effect of hydrocortisone on glycogen and protein turnover was reported in *A. testudineus* (Jameela & Oommen 1988). The present study was undertaken to examine the effects of bovine insulin and corticosterone on the turnover of a few biochemical indices and the activities of two enzymes associated with intermediary metabolism in a teleost, *Anabas testudineus*. Alloxan was also employed in the present study to substantiate the effect of insulin on intermediary metabolism.

Materials and Methods

Adult *A. testudineus* (body wt. 25-30g) of both sexes were used in the present study. The experiments were conducted during the post-spawning phase (September-October). The fish were kept (12 L: 12 D photoperiod) in large tanks at 28 ± 2°C and fed on commercial fish feed every alternate day.

The fish were acclimated to these conditions for at least two weeks prior to experiment.

Three sets of experiments were conducted independently. In the first experiment, the time-dependent effects of bovine insulin (Boots India Co. Bombay) were investigated. Thirty fish were divided into 5 groups of 6 each and fasted for 24 hr before experiment. Group 1 animals received 0.1 ml of saline and served as controls. Each animal of groups 2, 3, 4 and 5 received 2.0 U of insulin and were sacrificed for experimental analysis at 1, 6, 12 and 24 hr respectively after hormone administration. All injections were given i.p. at the same time of the day (5 AM).

The second set of experiment was designed to study the effect of alloxan with or without insulin on intermediary metabolism. Thirty fish were selected, comprising 5 groups of 6 each. Animals of group 1 consisted saline injected controls. Groups 2 and 3 animals were injected with 2 mg of alloxan monohydrate (Sigma Chemical Co., USA, diluted with glass distilled water) and sacrificed after 24 and 48 hr respectively. Each specimen of group 4 was administrated with 2.0 U insulin and sacrificed at 6 hr. Each fish of group 5 received an injection of 2 mg alloxan, and after 24 hr they were also injected with 2.0 U of insulin and sacrificed at 6 hr.

To study the dose-dependent effects of corticosterone (Sigma, USA) 18 fish comprising 3 groups of 6 each were used in the third set of experiment. Group 1 animals received 0.1 ml of propylene glycol daily for 5 days (hormonal vehicle) and served as controls. Each animal of group 2 was injected with 2 µg of corticosterone daily for 5 days (total 10 µg) and group 3 fish received 5 µg corticosterone daily for 5 days (total 25 µg / fish). The control fish in the third set were sacrificed on 6th day.

The fish were decapitated and the liver excised was kept on ice. Glycogen content of the tissue was estimated by Anthrone method (Seifter et al. 1950). The total protein and lipid contents of the liver were estimated according to the methods of Folin et al. (1969) and Bragdon (1951) respectively. The rest of the liver tissue was homogenized in 0.25M sucrose medium and the cytosolic fraction was separated by differential centrifugation (10,000 g) at 4°C. The supernatent was used for the estimation of the activities of glucose-6-phosphatase (Swanson 1955) and acid phosphatase (Lowry et al. 1954). Cytosolic protein was estimated by Biruet reaction using BSA as standard (Gornall et al. 1949).

Data were collected from 6 animals for each test. Comparison among sample means was made by one way classification of analysis of variance and student 't' test (Snedecor & Cochran 1967).

Results

Effect of Bovine Insuling in Normal Fish

Administration of 2.0 U bovine insulin in *A. testudineus* at various time intervals (1, 6, 12 and 24 hr after hormone injection) produced significant alteration in the turnover of biochemical constituents and the activity of enzymes table 1. The concentration of glycogen, protein and lipid increased with the increasing time and a maximum increase was observed at 24 hr after insulin administration. A time-dependent decrease in the activity of G-6-Pase and Ac-Pase was observed after 2.0 U of insulin administration.

Effects of Alloxan and Insulin

Injection of alloxan monohydrate (2mg/fish) significantly decreased the glycogen, and lipid

Table 1 *Time-dependent effect of bovine insulin on total glycogen, protein and lipid contents and the activity of glucose-6- phosphatase (G-6-Pase) and acid phosphatase (AC. Pase) in the liver of A. testudineus. Each value is mean ± SD of six fish.*

Animal Status	Glycogen (mg/100 mg wet tissue)	Protein (mg/100 mg wet tissue)	Lipid (mg/100 mg wet tissue)	G-6-Pase (n mol Pi/ Min/mg protein)	Ac-Pase (n mol Pi/ Min/mg protein)
Control	12.07±0.34	19.88±1.74	2.17±0.13	73.47±1.46	55.71±2.32
2.0U insulin,sacrificed after 1 hr	12.63±0.37	20.81±0.51	2.94±0.21*	67.89±3.77*	49.66±2.48**
2.0 U insulin sacrificed after 6 hrs	13.67±0.44**	21.74±0.51	3..75±0.27**	62.25±4.48**	3043±2.65**
2.0 U insulin sacrificed after 12 hrs	14.51±0.24**	23.61±0.51**	4.25±0.27**	51.23±0.12**	21.22±3.15**
2.0 U insulin sacrificed after 24 hrs	18.69±0.17**	25.54±0.59**	7.50±0.27*	49.33±1.87**	20.41±1.39**

* $p < 0.025$; ** $p < 0.001$

Table 2 *Effects of alloxan and bovine insulin on total glycogen, protein and lipid contents and the activity of G-6-Pase and Ac. Pase in the liver of A. testudineus. Each value is mean ± SD of six fish.*

Animal status	Glycogen (mg/100 mg wet tissue)	Protein (mg/100 mg wet tissue)	Lipid (mg/100 mg wet tissue)	G-6-Pase (n mol Pi/ Min/mg protein)	Ac-Pase (n mol Pi/ Min/mg protein)
Control	13.31±0.32	18.65±1.53	2.22±0.13	75.63±1.81	50.75±3.81
2 mg alloxan sacrificed after 24 hrs	8.24±0.15**	14.67±0.42**	1.25±0.27**	150.69±8.59**	72.91±5.12**
2mg alloxan sacrificed after 48 hrs	10.97±0.27**	18.09±0.42	1.77±0.28**	88.16±4.18**	98.93±7.62**
2.0 U insulin sacrificed after 6hrs	14.81±0.44*	22.58±0.57**	3.98±0.31**	62.19±3.18**	38.15±2.31**
2mg alloxan/24 hrs + 2.0 U insulin sacrificed after 6hrs	14.47±0.56[a]	21.74±0.51[a]	3.95±0.13[a]	64.32±3.60[a]	41.60±3.51[a]

* $p < 0.025$; ** $p < 0.001$; [a] $p < 0.0025$ when compared to 2 mg alloxan-treated fish

Table 3 *Effect of corticosterone on total glycogen, protein and lipid contents and G-6-Pase and Ac-Pase acivities in the liver of A. testudineus. Each value is mean ± SD of six fish.*

Animal status	Glycogen (mg/100 mg wet tissue)	Protein (mg/100 mg wet tissue)	Lipid (mg/100 mg wet tissue)	G-6-Pase (n mol pi/ Min/mg protein)	Ac. Pase (n mol Pi/ Min/mg protein)
Control	10.97±0.27	18.94±0.51	2.28±0.27	70.16±2.36	76.00±2.36
10 µg corticosterone sacrificed after five days	14.47±0.57**	22.51±0.48**	1.78±0.28	116.27±3.36**	37.21±1.75**
25 µg corticosterone sacrificed after five days	16.55±0.12**	24.68±0.42**	0.88±0.14**	133.51±2.22**	34.82±1.32**

** $p < 0.001$

contents at 24 and 48 hrs. Alloxan injection while decreasing the protein concentration at 24 hrs, produced no change at 48 hrs. The activity of G-6-Pase and Ac-Pase showed a significant increase at 24 and 48 hrs in alloxanized fish (table 2).

Injection of 2.0 U insulin to alloxan pretreated fish significantly increased the contents of glycogen, protein and lipid, when compared to alloxan-treated fish (table 2). Insulin injection decreased the activity of G6-Pase and Ac-Pase in alloxan pretreated fish (table 2).

Effects of Corticosterone

The concentration of liver glycogen and protein and the activity of G-6-Pase increased significantly after 10 and 25 µg corticosterone treatments. However the corticosterone treatments significantly decreased the total lipid content and the activity of Ac-Pase in the liver (table 3).

Discussion

The increase in the glycogen contents of liver in *A. testudineus* after insulin treatment may undoubtedly be due to the hypoglycemic action of insulin. Insulin exerts its hypoglycemic action by increasing the rate of glycogenesis in the liver and muscle (Fritz 1972). Hypoglycemic action of insulin is well documented in fishes (Deroos & Deroos 1979, Ablett et al. 1981). Insulin has a direct effect on carbohydrate metabolism of frog liver tissue (Packard & James 1976). Pigeon and rats when injected with insulin showed an enhanced synthesis of glycogen in the liver (Pilo & Patel 1978). Insulin activated glycogen synthetase in isolated perfused liver from normal rats and also stimulated incorporation of glucose into glycogen (Guyton 1991). The depletion of glycogen content after alloxan treatment reveals that alloxan causes glycogenolysis in the liver, which in turn correlates to the insulin insufficiency. Boquist (1980) demonstrated that necrosis of the pancreatic islet β-cells represents the ultimate effect of alloxan β-cytotoxicity and forms the basis of manifestation of diabetes. Exogenous glucose has been shown to activate glycogen synthetase in liver of diabetic rats (Fernandez-Novell et al. 1994).

Corticosterone causes a rise in liver glycogen content suggesting increased glycogenesis. In *Onchorynchus*, there is an elevation of liver glycogen with six fold increase in plasma corticosteroids during the migration-spawning phase (Chester-Jones et al. 1969, Matty 1985). It has been shown that the plasma cortisol concentration increased during cohort sampling, and the hepatic glycogen content of fish stocked at high density was significantly much lower than the control in rainbow trout (Leatherland 1993). An elevation of cortisol and glucose levels has been observed in brook trout during stress (Biron & Benfey 1994).

Injection of insulin and corticosterone increases the protein content at different intervals of time. This reflects the increased uptake of amino acids into cells favouring net protein synthesis. Insulin has a direct control on protein synthesis in fishes (Lewander et al. 1976). Insulin lowers plasma aminoacids in both the eel, *Anguilla anguilla* and northern pike, *Esox lucius* (Thorpe & Ince 1974, Ince 1983). Insulin stimulates synthesis of DNA (Mastick et al. 1994) and soluble proteins in isolated rat hepatocytes (Randil & Hansen 1980). Accumulation of m RNA concentration has been reported in human cultured cells by glucocorticoid administration (Lid et al. 1994).

A time-dependent increase in total lipid content after insulin injection suggests lipogenic effect of insulin in teleosts as has been observed in mammals (Willing et al. 1994). In the Carp, *Cyprinus carpio* it has been shown that insulin injection increases fat deposition in liver and red muscle (Matty 1985). In the present study corticosterone administration seems to decrease the total lipid content in the fish liver. A decrease in glucose tolerance and lipid metabolism has been observed in adrenalectomized mice (Blair et al. 1994).

G-6-Pase is responsible for contributing free glucose to the blood from liver glycogen pool and from other precursors of glucose-6-phosphate. An inhibitory effect of insulin on gluconegenesis has been suggested in this fish. Insulin administration decreased the activity of G-6-Pase in the rat liver (Smith et al. 1983). Corticosterone augments G-6-Pase activity revealing its gluconeogenic role in fish liver. It is well known that glucocorticoids increase gluconeogenesis by increasing the supply of amino acid substrate and by increasing key enzymes of the pathway (Guyton 1991).

Acid phosphatase is considered as the hallmark of lysosomes as it is engaged in the hydrolysis of protein. The anabolic effect of insulin and corticosterone on total protein seems to be correlated with decreased acid phosphatase activity, which in turn depends on the energy demand within the cell. Although alloxan decreases the acid phosphatase activity, insulin administration in alloxanized fish increases the activity which further confirms the increased amino acid formation.

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