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Research Article

Response of Skeletal Muscle Protein and Nucleic Acid Levels to Thyroxine Injection in Fish

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ABSTRACT

The effect of Thyroxine (T4) injection on certain aspects of protein metabolism was studied under constant temperature (26±1°C.), nutritional status same age group of fish, *Anabas testudineus* commercially available Thyroxine-L-sodium, T4 was given in the form of injection. Two dosages of hormone were selected (4µg/10g and 8 µg/10g body weight). For cumulative study, the fish received daily intraperitoneal injections of T4 for one and two weeks. Single injection effect was studied after 24, 48 & 72 hours. Skeletal muscle protein and RNA content showed positive response to the hormone treatment, whereas, DNA levels were unaltered.

1. Introduction

Thyroid hormones (T4 & T3) have a general stimulatory metabolic effect on tissues in vertebrates (Frieden & Lipner, 1971). However, the results of the studies in the lower vertebrates are equivocal. The action of thyroxine (T4) at the peripheral cellular level in fish largely remains unknown. As pointed out by Plisetskaya et.al. (1983), this problem requires further investigations. In view of paucity of clear information on the action of thyroid hormones at tissue levels in fish, the present study was undertaken. In the present investigation, skeletal muscle protein, nucleic acid contents were studied under T4 injection effect in *Anabas testudineus*.

2. Material and Methods

The fish Anabas testudineus were obtained locally. Fish weighing 18-24 g were selected and maintained at constant temperature of 26±1°c. They were fed with commercially available high quality Fish Food of 2% body weight (Protein - 32%, Crude Fat - 4%, Fibre - 5%). The fish were divided into three groups, two experimental and one control. For each experiment, triplicates were kept with corresponding control groups.

Commercially available sodium - L - thyroxine, T4 (Eltroxin) was dissolved in saline at a dosage of 4µg and 8µg in 0.05 ml of 0.6% saline per 10g body weight. The saline was made slightly alkaline with NaOH in order to dissolve the thyroxine. The hormone was administered daily intraperitoneally to the experimental fish and the control fish received the same volume of 0.6% saline, Cumulative injection effect of hormone was studied after one and two weeks and single injection effect was studied after 24, 48 and 72 hours. At the end of each experiment fish were killed and the skeletal muscle was dissected out and immediately weighed and taken for biochemical estimations. The total protein content was estimated by Lowry et.al. (1951) method. Nucleic acids were extracted by the method of Munro & Fleck (1966) and estimated by Orcinol method (RNA) and Diphenylamine method (DNA) (Plummer, 1979).

For all the experimental parameters, the data obtained were statistically analysed by using Student's t' test (Pillai & Sinha, 1968).

3. Results and Discussion

Total protein and RNA content of skeletal muscle were increased significantly in the fish under both the dosages of T4 injection and at all the durations. The increase was linear to time and dosage. The effectiveness of thyroxine injection was more under 8 µg/10g body weight dosage than 4 µg/10g body weight dosage (Tables-1 & 2). The DNA content of skeletal muscle did not alter significantly under both the dosages and at all the duration of hormone administration (Table-3).

A significant protein deposition in skeletal muscle showed the anabolic effect of the hormone. Matty et.al (1982) observed that Tilapia fry subjected to T4 immersion and single T4 injection to different age groups of fish showed enhancement in protein and RNA content. It is known that thyroid hormone actions were concentrated on transcription and translation

Table-1. Effect of Thyroxine (T_4) injection on skeletal muscle protein content in *Anabas testudineus* (n=6)

Dosage of Thyroxine (T4)		Single injection effect			Cumulative effect	
		24 hours	48 hours	72 hours	one week	two weeks
Control	Mean	116.540	116.780	116.890	121.110	127.140
	SE	±0.653	±0.432	±0.496	±0.133	±0.694
Experimental						
	Mean	116.850^{NS}	117.960^{NS}	120.010^{NS}	141.230***	161.320***
4 µg/10g	SE	±0.371	±0.751	±0.537	±0.170	±0.781
	%V	0.27	1.01	2.67	16.61	26.88
		117.130 ^{NS}	119.030 ^{NS}	121.100 ^{NS}	149.400***	170.031***
8 µg/10g		±0.06	±0.790	±0.422	±0.244	±0.694
		0.51	1.93	3.52	23.36	33.73

Values expressed as mg of protein /g.wet weight of tissue.
*** P<0.001.
NS- Not significant.

Table – 2. Effect of Thyroxine (T_4) injection on skeletal muscle RNA content in *Anabas testudineus* (n=6)

Dosage of Thyroxine (T4)		Single injection effect			Cumulative effect	
		24 hours	**48 hours**	**72 hours**	**one week**	**two weeks**
Control	Mean	0.988	0.985	0.990	1.005	1.020
	SE	±0.010	±0.010	±0.011	±0.012	±0.014
Experimental						
	Mean	1.010	1.029	1.225***	1.445***	1.900***
4 µg/10g	SE	±0.052	±0.034	±0.012	±0.014	±0.016
	%V	2.54	4.47	23.74	43.78	86.27
		1.023	1.041	1.320***	1.676***	2.180***
8 µg/10g		±0.048	±0.078	±0.011	±0.017	±0.012
		3.85	5.68	33.33	66.76	133.72

Values expressed as mg of RNA /g.wet weight of tissue.
*** P<0.001.
NS- Not significant.

Table-3. Effect of Thyroxine (T_4) injection on skeletal muscle DNA content in Anabas testudineus (n=6)

Dosage of Thyroxine (T4)		Single injection effect			Cumulative effect	
		24 hours	48 hours	72 hours	one week	two weeks
Control	Mean	0.610	0.610	0.610	0.550	0.530
	SE	±0.009	±0.007	±0.006	±0.010	±0.012
Experimental						
	Mean	0.620	0.630	0.610	0.640	0.545
4 µg/10g	SE	±0.013	±0.009	±0.021	±0.020	±0.025
		0.570	0.630	0.620	0.630	0.650
8 µg/10g		±0.023	±0.013	±0.019	±0.026	±0.024

Values expressed as mg of DNA /g.wet weight of tissue.
*** P<0.001.
NS- Not significant.

systems involving mRNA and ribosomes, the protein synthesizing units of the cells. The increased activity of RNA polymerase and nuclear RNA both found to proceed the incorporation of labelled amino acids i n to mitochondrial and microsomal proteins (Frieden & Lipner, 1971). The response to thyroxine to protein biosynthesis in the present study might be due to the acceleration of protein synthesizing machinery. The increased RNA content in the tissue further supports this observation.

The increased biosynthesis of RNA is likely to be due to increased DNA dependent RNA polymerase activity. The enhanced activity of this enzyme was found in rat liver nuclei after injection of T3 to thyroidectomized rats (Tata, 1970). Medda and Ray (1979) reported that T4 and its analogues increased the RNA and protein content of liver and muscle of lata fish. Sri Handayani et.al.(2005) revealed that 3,5,3' – triiodothyronine (T3) hormone treatment showed that the highest RNA concentration of muscle in *Giant gouramy*, *Osphronemus gouramylac*.

The unchanged levels of DNA in skeletal muscle of *Anabas testudineus* in the present investigation indicated that thyroxine injection does not alter the DNA metabolism. In the liver and muscle of lata fish, *Ophicephalus punctatus*, a similar observation was recorded (Ray & Medda, 1977).

It can be inferred from the present study that T4 injection experiments were effective in stimulating protein and RNA levels of skeletal muscle in fish.

Competing Interests

The authors have declared that no competing interests exist.

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