Thyroid Hormone-Induced Changes in Cytoplasmic and Mitochondrial Proteins of a Teleost

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The effect of triiodothyronine (T_3) on the cytoplasmic and mitochondrial protein contents were studied in the liver and skeletal muscle of a freshwater teleost. The fish exposed to thiouracil for 28 days showed 1.5–2 times reduction in the total protein contents of cytoplasmic and mitochondrial fractions. A single injection of T_3 to thiouracil exposed fish caused the earliest induction in the liver and skeletal muscle mitochondrial protein and the skeletal muscle cytoplasmic protein at 12 hr of lapses. However, the initial induction in the cytoplasmic protein of the liver was observed at 3 hr after T_3 treatment. The maximum inductions (1.5–3.2 fold) in the cytoplasmic and mitochondrial proteins of the liver and skeletal muscle were obtained at 18–24 hr following hormonal administration. Thereafter, the cytoplasmic and mitochondrial protein contents of both the tissues declined to their control levels within 36–48 hr of T_3 injection which reflected the half-life and turnover period of the induced proteins. These T_3 dependent inductions in the cytoplasmic and mitochondrial proteins of the liver (1.4–3.2 fold) and skeletal muscle (1.8–2.7 fold) were inhibited by actinomycin D and cycloheximide indicating T_3-induced *de novo* synthesis of the proteins. The induction in the cytoplasmic protein (3 fold) was almost double to that of the mitochondrial protein (1.6 fold) suggesting more synthesis of protein molecules in the cytoplasm for cellular and subcellular activities.

Introduction

Thyroid hormones play an important role in protein metabolism of animals. It is now well established that the action of thyroid hormone is dependent on the stimulation of DNA-dependent RNA synthesis directing protein synthesis in cell. The mechanism of thyroid hormone-induced responses has been documented in some vertebrate species (Tata, 1963, 1970; Oppenheimer and Dillmann, 1978; Baxter *et al.* 1979; Donaldson *et al.*, 1979; Schultz *et al.*, 1988; Umesono *et al.*, 1988). Though there are some major differences about the effects of thyroid hormones in vertebrates, basically similar mode of thyroid hormone action may persist at the cellular level throughout the vertebrate phylogeny (Higgs *et al.*,1982). Some evidences favour that T_3 is also an active hormone in fishes (Donaldson *et al.*, 1979; Van der kraak and Eales, 1980; Omeljaniuk and Eales, 1985; Eales, 1995).

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The protein synthesis and deposition in fishes have been studied in relation to adaptation, feeding and nutrition (Haschemeyer, 1978; Mommsen *et al.*, 1980; Fauconneau, 1985). The seasonal changes in the cytoplasmic and mitochondrial proteins have been reported in catfish (Tripathi and Shukla, 1991). Thyroid hormones may also be used as an anabolic agent in fish culture (Higgs *et al.*, 1982). Nevertheless no information exists on the thyroid hormone-induced changes in proteins of freshwater fishes. Therefore, the present investigations were carried out to analyse the effects of T_3 on the cytoplasmic and mitochondrial protein contents of the liver and skeletal muscle of a freshwater teleost, *Clarias batrachus.* The study on mitochondrial protein was considered because the thyroid hormone-induced calorigenic effects occur through various enzymatic (protein) changes in the mitochondria.

Materials and Methods

Animal and treatment

Healthy and adult specimens of either sex of *Clarias batrachus*, weighing 70–75 g having a body

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length of 18–19 cm, were collected from local ponds during the months of November to February. They were acclimatized to the laboratory conditions for two weeks prior to experimentation and fed on minced goat liver on alternate day. The experimental fish were divided into following five groups:

(A) Normal or untreated fish, which were kept separately for 28 days before sacrifice.

(B) Thiouracil exposed fish which were exposed to 0.4% thiouracil for 28 days and injected (0.2 ml) intraperitoneally alkaline saline (pH 8.4)

(C) Thiouracil plus T_3 treated fish, which were indentically exposed to thiouracil for 28 days and then injected (0.2 ml) intraperitoneally with 20 μg of 3, 5, 3'-triiodo-L-thyronine (T_3) (dissolved in alkaline saline) per 100 g body mass. The three other doses of T_3 (5 μg , 10 μg, 30 μg) were also tested in a pilot experiment but 20 μg was found best in increasing protein content.

(D) Thiouracil plus actinomycin D (ACT) plus T_3 treated individuals.

(E) Thiouracil plus cycloheximide (CHX) plus T_3 treated fish.

The actinomycin D (20 μg per 100 g body mass) and cycloheximide (200 μg per 100 g body mass) were given one hr prior to T_3 injection. The cytoplasmic and mitochondrial protein contents were measured at different time intervals (0 to 48 hr) after the administration of T_3. The effects of actinomycin D and cycloheximide were recorded at 18 h after T_3 administration.

Tissue preparation and subcellular fractionation

The freshwater catfish, *Clarias batrachus*, were sacrificed and the liver as well as skeletal muscle were removed. A 10% homogenate (w/v) was prepared in 0.25 M buffered sucrose solution (0.1 M sodium phosphate buffer, pH 7.4, containing 0.25 M sucrose) using a Potter – Elvehjem homogenizer fitted with teflon pestle. The homogenate was centrifuged at 700×*g* for 15 min. The supernatant was decanted and centrifuged at 12,100×*g* for 20 min to get the mitochondrial pellet. The subsequent supernatant thus obtained was recentrifuged at 30,000×*g* for 30 min and the resulting supernatant was taken as the cytoplasmic fraction for the estimation of cytoplasmic protein.

Extraction of mitochondrial protein content

The mitochondrial pellet was washed thrice in 0.25 M buffered sucrose and each washing was followed by centrifugation at 12,100×*g* for 15 min. Finally, the mitochondrial pellet was suspended by shaking in 2 ml of 0.1 M sodium phosphate buffer containing 1% triton X-100 (v/v) for 30 min. It was again centrifuged at 30,000×*g* for 30 min and the resulting supernatant was taken as mitochondrial fraction for the estimation of mitochondrial protein.

Protein estimation

The protein contents of both the cytoplasmic and the mitochondrial fractions were determined by folin method (Lowry *et al.*, 1951) using a linear standard curve of bovine serum albumin. The total protein content was expressed as mg xg^{-1} wet wt. (mass) of the tissue.

Statistical analysis

Wilcoxon's test for paired comparisons (Bailey, 1995) was employed to acertain the level of significances for the data given in Table II.

Results

Time-course effect of T_3 on protein contents

The total cytoplasmic protein content of the liver increased (24%) at 3 hr reaching maximum at 18 hr of T_3 injection. This level was maintained till 24 hr and then declined gradually. The protein content at 42 hr was equal to the control value (Table I). However, the total cytoplasmic protein in skeletal muscle increased (69%) only at 12 hr and it was found maximum at 24 hr in response to a single injection of T_3 to the thiouracil exposed fish. This peak value remained constant till 30 hr and then declined to the control level at 42 hr of T_3 treatment (Table I).

A single injection of T_3 showed the earliest increase (31%) in the total mitochondrial protein content of the liver at 12 hr of lapse and gradually attained the peak level at 24 hr. This high profile was maintained up to 30 hr and then declined to its control level within 36 hr of T_3 administration (Table 1). Likewise the mitochondrial protein of the skeletal muscle increased (44%) at 12 hr showing maximum induction at 24 hr of T_3 treatment.

Table I. The effect of 3,5,3'-triiodo-L-thyronine (T_3) on total cytoplasmic (cP) and mitochondrial (mP) protein (mg x g^{-1} wet mass) at different time intervals from the liver and skeletal muscle of the freshwater catfish, *C. batrachus*[a]. Each datum represents mean ± SEM of four individuals (n=4).

Duration	Liver		Skeletal muscle	
[hr]	cP [mg x g^{-1}]	mP [mg x g^{-1}]	cP [mg x g^{-1}]	mP [mg x g^{-1}]
0.0 (Control)	29.379 ± 0.955	4.517 ± 0.393	14.808 ± 1.136	0.424 ± 0.028
0.5	28.993 ± 0.514	4.442 ± 0.322	15.068 ± 0.911	0.427 ± 0.029
1.0	30.156 ± 1.636	4.473 ± 0.320	15.254 ± 0.404	0.414 ± 0.003
2.0	29.271 ± 1.152	4.509 ± 0.127	14.642 ± 0.499	0.427 ± 0.035
3.0	36.560 ± 1.508	4.602 ± 0.255	16.236 ± 0.415	0.482 ± 0.024
6.0	47.565 ± 2.295	5.116 ± 0.207	17.715 ± 0.895	0.530 ± 0.035
12.0	67.674 ± 3.439	5.898 ± 0.274	25.040 ± 0.668	0.611 ± 0.021
18.0	93.505 ± 4.731	6.389 ± 0.412	40.702 ± 1.391	0.909 ± 0.021
24.0	91.456 ± 4.611	6.581 ± 0.246	40.815 ± 1.005	0.938 ± 0.020
30.0	76.438 ± 4.703	5.929 ± 0.291	38.431 ± 0.501	0.828 ± 0.031
36.0	65.880 ± 1.274	5.118 ± 0.176	27.716 ± 0.473	0.765 ± 0.059
42.0	31.937 ± 2.271	4.580 ± 0.233	15.757 ± 0.391	0.574 ± 0.028
48.0	31.073 ± 2.021	4.633 ± 0.263	15.433 ± 0.552	0.459 ± 0.012

[a] Fish were exposed to thiouracil for 28 days and they were injected T_3.

It remained somewhat constant till 36 hr. The protein content decreased afterwards and became same as that of the control level at 48 hr of T_3 injection to thiouracil exposed fish (Table I).

Effect of inhibitors on T_3 induced changes in protein contents

The exposure of thiouracil to the catfish for 28 days caused 2.1-times reduction in the total cytoplasmic protein content of the liver. T_3 induced an increase (3.2 fold) in the protein level which was suppressed by the administration of actinomycin D or cycloheximide (Table II). Similarly, the treatment of thiouracil exhibited 1.5-times decrease in the total mitochondrial protein of the liver. A single injection of T_3 to the thiouracil exposed individuals caused 1.4-fold increase in the protein content. This T_3-dependent increase was completely suppressed by the administration of either actinomycin D or cycloheximide (Table II).

The exposure of the fish to thiouracil reduced the total cytoplasmic protein content of the skeletal muscle by 1.5 times as compared to the protein content of the normal individuals. Administration of T_3 to these thiouracil exposed fish produced an increase (2.7 fold) in its level and the increase was blocked by the injection of actinomycin D or cycloheximide (Table 2). Similary, the exposure of thiouracil reduced the mitochondrial protein content of the skeletal muscle. The administration of a single dose of T_3 to the thiouracil exposed fish produced 1.8-fold increase in the protein content. This T_3-dependent induction in mitochondrial protein was inhibited by the treatment of actinomycin D or cycloheximide (Table II).

Discussion

The treatment of thyroid hormone (T_3) to thiouracil exposed fish gradually increased the total cytoplasmic and mitochondrial proteins of the liver and skeletal muscle (Table I). These protein contents in both the tissues peaked around 18–24 hr and remained maintained up to 24–30 hr of hormonal administration. The mitochondrial protein of the liver and skeletal muscle and the cytoplasmic protein of the skeletal muscle showed the earliest inductions (31–69%) at 12 hr of T_3 injection. However, the cytoplasmic protein of the liver exhibited the earliest induction (24%) already after 3 hr of T_3 treatment. The initial inductions in the cytoplasmic and mitochondrial protein contents followed a gradual trend of increase in protein concentrations. There were approximately 3-fold maximum induction in the concentration of cytoplasmic protein of the liver and skeletal muscle. By contrast the total mitochondrial proteins in

Table II. The effect of actinomycin D (ACT) and cycloheximide (CHX) on 3,5,3'-triiodo-L-thyronine (T_3) induced increase in total cytoplasmic (cP) and mitochondrial (mP) protein (mg x g^{-1} wet mass) from the liver and skeletal muscle of the freshwater catfish, *C. batrachus*[a]. Each datum represents mean ± SEM of five individuals (n=5).

Duration	Liver		Skeletal muscle	
[hr]	cP [mg x g^{-1}]	mP [mg x g^{-1}]	cP [mg x g^{-1}]	mP [mg x g^{-1}]
Normal	60.162 ± 2.482[b]	6.858 ± 0.179[g]	21.966 ± 0.692[l]	0.609 ± 0.048[q]
Thio+Saline	28.260 ± 0.648[c]	4.573 ± 0.210[h]	14.624 ± 0.337[m]	0.449 ± 0.043[r]
Thio+T_3	89.814 ± 2.325[d]	6.241 ± 0.205[i]	39.417 ± 1.000[n]	0.823 ± 0.045[s]
Thio+ACT+T_3	20.356 ± 1.835[e]	3.702 ± 0.164[j]	10.881 ± 0.791[o]	0.386 ± 0.030[t]
Thio+CHX+T_3	18.241 ± 1.451[f]	3.046 ± 0.236[k]	11.965 ± 0.409[p]	0.408 ± 0.017[u]

[a] Fish were exposed to thiouracil (Thio) for 28 days and were given T_3, or ACT + T_3, or CHX + T_3. Animals were sacrificed after 18 h of T_3 administration.
The data were statistically significant ($P<0.05$) after comparing b&c, g&h, l&m, q&r, b&d, l&n, q&s, c&d, h&i, m&n, r&s, d&e, i&j, n&o, s&t, d&f, i&k, n&p, and s&u. However, the comarison between g&i was statistically non-significant.

both the tissues showed only 1.6-fold induction in response to T_3 administration. This supports the well established fact that T_3 basically enhances protein synthesis in cytoplasm and only a fraction of these newly synthesized proteins are transported to mitochondria (Kozak, 1983; Granner, 1990). It is the reason why the apparent induction in the cytoplasmic proteins is more than those of the mitochondrial proteins in response to T_3 treatment. After attaining the maximum induction, both the cytoplasmic as well mitochondrial proteins declined to their control levels within 36–48 hr of T_3 injection. This clearly suggests that most of the T_3-induced protein molecules were degraded and utilized within 42 hr.

Administration of a single dose of either actinomycin D (transcriptional inhibitor) or cycloheximide (translational inhibitor) to thiouracil exposed fish one hr prior to T_3 injection completely suppressed/blocked the increase in cytoplasmic and mitochondrial protein contents of the liver and skeletal muscle (Table II). The inhibition in new protein synthesis by actinomycin D and cycloheximide in T_3 administered individuals inferred the T_3 induced *de novo* synthesis of the cycloplasmic and mitochondrial proteins in the catfish. This may be substantiated by some important reports in other vertebrates (Tata, 1963, 1970; Oppenheimer and Dillmann, 1978; Donaldson *et al.*, 1979; Baxter *et al.*, 1979; Schultz *et al.*, 1988). Therefore, T_3 may be used as a good inducer of protein synthesis in fishes and thus may be employed as an anabolic agent in fish culture.

Bailey, N. T. J. (1995), Statistical Methods in Biology. Cambridge University Press, Cambridge. pp. 172–189.

Baxter J. D., Eberhardt N. Z., Apriletti J. W., Johnson L. K., Ivarie R. D., Schachter B. S., Morris J. A., Seeburg P. H., Goodman H. M., Latham K. R., Polansky J. R. and Martial J. A., (1979), Thyroid hormone receptors and responses. Rec. Progr. Horm. Res. **35**, 97–153.

Donaldson E. M., Fagerlund U. H. M., Higgs D. A. and Mc Bride J. R. (1979), Hormonal enhancement of growth. In: Fish Physiology (Eds. Hoar W. S., Randall D. J. and Brett J. R.). Vol. III, Academic Press, New York. pp. 455–597.

Eales, J. G. (1995), Regulation and measurement of thyroidal status in fish. Neth. J. Zool. **45**, 175–180.

Fauconneau B. (1985), Protein synthesis and protein deposition in fish. In: Nutrition and Feeding in Fish (Eds. Cowey C. B., Mackie A. M. and Bell J. G.). Academic Press, London. pp. 17–46.

Granner, D. K. (1990), Thyroid hormones. In: Harper's Biochemistry (Eds. Murray R. K., Granner D. K., Mayes P. A. and Rodwell V. W.). Appleton and Lange, Connecticut. pp 487–491.

Haschemeyer A. E. V. (1978), Protein metabolism and its role in temperature acclimatiion. In: Biochemical and Biophysical Perspectives in Marine Biology (Eds. Marlins D. C. and Sargent J. R.), Vol. **4**. Academic Press, New York. pp. 30–84.

Higgs D. A., Fagerlund U. H. M., Eales J. G. and Mc Bride, J. R. (1982), Application of thyroid and steroid hormones as anabolic agents in fish culture. Comp. Biochem. Physiol. **73B**, 143–176.

Kozak, M. (1983), Comparison of initiation of protein synthesis in prokaryotes, eukaryotes and organelles. Microbiol. Rev. **47**, 1–9.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951), Protein measurement with the folin phenol reagent. J.Biol. Chem. **193**, 265–275.

Mommsen T. P., French C. J. and Hochachka P. W. (1980), Sites and patterns of protein and amino acid utilization during the spawning and migration of salmon. Can. J. Zool. **58**, 1785–1799.

Omeljaniuk R. J. and Eales J. G. (1985), Immersion of rainbow trout in 3,5,3'-triiodo-L-thyronine (T_3): Effect on plasma T_3 levels and hepatic nuclear T_3 binding. Gen. Comp. Endocrinol. **58**, 81–88.

Oppenheimer J. H. and Dillmann W. H. (1978), Nuclear receptors for triiodothyronine : A physiological perspective. In: Receptors and Hormone Action – (Eds. O'Malley B. W. and Birnbaumer L.) Vol. 3. Academic Press, New York. pp. 1–34.

Schultz J. J., Price M. P. and Frieden E. (1988), Triiodothyronine increases translatable albumin messenger RNA in *Rana catesbeiana* tadpole liver. J. Exp. Zool. **247**, 69–75.

Tata J. R. (1963), Actinomycin inhibits thyroid hormone action. Nature **197**, 1167–1168.

Tata J. R. (1970), Regulation of protein synthesis by hormones. In: Biochemical Actions of Hormones – (Eds. Litwack G.), Vol. 1. Academic Press, New York. pp. 89–133.

Tripathi G. and Shukla S. P. (1991), Cytoplasmic and mitochondrial proteins in a freshwater catfish under normal and experimental conditions. Arq. Biol. Technol. **34**, 207–216.

Umesono K., Giguere V., Glass C. K., Rosenfeld M. G. and Evans R. M. (1988), Retinoic acid and thyroid hormone induce gene expression through a common responsive element. Nature **336**, 262–264.

Van der Kraak G. J. and Eales J. G. (1980), Saturable 3,5,3'-triiodo-L-thyronine-binding sites in liver nuclei of rainbow trout (*Salmo gairdneri* Richardson). Gen. Comp. Endocrinol. **42**, 437–448.