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# Effect of propylthiouracil (PTU) on serumthyroxin ($T_4$) and triiodothyronine ($T_3$) concentration in mature tropical toad, Bufomelanostictus

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**Abstract**

**Background:** *The variations in results regarding plasma concentrations of thyroid hormone of mature amphibia are related to species and season as well as to other physiological and environmental factors such as sex, nutrition, photoperiod or temperature. Studies of seasonal changes in circulating thyroid hormone levels in amphibia have disclosed changing patterns. Plasma $T_4$ level was high in a period before the breeding season in toads and frogs. $T_3$ is more potent hormone than $T_4$ in amphibia. The deiodination activity in liver was low before metamorphosis but showed a quick, transient rise during metamorphosis. The deiodination activity in liver reduced during post metamorphic growth but owing to the growth of the organ the total activity did not decrease significantly. In mammals, administration of 6-n-propylthiouracil (PTU) blocks most of the $T_4$ to $T_3$ conversion in liver and other tissues and abolishes the effect of exogenously administered $T_4$ .In tadpoles, undergoing metamorphosis, conversion of $T_4$ to $T_3$ blocked by PTU.*

**Materials and Methods:** *Female toads were procured locally and acclimatized into laboratory conditions. The animals under experimental group were treated with PTU as per experimental protocol. The control groups were maintained and treated with vehicle. Both groups were sacrificed and blood was collected. The radioimmunoassay (RIA) was performed to record the data of serum $T_4$ and $T_3$.*

**Results:** *The results from our study showed significant differences in serum $T_4$ and $T_3$ concentration between control and PTU treated group. Results showed that both serum $T_4$and $T_3$concentrations in mature toad decreased after treatment with PTU. These findings were indicating the inhibitory action of PTU on the iodide metabolism particularly transformation of iodide into its ionic form, its entry into the follicular cells, iodination of tyrosil residues and subsequent deiodination of $T_4$ to $T_3$.*

**Conclusion:** *The similarities of the thyroid hormone action found in mature toad in the study with those of other mammalian and non-mammalian vertebrates supports evolutionary conservancy of the nature of thyroid hormone action.*

**Keywords:** *Thyroid hormone ($T_4$ and $T_3$), Deiodinase enzyme, Propylthiouracil (PTU), Radioimmunoassay (RIA).*

## Introduction

The basic mechanism of action for thyroid hormone is common to all species because many aspects of thyroid hormone action in amphibia are similar to those proposed for mammalian and non-mammalian species and poikilotherms such as fish

[1,8,10,11,12]. The results from earlier studies show that $T_4$ action on total protein and RNA content, cytosolic malic enzyme activity, mitochondrial alpha glycerophophate dehydrogenase activity and crude and microsomal $Na^+ K^+$ ATPase activities are inhibited in only PTU treated and PTU+$T_4$ treated mature toads (3,4,5 and 6). On the basis of such observations it has been suggested that probably synthesis of thyroid hormones and also conversion of $T_4$ to $T_3$ has been inhibited. So attempt has been made to study the serum concentrations of $T_4$ and $T_3$ in toads pre-treated with PTU, by radioimmunoassay (RIA).

**Aims and Objectives**

1. To get an idea about the concentration of thyroid hormone, if any, in the serum of mature toad.
2. To understand whether $T_3$ is the potent hormone in mature toad like what it is in other mammalian or non-mammalian vertebrates.
3. To know the mechanism of action of propylthiouracil (PTU) in mature toad.

**Materials and Methods**

All the experiments were performed after receiving ethical clearance from the concerned authority.

**A. Treatment of the animals**

Mature female toads (body wt. 30-40 gm.) were procured and maintained in laboratory condition at a room temperature around $25^0$C. The toads were divided into control and treated groups. Each group contained eight animals. A group of toads were injected daily with single dose of PTU (10µg/g) for six consecutive days (2 and 9). PTU was procured from Sigma Chemical company, USA. The control animals received same amount of vehicle (alkaline 0.65% NaCl solution, pH 9) only. All the animals were sacrificed after 24 hours of the last injection of PTU.

**B. Collection of the serum samples**

Blood was taken directly from the heart of toads with the help of a glass van syringe fitted with 22 gauge needle. The blood was transferred to conical glass centrifuge tubes, containing no anticoagulant. The tubes were kept at room temperature for 30 minutes and the blood was allowed to clot. The tubes were centrifuged at 3000 × g for 10 minutes. After centrifugation the serum was aspirated out with the help of Pasteur pipette and stored at – $70^0$C for the assay.

**C. Reagents for Radioimmunoassay of $T_3$ and $T_4$**

Radioimmunoassay (RIA) of the thyroid hormone ($T_3$ and $T_4$) was performed with the help of direct assay RIA kit, supplied by Board of Radiation and Isotope Technology, Mumbai, India.

The RIA kit contained

i.$T_4$and $T_3$ antiserum

ii. $^{125}$I-$T_4$ and $^{125}$I-$T_3$

iii. $T_4$ and $T_3$ standard solution

iv. Precipitating agent (Polyethylene Glycol, PEG)

a.22% PEG in 1% NaCl as precipitating agent for $T_4$ RIA

b.12% PEG in 1% NaCl as precipitating agent for $T_3$ RIA

Assay buffer for $T_4$ and $T_3$ RIA

The assay buffer contain 0.14 M Trishydroxy-methyl aminomethane buffer with 0.1% gelatin. The pH of the buffer was 8.6.

**D. Procedure of the radioimmunoassay (RIA) of $T_4$ and $T_3$**

Reagents and samples were brought to room temperature ($25^0$C) and mixed thoroughly. The zero standard was taken into the non-specific binding (NSB) tubes.

Other standards (0.15, 0.3, 0.6, 1.2 and 2.4 ng/ml for $T_3$ and 2.5, 5, 10, 20 µg/dl for $T_4$) , respective antiserum for $T_3$ and $T_4$ , assay buffers, and serum samples for assay were taken in different RIA tubes. $^{125}$ I-$T_4$ and $^{125}$I–$T_3$ were added to each tube to assay $T_4$ and $T_3$ respectively and mixed well. The assay mixture was then incubated at room temperature for 75 minutes and 180 minutes for $T_4$

and $T_3$ respectively. After incubation, polyethylene glycol (PEG), the precipitating agent, mixed well to precipitate the antigen – antibody complex completely. Analysis of all the samples carried out in duplicate. The samples were then centrifuged at 3000×g for 20 minutes to precipitate antigen-antibody complex. The tubes were decanted completely and carefully without disturbing the precipitate except the tubes kept for the total count.

The counts of the pellets were recorded in a Beckman Gamma Counter for one minute (count per minute, cpm).

The sensitivity of the $T_4$ and $T_3$ assay was 0.5 µg /dl and 0.24ng /dl respectively of the samples, based on 90% $B/B_0$ intercept where B is the corrected average counts of the standard/samples and $B_0$ is the corrected average counts of zero standard.

Significance of the differences in mean hormone concentration between groups was tested by performing analysis of variance (ANOVA) followed by Duncan's multiple range test. Values of P less than 0.05 were taken as significant.

**Results**

Values of serum $T_4$ and $T_3$ concentrations in control and PTU treated toads were mentioned in Table – 1.

The values of serum $T_4$ and $T_3$ concentrations of control group of toads were 0.55± 0.08 µg/dl (n=8) and 0.67±05 ng/ml (n=8) respectively. The values of serum $T_4$ and $T_3$ concentrations in PTU treated group of toads were 0.30±0.05 µg/dl (n =8) and 0.28± 0.04 ng/ml (n=8) respectively. Compared to the control, the values of serum $T_4$ and $T_3$ concentrations of PTU treated toads were significantly low.

**Table -1** Serum $T_4$ and $T_3$ concentrations in control and PTU treated toads

| Treatment Groups | $T_4$ conc (µg./dL) | $T_3$ conc (ng/dL) |
|---|---|---|
| **Control (Mean ± SE)** | 0.55± 0.08 | 0.67±0.05 |
| **PTU Treated (Mean ± SE)** | 0.30±0.05[a] | 0.28±0.04[a] |

Each group contained 8 animals
SE- Standard Error, p value –a= P <0.05
Where 'a' denotes comparison between control and PTU treated group.

**Discussion**

In numerous test systems, it has been postulated that the biological activity of thyroid hormone is mostly dependent upon the conversion of $T_4$ to $T_3$ by deiodinase enzymes localized in the liver, kidney and brain[7] because $T_3$ having the greatest biological activity while reverse $T_3$ (r-$T_3$) is devoid of activity. Thus $T_4$ has been presumed to be the inactive precursor or prohormone[7]. The quantity of the production of active hormone $T_3$ is regulated at the level of the peripheral tissues namely in target organs. Modulation of the conversion of $T_4$ to $T_3$ may be happened by pharmacologic agent like propylthiouracil (PTU) which is a potent inhibitor of iodothyronine deiodinase. Apart from the deiodination process that is inhibited by PTU, the same has got also inhibitory effect on the biosynthesis of the hormone since PTU inhibits the transformation of iodide into organic iodine and the coupling of iodotyrosines. The inhibitory effects cannot be reversed by large doses of iodide[7].

Results from the study suggest that serum $T_4$ and $T_3$ concentrations decreased in PTU treated toads in comparison to the control (untreated toads). It is also giving an idea about the endogenous serum $T_4$ and $T_3$ levels of toad and at the same time suggesting that conversion of $T_4$ to $T_3$ takes place in this animal. It has been stated earlier that the liver nuclei of mature toad contain high affinity saturable $T_3$ binding sites[9]. So, it is also expected that the free thyroid hormone should be preferentially distributed in different organs of toad according to number and capacity of binding sites.

**Conclusion**

The present study throws light on thyroid hormone responsiveness of mature toad, Bufomelonostictus. It also suggest that $T_4$ to $T_3$

conversion was blocked by PTU treatments and the biological effects of $T_4$ as judged by changes in serum $T_3$ level were counteracted by PTU. The results lead us to conclude that $T_4$ may act as a prohormone for $T_3$ in toad.

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