Effect of propylthioureacil (PTU) on serumthyroxin (T₄) and triiodothyronine (T₃) 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 T₄ level was high in a period before the breeding season in toads and frogs. T₃ is more potent hormone than T₄ 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 T₄ to T₃ conversion in liver and other tissues and abolishes the effect of exogenously administered T₄. In tadpoles, undergoing metamorphosis, conversion of T₄ to T₃ 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 T₄ and T₃.

Results: The results from our study showed significant differences in serum T₄ and T₃ concentration between control and PTU treated group. Results showed that both serum T₄ and T₃ 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 T₄ to T₃.

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 (T₄ and T₃), 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.
The results from earlier studies show that T4 action on total protein and RNA content, cytosolic malic enzyme activity, mitochondrial alpha glycerophosphate dehydrogenase activity and crude and microsomal Na + K+ ATPase activities are inhibited in only PTU treated and PTU+T4 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 T4 to T3 has been inhibited. So attempt has been made to study the serum concentrations of T4 and T3 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 T3 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°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°C for the assay.

C. Reagents for Radioimmunoassay of T3 and T4
Radioimmunoassay (RIA) of the thyroid hormone (T3 and T4) 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. T4 and T3 antiserum
ii. 125I-T4 and 125I-T3
iii. T4 and T3 standard solution
iv. Precipitating agent (Polyethylene Glycol, PEG)
   a. 22% PEG in 1% NaCl as precipitating agent for T4 RIA
   b. 12% PEG in 1% NaCl as precipitating agent for T3 RIA
Assay buffer for T4 and T3 RIA
The assay buffer contain 0.14 M Trishydroxymethyl aminomethane buffer with 0.1% gelatin. The pH of the buffer was 8.6.

D. Procedure of the radioimmunoassay (RIA) of T4 and T3
Reagents and samples were brought to room temperature (25°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 T3 and 2.5, 5, 10, 20 μg/dl for T4), respective antiserum for T3 and T4, assay buffers, and serum samples for assay were taken in different RIA tubes. 125I-I-T4 and 125I–I–T3 were added to each tube to assay T4 and T3 respectively and mixed well. The assay mixture was then incubated at room temperature for 75 minutes and 180 minutes for T4.
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.

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

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>T_4 conc (μg/dL)</th>
<th>T_3 conc (ng/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Mean ± SE)</td>
<td>0.55± 0.08</td>
<td>0.67±0.05</td>
</tr>
<tr>
<td>PTU Treated (Mean ± SE)</td>
<td>0.30±0.05</td>
<td>0.28±0.04</td>
</tr>
</tbody>
</table>

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 T₄ as judged by changes in serum T₃ level were counteracted by PTU. The results lead us to conclude that T₄ may act as a prohormone for T₃ in toad.

References
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