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REGULATORY ROLE OF THYROXINE ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN INDIAN TOAD

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ABSTRACT

A review of the hormonal control of G6PDH activity reveals that much less work has been done on the effect of thyroxine on the above enzyme. This is probably because of, equivocal consideration of thyroxine as a metabolic hormone for its adaptive role in all the metabolic processes and its primary involvement in glycogenolysis and gluconeogenesis. Much less attention has, so far been paid to the enzymes of HMP-shunt, despite the fact that this pathway also provides substrates such as fructose-6-phosphate and glyceraldehydes-3-phosphate to gluconeogenesis. Precisely why, the present work has been designed to study the effect of the hormone thyroxine *in vivo,* on the activity of the enzyme G6PDH in liver of Indian toad, Bufo *melanostictus.* Thyroxine caused significant inhibition of G6PDH activity *in vivo* in liver of toads at two different dose levels.

INTRODUCTION

The thyroid hormone, thyroxine (T_4) and triiodothyronine (T_3) are tyrosine based hormones produced by the thyroid gland. Iodine is an important component in the synthesis of thyroxine. The thyronines act on the body to increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness. The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat and carbohydrate metabolism, affecting how human cells use energetic compounds. Numerous physiological and pathological stimuli influence thyroid hormone synthesis.

Thyroid hormones, both L-thyroxine (T_4) and L-triiodothyronine (T3), have been documented to have profound effects on growth, differentiation, development (Wolf and Wolf, 1964; Cohen, 1970; Frieden and Just, 1970; Barker, 1971; Greenberg *et al.,* 1974; Hoch, 1974; Tata, 1974;), possible immunologic responses (Ludin, 1958), calorigenesis (Ismail-Beigi and Edelman, 1971), regulation of enzyme activity (Wolf and Wolf, 1964) and control of pituitary hormones, in particular, thyrotropin (Florsheim, 1974), growth hormone (Solomon and Greep, 1959) and prolactin (Snyder *et* al., 1973). The studies show that the thyroid hormones, which has remarkable and dramatic influences on the key metabolic processes during growth, development and differentiation.

It is evident from the results that thyroxine treatment enhances both the cytoplasmic area and nuclear area of the liver cells and also proteins, RNA and DNA contents of liver of rat, toad and fish of all age groups, excepting the DNA content of liver of adult toad and fish. The increase in dose of T4 generally causes increase in the responsiveness of the animals with respect to all these parameters. The most marked effects in these respects have been found with 1 µg of T4 per g. The interesting fact emerged from the results of the rate of T_4-induced increase of liver protein, RNA and DNA is that the poikilothermic vertebrates (fish.or toad) are more responsive to anabolic dose of T4 (I µg/g) than homeotherms (rat). The hibernating toads fail to respond to thyroxine treatment.

Glucose-6-phosphate Dehydrogenase is a cytosolic enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cell (such as erythrocytes) by maintaining the level of the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH). This cytoplasmic enzyme is widely distributed in microorganisms, plants and animals. In animal tissues, it is of little importance in brain and skeletal muscle but is of more significant in kidney, cornea, liver, lungs, testis, adrenal gland, lactating mammary gland and red blood cell. In kidney the percentage of carbohydrate metabolized through pentose phosphate pathway is about 20% (Thrope *et al.,* 1970). In two species of fish, the liver and kidney were found to contain a significantly higher activity of enzyme as compared to other body tissues . The kinetic properties of G6PD in these species of poikilothermic vertebrate were more or less similar to those of the enzyme extracted from rat. G6PDH is an important enzyme since it serves as a source of NADPH. Synthesis of glutamate by glutamate Dehydrogenase in liver.

1977; Mamaev *et al.,* 1977; Marc and Portet, 1981; Szepsi and Kamara, 1986; Mayes, 1988) and hormonal conditions (Gracia and Holten, 1974; Aruldhas *et al.,* 1982; Martin *et al.,* 1985; Nirot *et al.,* 1985; Fritz and Kletzien, 1987; Haghighi *et al.,* 1989; 1994; 2005; Peragon *et al.,* 1989; Sabrasua *et al.,* 1989; Haghighi and Ghanbari, 1991; Fernando *et al.,* 1992).

AIM OF THE PRESENT STUDY

The survey of foregoing literature indicates that works pertaining to hormonal changes in G6PDH activity in tissues of poikilothermic animals are rather meager. In amphibian species, particularly such studies are completely lacking. The enzyme has the significant adaptive values as an alternate bypass mechanism in carbohydrate metabolism and the fact that this route is involved in the production of NADPH required for reductive biosynthesis and indirectly involved in constant supply of pentose–phosphate necessary for nucleotide synthesis, the present work is aimed to study the role of thyroxin on the activity of the above enzyme in liver of common Indian toad, *Bufo melanostictus.*

WORKDONE IN PRESENT STUDY

The following parameters of liver of India toad were estimated after 3 hr. thyroxine treatment under laboratory condition.

I) Total protein content
II) Effect of the hormone thyroxine in altering the activity of G6PDH, the key enzyme in ppp.

MATERIAL AND METHODS

A)Animal:

Adult Bufo melanostictus, the common Indian toads of either sexes (Body wt. range 20-60 gms., S-V length range 5.5-8.0 cms.) were collected from Berhampur locality (19° 19' 30" N, 84° 47' 45" E, Ht. 12.8 meters from the mean sea level) near Bay of Bengal during December February to July 2013 for the present study.
These animals belong to: Class -- Amphibia, Sub-class – Anura, Order – Procoela, Family – Bufonidae, Genus – Bufo and Species – melanostictus

E)Animal sacrifice and tissue processing:

The animals were pithed by piercing a pointed needle just posterior to occipital region after 3 hrs of treatments. Immediately the liver was dissected out and transferred to petridish containing ice cold amphibian ringer (KCI 140 mg, NaCI 6.5g, $NaHCO_3$ 100mg / litre, P^H 7.4) to clear the adherent materials. The tissue was quickly soaked by Whatman filter paper No.40 and weighted. Then the tissues were taken for estimation of biochemical parameters.

G) Biochemical estimation:

The following biochemical estimations were carried on: Assay of G6PDH (Ec.1.1.1.49) activity: The G6PDH was assayed following the method of Ells and Kirkman (1961).

The assay mixture was taken in two tubes. One was designated as control tube and the other one was experimental. The control tube was consisted of 1.25 ml of 0.5 M Tris buffer (P^H 7.5), 0.1 $_{MI}^{of\ I\ M}$ ^{Mg}C12, 0.5ml of 0.01% 6-Dichlorophenolindophenol (DCIP-disodium salt), 0.25m1 of 0.01M Nicotinamide Adenine Dinucleotide Phosphate ($NADP^+$), 0.25ml of Phenazine Methosulphate (PMS, 0.05mg/ml) and 0.9ml of distilled $_{H20.}$ The experimental tube contained lml Tris buffer and 0.25ml of 0.02M glucose-6phosphate prepared in Tris buffer $^{(PH}$ 7.5) along with the other reactant same as that of the control tube. Both of the tubes were incubated at 39°C for 10 minutes for temperature calibration followed by addition of 0.25ml of tissue extract to both the tubes and were again incubated at the same temperature for 30 minutes. After incubation, the optical density readings of the samples were taken in the UVNIS spectrophotometer (Systronics, model-119) at 620nm against a water blank adjusted to zero. The reduction of DCIP was estimated from a standard curve prepared with DCIP (LOBA). The enzyme activity was expressed as tg of DCIP reduced/gm tissue wet wt. and per mg protein.

Table – I: In vivo effect of Thyroxin (dose: 0.5g and 2g/gm body wt.) on the G6PHDN activity (g DCIP reduced/gm tissue wet wt.) in liver homogenates of toad, Bufa melanostictus. Values for G6PDH activity are g DCIP reduced/gm tissues wet wt. and per mg protein. Values for total protein content are mg protein/gm tissue wet wt; Mean SEM. Numbers in parenthesis indicate sample size. NS not significant at 0.05 confidence level.

Experimental condition	G6PDH activity		Mg Protein/gm tissue wet wt.
	g DCIP reduc3d/gm tissue wet wt.	g DCIP reduced/mg protein	
Control P	386.43 10.02 (10) <0.001	6.216 0.161 (10) <0.001	62.16 2.18 (10) <0.001
Thyroxin Treated	239.32 6.212 (10)	3.705 0.097 (10)	64.58 3.08 (10)
% of change	+ 38	+40	+3

Table – II : In vivo effect of Thyroxin (2g/gm body wt.) on the Glucose-6-phosphate Dehydrogenase activity and total protein content in liver of Indian toad, Bufo melanostictus. Values of G6PDH activity are g DCIP recued/gm tissue wet wt. and per mg protein. Values for total protein content are mg protein/gm tissue wet wt; Mean SEM. Number in parentheses indicates sample size. NS not significant at 0.05 confidence level.

Experiment al condition	G6PDH activity		Mg Protein/gm tissue wet wt.
	g DCIP reduced/gm tissue wet wt.	g DCIP reduced/mg protein	
Control P	386.43 9.67 (10) <0.001	6.082 0.152 (10) <0.001	63.212 2.76 (10) <0.01
Thyroxin Treated	168.96 4.254 (10)	2.507 0.063 (10)	67.381 2.53 (10)
% of change	+ 56	+58	+6

SUMMARY OF RESULTS

Glucose-6-phosphate Dehydrogenase is an adaptive enzyme supplying substrates for Gluconeogenesis, glycolysis, synthesis of nucleotides of the hormone thyroxine on the levels and activity of the above enzyme in animals, the present work is undertaken to study the possible role of thyroxine hormone in modifying the activity of G6PDH in liver of Indian toad, *Bufo melanostictus.* The results obtained (Table-1 and 2) *in vivo* are summarized below:

In Vivo effect of thyroxine on enzyme activity-

- Thyroxine caused significant inhibition of G6PDH activity *in vivo* in liver of toads at two different dose levels. The degree of decrease in the enzyme activity was seen dependent on the dose of the hormone administrated.
- The total protein content in liver homogenates of thyroxine treated toads did not show any significant change at lower dose (0.5µg/gm body) but there is a significant change at higher dose (2µg/gm body wt).

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