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# CHANGES IN GPT ACTIVITY IN TISSUES OF INDIAN TOAD FOLLOWING TESTOSTERONE TREATMENT

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**ABSTRACT**

Testosterone is the dominant hormone in the fetal and neonatal rat, but the testes make only androsterone soon after birth. The ability to produce testosterone is restored at puberty and continues throughout life. Therefore, the present study is designed to assess the role of testosterone in changing some aspects of transaminase activity in the liver and kidney of Indian toad. Following treatment of testosterone in vivo for 5 days, the activity of GPT enzyme decreased significantly in liver tissue of toads when expressed in gm tissue basis.

Human derive all their steroid hormones from cholesterol. Two classes of steroid hormones are synthesized in the cortex of the adrenal gland. Mineral corticoid, which control the reabsorption of inorganic ions ($Na^{+}$, $Cl^{-}$ and $HCO_3$ ) by the kidney and glucocorticoids, which help regulate gluconeogenesis and reduce the inflammatory response. Sex hormones are produced in male and female gonads and the placenta. They include progesterone, which regulates the female reproductive cycle, and androgens (such as testosterone) and estrogens (such as estradiol), which influence the development of secondary sexual characteristics in males and females, respectively. Steroid hormones are effective at very low concentration and are therefore synthesized in relatively small quantities. In comparison with the bile salts, their production consumes relatively little cholesterol.

Synthesis of steroid hormones requires removal of some or all of the carbons in the "Side chains" on C27 of the D-ring of cholesterol, side chains removal takes place in the mitochondria of steroidogenic tissues. Removal involves the hydroxylation of two adjacent carbons in the side chain ($^{C}$20 and C22) followed by cleavage of the bond between them. Formation of the various hormones also involves the introduction of oxygen atoms. All the hydroxylation and oxygenation reactions in steroid biosynthesis are catalyzed by mixed function oxidases that use NADPH, 02 and mitochondria) cytochrome P-450.

The large group of steroid, retinoic acid (retinoid) and thyroid hormones exert atleast part of their effects by a mechanism fundamentally different from that of other hormones, they act in the nucleus to alter gene expression.

Out of these all above steroid hormones testosterone the male sex hormone secreted by the leydig cells (interstitial cells) of testes and sometimes from the adrenal cortex in a very less amount. It's primary site of action is the testes along with other accessory male sex organs. Testosterones stimulates spermatogenesis (Dodd, 1960; Lofts *et al.,* 1966).

Lack of testosterone reduced the secretory activity of epididymis, ductus deferences and accessory glands of reproduction in slender loris (Manjula . and Kadam, 1980). A higher concentration of testosterone *in vitro* inhibited the oxygen consumption of ventral prostate (Bern, 1953). There was a 100% increase in the level of S-adenosyl – L-methionine decarboxylase in the prostate of the castrated rats. (Pitot and Yatvin, 1973).

*In Vitro* administration of 3H testosterone to the caput and cauda epididymis of rat caused a higher level of dihydrotestosterone in the Caput segment then the cauda segment (Foldesy, 1979).

Testosterone administration to prepubertal rabbit and rhesus monkey increased the RNA, DNA, total protein, sialic acid, glycogen, glycery 1phosphory Icho line, alkaline and acid phosphatase conent in vas deferens. More ever, there was evidence of tissue growth and secretory functions (Singh, 1983).

There was a significant fall in DNA/RNA ratio in the seminal vesicle and prostate following testosterone treatment (Dani *et al.,* 1979).

Testosterone is the dominant hormone in the fetal and neonatal rat, but the testes make only androsterone soon after birth. The ability to produce testosterone is restored at puberty and continues throughout life.

Similar observations have been made in other species, and these ages related changes may also occur in humans (Murray *et al., 1988).*

Fluctuations in tissues levels of ATP and pyridine nucleotide (Ritter, *1966)* and increase in both pulse labeling of various RNA fractions (Greenman *et al., 1965)* and polyribonucleotide synthesis by isolated nuclear preparations (Liao *et al., 1965)* has been reported to occur within 1 hour after injection of testosterone to ovariectomized animals.

Testosterone increases cellular mitosis and growth of many somatic tissues such as epiphysial cartilages, long bones, skeletal muscles, kidneys, liver and skin (Das, 1995).

Glycogen level significantly increases where as lactate and pyravate level decrease significantly in liver of *24* hours testosterone treated Indian toad *Bufo melanostictus* (Behera, *2008).*

$Na^{+}$-$K^{+}$-ATPase level increased significantly in liver and muscle homogenates of *24* hours *in vivo* and *in vitro* testosterone treatment of Indian toad *Bufo melanostictus* .

**Effect of testosterone on enzyme activity**

Administration of testosterone caused an induction of phosphofructokinase activity in the prostate and vehicles of young rats (Singhal, 1967). The activity of the enzyme phosphodiesterase in the epididymis has been shown to increase upon testosterone treatment (Holtz, 1980). An interesting isolated effect seen is the marked lowering of carnitine acetyl transferase, which decreases by 80-90% in the epididymis, 21 days after castration of rat (Marquis and Fritz, 1965).

Testosterone increases the phosphofructokinase in the seminal vesicles (Singhal and Valadares, 1968). On the other hand phosphohexoisomerase under similar condition does not change at all (Kitay *et al.,* 1966).

**AIM OF THE PRESENT STUDY**

The foregoing review of literature indicated that the effect of steroid hormones (especially that of testosterone) indicated

changes in transaminase activity, are limited to mammalian species. No work has so far been done on the effect of the above hormone in amphibians. These animals specially those have left water, generally inhabit environments that are hostile to their basic physiology. Nevertheless, these organisms by combinations of many unique morphological structures, physiological adaptation and behavioural response have made them selves well suited to nearly all terrestrial habits.

Therefore, the present study is designed to assess the role of testosterone in changing some aspects of transaminase activity in the liver and kidney of Indian toad, *Bufo melanostictus,* a representative of *Poikilothermic vertebrates.*

## MATERIALS AND METHODS

### D.Collection of Tissues

The animals were pitched by piercing a pointed needle just posterior to occipital region. The liver and kidney were dissected out and transferred to a petridish containing cold Amphibian ringer (KCl140 mg, NaCI — 6.5gm, $^{CaC1}2$ — 120 mg, $NaHCO_3$ — 100mg, making up the volume 1 litre, pH-7.4) inside an ice box. The adherent connective tissues, blood vessels and nerve fibres were removed. The tissues were then blotted off on a Whatman filter paper, weighed individually (Anamed electronic balance, M-300DR) and taken for biochemical estimation.

### Assay of Enzyme Activity *in vivo*

The activities of the enzymes, Glutamate pyravate transaminase were estimated following essentially the method of Reitman and Frankel (1957) as described by Oser (1965).

The Assay mixture contained 0.5 ml of Potasium phosphate buffer (20 mM $_{KH2PO4}$ and 80 mM $_{K2HPO4)}$ pH = 7.4 and 0.5 ml of GPT substrate (0.292 gm of a – ketoglutaric acid and 17.8 gm of DLalanine dissolved in 1000 ml of buffer solution pH – 7.4). The mixture was kept in constant temperature water bath at 39°C for 10 minutes. After temperature calibration 0.5 ml of 5% homogenate prepared from liver and kidney was added to it. Simultaneously a control without substrate and blank without the homogenate were run for correction of endogenous activity.

### Statistical Analysis

To evaluate the statistical significance of the data student's t-test was used.

$$t = \frac{(\bar{x}_1 - \bar{x}_2)\sqrt{\frac{N_1 N_2}{N_1 + N_2}}}{\sqrt{\frac{(N_1 - 1)S_1^2 + (N_2 - 1)S_2^2}{N_1 + N_2 - 2}}}$$

### The formula used:

Here S, and S2 represent standard deviation of two different sets of data and N, and N2 stand for the number of cases in each step, Y, and Y2 being the respective means. The confidence level (P) is found out from 't' table (Abramoff and Thomson, 1966; Bishop 1966). 'P' values at 0.05 level and below were considered significant, at 0.10 level as marginally significant and above 0.10 level as not significant (NS) statistically.

## RESULTS

### *1.In vivo* effect of Testosterone on Transaminase activity

### (a)Liver

Following *in vivo* testosterone (10 µg/g body wt.) treatment for 5 days, the activity of the enzyme glutamate pyruvate transaminase (GPT) significantly decreased in liver of toad when expressed in gm tissue basis ($p < 0.05$, Table – I, Fig. 1).

### (b)Kidney

*In vivo* treatment of testosterone (10 µg/g body wt.) for 5 days, induced a significant decrease in the activity of glutamate pyruvate transaminase (GPT) in kidney of toads when expressed per gm tissue basis ($p < 0.001$, Table – I, Fig. 1).

### *2.In vitro effect* of Testosterone on Enzyme Kinetics

### (a) Liver

The substrate saturation curve taking the substrate concentrations along x-axis and enzyme activity along y-axis in the liver homogenate of toads following testosterone treatment to the homogenate mixture showed a marginal decrease of activity with V,,,,, (Controlled) – 13977 units and V,,,$_a$,, (Treated) – 12749 units at 2.OmM. But the Michaelis constant in both treated and controlled tissue is same having $K_m$ value 0.29 mM (Table – II, Fig. – 2).

After attaining the maximum velocity, on subsequent increase of substrate concentrations, the enzyme showed decrease in activity.

### (b) Kidney

The substrate saturation curve for the enzyme in the kidney homogenates of toads following the same treatment showed $^{V}$maxaxvalue (Controlled – 12630.4 units, Treated – 12089.6 units) at 0.6 mM of substrate concentration. Michaelis constant in controlled and treated tissue is same having $K_m$ value 1.2 mM (Table – III, Fig. -3).

After attaining the maximum velocity, on subsequent increase of substrate concentrations, the enzyme showed a marginal decrease in activity.

**Table-I :** *In vivo* effect of testosterone (10 µg/g body wt.) on GPT activity in liver and kidney homogenates of toads. Values are units of activity/gm tissue/hr. (Mean ± SEM) Numbers in parentheses indicate sample size.

| Experimental condition | Liver | Kidney |
|---|---|---|
| | 4685.090 | 5565.090 |
| Control | | |
| | 114.262 | 121.286 |
| | (11) | (11) |
| P | < 0.05 | < 0.001 |
| | 4350.545 | 4472.727 |
| Treated | | |
| | 109.706 | 217.548 |
| | (11) | (11) |
| % of change | 7.140 | 19.628 |

**Table - II :** Effect of *in vitro* testosterone treatment on the activity of enzyme GPT in liver of toad of various concentration of substrate. Values are units of activity/gm tissue/hr (Mean ± SEM) Numbers in parentheses indicate sample size.

| Condition | Substrate concentrations | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.2 MM | 0.4 Mm | 0.6 mM | 0.8 mM | 1.0 mM | 1.2 mM | 1.4 mM | 1.6 mM | 1.8 mM | 2.0 mM | 2.2 mM | 2.4 mM | 2.6 mM | 2.8 mM |
| | 5856 | 7992 | 8792 | 11040 | 11616 | 12072 | 12256 | 12787.2 | 13000 | 13977 | 8659.2 | 7001.6 | 5913.3 | 5651.2 |
| Control | 502.91 | 716.30 | 485.01 | 753.98 | 386.43 | 227.54 | 316.40 | 480.20 | 920.49 | 940.20 | 631.38 | 588.90 | 682.47 | 535.85 |
| | (4) | (4) | (4) | (4) | (4) | (4) | (10) | (10) | (7) | (7) | (10) | (10) | (10) | (10) |
| % Change | 5.46 | 6.60 | 1.00 | 9.71 | 3.71 | 5.5 | 1.72 | 4.32 | 12.52 | 1.06 | 2.80 | 7.81 | 10.33 | 8.04 |
| | 5536 | 7464 | 8704 | 9968 | 11184 | 11408 | 12044.8 | 12233.6 | 12482 | 12749 | 8416 | 6454.4 | 5302.4 | 5196.8 |
| Treated | 84472 | 896.03 | 704.24 | 293.43 | 676.99 | 757.09 | 349.95 | 448.60 | 947.10 | 1121.61 | 373.23 | 608.81 | 687.48 | 536.489 |
| | (4) | (4) | (4) | (4) | (4) | (4) | (10) | (10) | (7) | (7) | (10) | (10) | (10) | (10) |

**Table - III :** Effect of in vitro testosterone treatment on the activity of enzyme GPT in kidney of toad of various concentration of substr ate. Values are units of activity/gm./tissue/hr. (Mean ± SEM) Numbers in $^{P}$arentheses indicate sam$^{P}$le size.

| Condition | Substrate concentrations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.2 rnM | 0.4 mM | 0.6 mM | 0.8 rnM | 1.0 mM | 1.2 mM | 1.4 mM | 1.6 mM | 1.8 mM | 2.0 mM |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | 10771.2 | 12012.8 | 12630.4 | 12236.8 | 11817.6 | 11494.4 | 11292.8 | 10912 | 10633.6 | 10150 |
| | 433.86 | 486.26 | 427.74 | 356.47 | 334.94 | 329.44 | 408.26 | 363.42 | 213.96 | 262.19 |
| | (10) | (10) | (10) | (10) | (10) | (10) | (10) | (10) | (10) | (10) |
| **% Change** | 2.76 | 4.76 | 4.28 | 4.52 | 3.49 | 2.78 | 0.99 | 0.14 | 1.62 | 1.35 |
| **Treated** | 10473.6 | 11440 | 12089.6 | 11683.2 | 11404.8 | 11174.4 | 11180.8 | 10896 | 10460.8 | 10012.8 |
| | 548.60 | 414.85 | 391.61 | 341.89 | 325.39 | 322.24 | 220.99 | 202.65 | 285.80 | 284.32 |
| | (10) | (10) | (10) | (10) | (10) | (10) | (10) | (10) | (10) | (10) |

## SUMMARY OF THE RESULTS

The present work has been under taken to assess the activity profile on the enzyme glutamate pyruvate transaminase (GPT) in the liver and kidney tissues in vivo and the study of enzyme Kinetics in *vitro* following treatment of testosterone.

The results obtained are summarised as follows

(1) Following treatment of testosterone in *vivo* for 5 days, the activity of GPT enzyme decreased significantly in liver tissue of toads when expressed in gm tissue basis.

(2) Similar treatment of testosterone showed a significant decrease in the activity of GPT in Kidney tissue of toads when expressed in gm tissue basis.

(3) Following treatment of testosterone in *vitro* the substrate saturation curve for the enzyme in liver showed an decreased Vmax value in treated tissue as compared to the controlled tissue. However, the K,,, value is same in both treated and controlled

(4) (4) Similar treatment of testosterone to the homogenate mixture, the substrate saturation curve for the enzyme in Kidney showed an decreased $V_{m,,}$ value in treated tissue as compared to the controlled tissue.

(5) Here also the $_{t;v}$ value is same in both treated and controlled tissue showing non-competitive inhibition in both liver and kidney.

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