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IN VITRO EVALUATION OF EFFECT OF THYROXIN ON PROTEIN, TOTAL ATPASE AND ACETYL CHOLINESTERASE ACTIVITIES IN KIDNEY AND LIVER TISSUE OF ALBINO RAT

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ABSTRACT

In vitro evaluation of effect of thyroxin on protein, ATPase and AChE in liver and kidney tissue of rats was investigated. Among the two groups of rats, one group was injected subcutaneously with 200µl of thyroxine / 50g of body weight whereas the other group served as control. The animals were vivisected with anesthesia after intervals of 24, 48, 72 and 96 hours. The activity of ATPase was found to be 0.0012µ mol/gram/minute in the liver of control rats. Further the activity decreased in the rats injected with thyroxine. The values found to be 0.002µ mol/gram/minute in 24hours, 0.0029 in 48hours, 0.0033 in 72hours, 0.003 in 96 hours and 0.0031 in 120 hours of treatment. The ATPase activity in Kidney of control rats was found to be 0.0023µ mol/g/min whereas, in the rats injected with thyroxine, the values found to be 0.0029µ mol/gram/minute in 24hours, 0.003 in 48hours, 0.004 in 72hours, 0.002 in 96 hours and 0.0016 in 120 hours of treatment. The activity of AChE in Liver was found to be 308.39µ mol/gram/minute in the liver of control rats. Further the activity decreased in the rats injected with thyroxine. The values found to be 114.26µ mol/g/min in 24hours, 287µ mol/gram/minute in 48hours, 639.0µ mol/gram/minute in 72hours, 240.0µ mol/gram/minute in 96 hours and 178.3µ mol/gram/minute in 120 hours of treatment. The AChE activity in Kidney of control rats was found to be 393.12µ mol/g/min whereas, in the rats injected with

thyroxine, the values found to be 202.73µ mol/gram/minute in 24hours, 234µ mol/gram/minute in 48hours, 359.6µ mol/gram/minute in 72hours, 371µ mol/gram/min in 96 hours and 208.8µ mol/gram/minute in 120 hours of treatment. The experimental results showed that the Protein, ATPase, AChE activity in the liver and kidney fluctuates in the thyroxine treated rats compared to control.

KEYWORDS: Thyroid, acetyl cholinesterase, ATPase, Kidney, Liver.

INTRODUCTION: Thyroxine (Na) is the major hormone elaborated by the thyroid gland. Chemically it is L-3, 5, 3', 5'-tetraiodothyronine. Its chief function is to increase the rate of cell metabolism. It also essential for the central nervous system maturation and regulates a number of other functions. It mainly used in the treatment of hypothyroidism. Thyroxin is a hormone secreted in thyroid gland which helps in regulating the growth and controls the metabolism in animals. The thyroid hormones are crucial determinants of normal development and metabolism, especially in the central nervous system. Lack of thyroxin causes decrease in the metabolism of all cells that is clearly visible by a decrease in nucleic acid and protein synthesis. The studies revealed that thyroid hormone enhances diastolic relaxation in the heart accompanied by an increased Ca pumping ability in the sarcoplasmic reticulum [1, 2]. Thyroid hormones were found to stimulate incorporation with an ATP generating system (ATP, phosphoenol pyruvate, and pyruvate kinase) while increasing ATPase activity approximately 100% [3].Total TH concentration in serum is normally kept at a level proportional to the concentration of carrier proteins, and appropriate to maintain a constant free hormone level. Most carrier protein dependent alterations in total hormone concentration in serum are due to quantitative changes in the hormone- binding proteins and less commonly to changes in affinity for the hormone. Since a wide fluctuation in the concentration of TH carrier proteins does not alter the hormonal economy or metabolic status of the subject [4], their function is open to speculation. In blood, thyroxine (T4) and triiodothyronine (T3) circulate bound to thyroid hormone binding proteins (THBP) viz., thyroxine binding globulin (TBG), albumin and tranthyretin (TTR). The bound thyroid hormones (bTH) serve as an extrathyroidal reservoir of the hormones ensuring that an uninterrupted supply of thyroid hormones is available to various target tissues of the body [5,6,7]. Changes in thyroid state have been well documented to affect the sensitivity of the heart to Cardiac glyco- sides. At the cellular level, thyroid hormone has been shown to increase Na, K-TPase activity and the number of Na, K- ATPase sites in several types of tissue, including

heart cells. Thyroid hormones are involved in regulating AChE activity in neural cells: AChE activity is decreased in the brains of neonate thyroidectomized rats [8], and triiodo-L - thyronine (T3) increases AChE activity in primary neuronal cultures initiated from fetal rat brains [9]. Hence in the present study, the effect of different concentration of thyroxin on protein ATP and AChE was investigated at duration of time viz., 24, 48, 72 and 96 and 120 hours of treatment.

MATERIALS AND METHODS

Test Animal: Male Rattus Norwegicus (Albino rats) rats weighing around 150 grams at the age of one month old were used in this study. The animals were collected from GYAN research foundation, Bangalore, and housed in polypropylene cages under hygienic conditions and feedings were done using rat pellet diet (Hindustan Lever Limited) and water *ad libitum*.

Chemicals Used: Enzymes ATP and AChE were obtained from Hi-Media, Commercially available analytical grade Tris base, Sucrose, EDTA etc were obtained from Hi-Media laboratories Pvt. Ltd., India, Folinceocalteau reagent (FCR) were procured from Merck India, Pvt. Ltd.

Treatment of rats with thyroxine: The 28-30 days age albino rats, Rattus Norwegicus were collected and they were divided into two groups. Each group consists of ten test rats. One group was injected subcutaneously with 200µl of thyroxine whereas the other group of rats without any treatment of thyroxine served as control. All the rats were vivisected with anesthesia after intervals of 24, 48, 72, 96 and 120 hours respectively. After the treatment of rats with thyroxin and anesthesia, the liver and kidney tissues were removed and subjected for the analysis of Protein content, total ATPase and AChE (A-C) [10].

Estimation of Protein: Liver and kidney were removed from treated rats and stored in 0.9% saline. 2.0% tissue homogenates was prepared from liver and kidney tissue in 0.13M Tris-Sucrose buffer (pH 7.4). 0.2 ml of tissue homogenate was mixed with 5ml of copper sulphate reagent which was incubated for 10 minutes and 1:2 Folin-Phenol reagent which was incubated for 15 minutes. After the treatment, the optical density was read at 660nm against the blank [11].

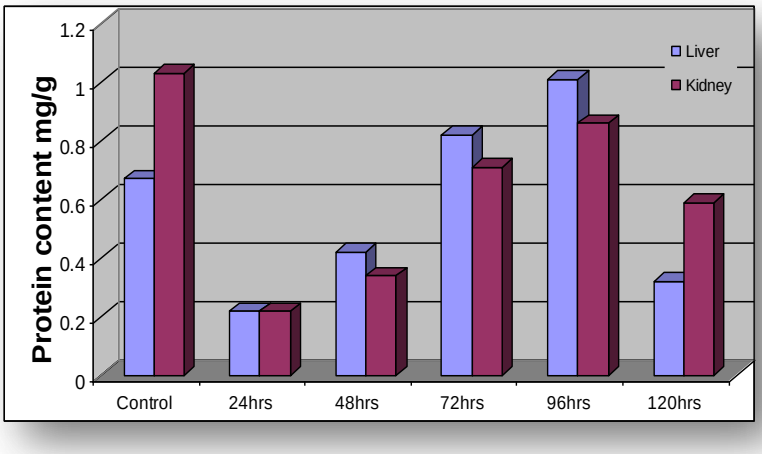
Estimation of Total ATPase: 2.0 % tissue homogenate (w/v) of liver and kidney tissues was prepared separately in 0.2 molar ice cold sucrose solution containing 5 mM EDTA and 0.01M imidazole. The obtained homogenate was centrifuged at 3000 rpm for 10 minutes and supernatant was taken which is crude enzyme and used for the assay of ATPase and its activity. The activity of ATPase is expressed as µM pi liberated per milligram of protein per hour [12].

Estimation of Total AChE: 2.0 % tissue homogenate (w/v) was prepared in phosphate buffer solution. The obtained homogenate was centrifuge for 2 minutes at 3000 rpm. The supernatant was collected and 1 ml of supernatant was taken and added with 1 ml of buffer substrate, mixed well and incubated at room temperature for 2 minutes. 2 ml of alkaline hydroxylamine hydrochloride and 1 ml 4N HCl, 1ml ferric chloride solution was added to homogenate. The optical density of the treated homogenate was read at 440 nm using blank. The values are expressed in µm/mg protein per hour [13].

RESULTS

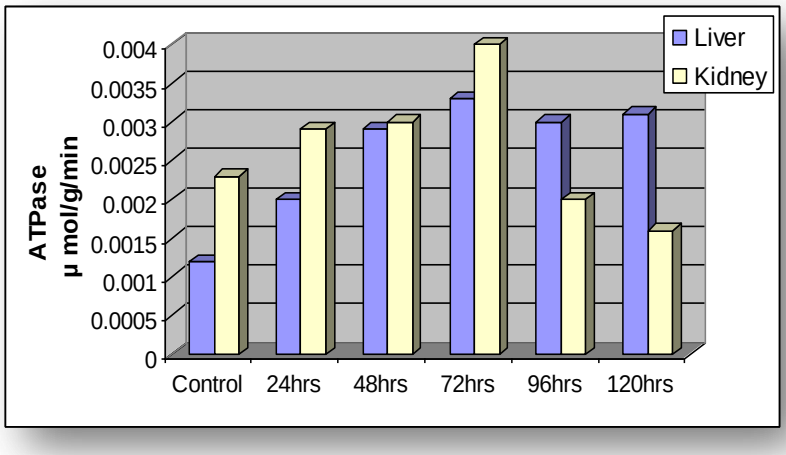
Estimation of Protein: The protein content in control rat was found to be 0.67 mg per gram wet weight in liver tissue. When the rats are injected with thyroxine, the total protein content was 0.22mg/gram in 24 hours, 0.42 mg/gram in 48 hours, 0.82mg/gram in 72 hours, 1.01mg/grams in 96 hours and 0.32mg/grams in 120 hours respectively. In kidney tissue, the protein percentage was 1.03mg/gram in control. In treated rats the protein content was 0.22mg/gram in 24hours, 0.34mg/gram in 48 hours, 0.71mg/gram in 72 hours, 0.86 mg/gram in 96 hours and 0.59mg/gram in 120 hour respectively (Figure 1).

Figure 1: Effect of Thyroxine on protein content of liver and kidney tissue of rats.



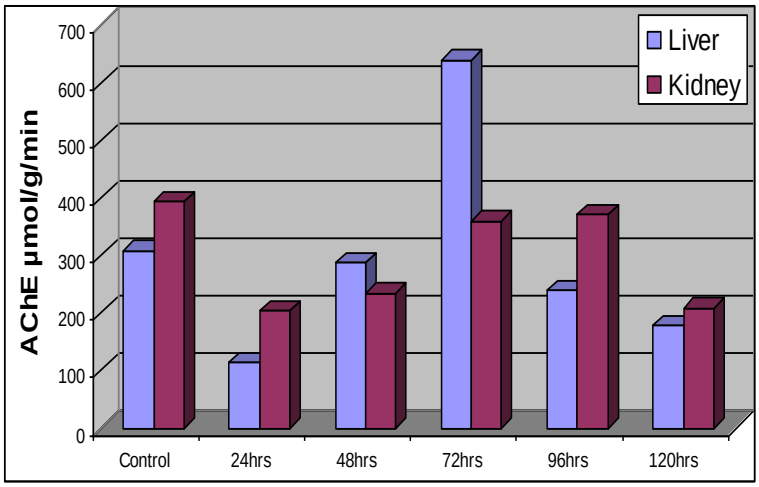
Estimation of Total ATPase: The activity of ATPase was 0.0012µ mol/gram /minute in the liver tissue of control rats. In thyroxine treated rats , the activity of ATPase was decreased and recorded 0.002 µ mol/gram/minute in 24 hours, 0.0029 µ mol/gram/minute in 48hour , 0.0033 µ mol/gram/minute in 72 hour, 0.003 µ mol/gram/minute in 96 hour and 0.0031 µ mol/gram/minute in 120 hours of treatment. In Kidney tissue, the activity of ATPase was 0.0023µ mol/gram/minute in control. In treated rats, the ATPase content was 0.0029µ mol/gram/minute in 24 hours, 0.003 in 48hours, 0.004 in 72hours, 0.002 in 96 hours and 0.0016 in 120 hours of treatment (Figure 2).

Figure 2: Effect of Thyroxine on ATPase content of liver and kidney tissue of rats.



Estimation of Total Ache: The activity of AChE in control rat was 308.39µ mol/gram/minute in the liver. The activity decreased in the rats injected with thyroxine and recorded 114.26µ mol/gram/minute in 24 hours, 287µ mol in 48hours, 639.0µ mol in 72hours, 240.0µ mol in 96 hours and 178.3µ mol/gram/minute in 120 hours of treatment. The AChE activity in Kidney of control rats was found to be 393.12µ mol whereas, in the rats injected with thyroxine, the activity was 202.73µmol in 24hours, 234µ mol in 48hours, 359.6µmol in 72 hours, 371µmol in 96 hours and 208.8µmol/gram/minute in 120 hours respectively (Figure 3).

Figure 3: Effect of Thyroxine on AChE content of liver and kidney tissue of rats.



DISCUSSION

Proteins are heterogeneous group of macromolecules having diverse physiological functions.They are colloidal in nature, not diffusible and contain high molecular weight. When denatured the protein loses its biological nature. Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in every process within cells. Proteins play an important role in the body construction and cell metabolism. It serves as structural components as biocatalysts, as hormones, as depositors for the characteristic genetic information of the species. Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins also have structural or mechanical functions. ATPases are a class of enzymes that catalyze the decomposition of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a free phosphateion. This dephosphorylating reaction releases energy, which the enzyme (in most cases) harnesses to drive other chemical reactions .The presence of ATPase was first demonstrated in the crab's myelinated nerves, which are activated by sodium, magnesium and potassium ions. Some such enzymes are integral membrane proteins (anchored within biological membranes), and move solutes across the membrane. (These are called transmembrane ATPases). Transmembrane ATPases imports many of the metabolites necessary for cellmetabolism and export toxins, wastes, and solutes that can hinder cellular processes. An important example is the sodium-potassium exchanger (or Na+/K+ATPase), which establishes the ionic concentration balance that maintains the cell potential. Another example is the hydrogen potassium ATPase (H+/K+ATPase or gastric proton pump) that acidifies the contents of the stomach. AChE is an enzyme that is essential for the normal functioning of the central and peripheral nervous system and is widely distributed in the

neural and non-neural tissues. The thyroid hormones (THs) are crucial determinants of normal development and metabolism, especially in the central nervous system. Acetyl cholinesterase in one of the hydrolytic enzyme that modulate the amount of neurotransmitter substance acetylcholine at neuron junctions [2].

CONCLUSION

From the above experiment it can be concluded that, the treatment of thyroxine to liver and kidney issue in rats has effect in protein, ATPase and AChE. The dose response of kidney and liver was considered to be more effect on the changes in cellular disturbances. The percentage of protein, ATPase and AChE is completely varied in dose of treatment and found in decreasing the components. Hence from the present observation, it can be concluded that a thorough investigation is necessary to identify the thyroid concentration which is responsible for decrease and increase in protein, ATPase and AChE activity.

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