ORIGINAL RESEARCH PAPER

Physiology

KEY WORDS: Thyroxine,Na+-K+-ATPase

DOSE DEPENDENT REGULATION OF THYROXINE ON Na+-K+-ATPase ACTIVITY IN INDIAN TOAD

Shradhanjali Prusty Berhampur university,Berhampur, Odisha.

Bibhupada Mahapatra* MKCG Medical College,Berhampur,Odisha. *Corresponding Author

Gitanjali Mishra Berhampur university,Berhampur, Odisha.

ABSTRACT

Of the endocrine glands in the body, the important gland is thyroid gland. Because of maintenance of constancy of the internal environment. This gland secretes T_3 and T_4 (thyroxine). This hormone is amino acid derived hormone, they are solubilised and transported in the plasma by specific carrier protein and exert their action intracellularly after penetrating the plasma membrane of the target cell. It is shown that thyroid hormones stimulate Na^+-K^+-ATPase of plasma membrane to bring about an enhanced substrate influx and thus accelerate the rate of oxygen consumption in tissues. The present study aims to find out the effect of thyroxine on Na^+-K^+-ATPase activity in liver of Indian-toad; *Bufo melanostictus.* The total protein content in liver homogenate of thyroxine treated toads did not show any significant change at the dose of 0.5µg/gm but increased significantly($P<0.01$) at the dose of 2 µg/gm body tissue wet wt/hr.

INTRODUCTION

Integration and coordination requires for survival of multicellular organism. The requirements are fulfilled by a form of intercellular communication in which chemical signals (messengers) released by one cell evoke a receptor mediated response in another. There are 2 types of chemical messengers (a) Hormones (b) neurotransmitters.

Hormones are the chemical substance usually defined as messengers that are secreted by various endocrine glands, released into interstitial space and transported into the bold to various target organs where they regulate a variety of physiological and metabolic activities in vertebrate body. Neurohormones convey signals from one neuron to another and reach their target sites, travel within a very short distance.

The characteristics of the endocrine system are that a balance state of feedback regulation is normally maintained among the various glands. They are known to act at different levels in biological regulatory mechanisms involved in homeostasis. Homeostasis signifies maintenance of dynamic equilibrium of milieu interior at a particular physiological range. Hormones are secreted from endocrine glands. It plays vital roles both anabolic and catabolic in the metabolic processes of animals.

1.Of the endocrine glands in the body, the important gland is thyroid gland. Because of maintenance of constancy of the internal environment. This gland secretes T_3 and T_4 (thyroxine). This hormone is amino acid derived hormone, they are solubilised and transported in the plasma by specific carrier protein and exert their action intracellularly after penetrating the plasma membrane of the target cell.

Physiological Role of Thyroid Hormone:

The thyroid hormones, Thyroxine (T_4) and Triiodothyronine (T_3) which appeared only in vertebrates during the course of biochemical evolution has attracted the attention and fascination of biologist and medical scientists alike since a long for their involvement primarily in the control of cell multiplication (Rudland and Jumeneez, 1976; Gibson *et al.,* 1978). Although principally anabolic in nature, they have long been considered to have regulatory influences on almost all the metabolic processes. Nevertheless, the exact mechanism of their action at subcellular and molecular level has been a subject of intensive debate in recent years. Chemically, they are iododerivatives of amino acid tyrosine and are relatively hydrophobic and lipophilic. They are solubilised and transported in the plasma by specific carrier proteins (TBP), which hold them partly hidden in hydrophobic pockets of the peptide chain. They have long half lives and exert their action intracellularly after penetrating the plasma membrane of the target cells (Kochupillai and Ramalingaswama, 1980). A number of classical models have been proposed for a complete or partial explanation of their mechanism of action.

(a)The nuclear transcription model proposes binding of thyroid hormones to high affinity, low capacity effector loci in the nuclear chromatin to elicit an increased transcription rate that expresses itself in the translation and synthesis of specific cytoplasmic proteins possibly some enzymes, which bring about the altered metabolic rate following thyroid hormone action. Thyroid hormones act at many sites within the cell (Davis and Davis, 1997) and their major influence is at the genomic level via a group of nuclear receptors, which regulate the expression of numerous target genes (Green and Chambon, 1986; Munoz and Bernal, 1997).

(b)The mitochondrial activation model proposes mitochondria as the most appropriate subcelluar organelles or thyroid hormone action. These organelles are considered to be the logical site examine or the action. These organelles are considered to be the logical site to examine or the action of thyroid hormones for their crucial role in energy metabolism and calorignenesis. Strong suggestive evidence for mitochondria as the site of thyroid hormone action has recently been obtained in the demonstration of high affinity, saturable T_3 binding receptors in the inner mitochondrial membrane. The discovery of such receptor in and around the site for oxidative phosphorylation gives greater credibility to this model. It is also interesting the these mitochondrial T_3-receptors have been detected only in organ such as liver, kidney and skeletal muscles that are quite responsive to T_3 but not in organs like adult brain that are unresponsive to this hormone. It is suggested that T_3 but not in organs like adult brain that are unresponsive to this hormone. It is suggested that T_3binds to specific integral inner lipoprotein macromolecules in the mitochondrial inner membrane to induce a conformation change so as to enhance oxidative phosphorylation.

(c)Being iodo-derivatives of tyrosine, these hormones may enter into the metabolic pathway of this amino acid to bring about important modification in the metabolism of catecholamine and proteins. It has been demonstrated, for example, that both T_3 and T_4get themselves incorporated to short lived proteins of developing organisms and such incorporation of halogenated amino acid led to an increased turnover rate of such proteins with secondary stimulation of protein synthesis. Likewise, entry of thyroid hormones into catecholamine biosynthesis pathways may well lead to the production of analogous andrenergic neurohormones leading to sympathetic hyperfunction.

(d) It is shown that thyroid hormones stimulate Na^+-K^+-ATPase of plasma membrane to bring about an enhanced substrate influx and thus accelerate the rate of oxygen consumption in tissues. Since normally, about 40% of the oxygen consumption of the body is utilized for energy dependant ionic fluxes across the plasma membrane mediated by this enzyme, this may well be considered to a final common pathway for all the expenditures in response to thyroid hormone action. The thyroid hormone induced increment Na^+-K^+-ATPase activity seems to be an increase in the number of enzyme molecules on the cell membrane. Precisely why, the nuclear transcription may well be the underlying mechanism for this action of thyroid hormones.

Dayton *et al.,* (1960) reported an increased in fatty acid synthesis in ratz fed with tyroxine powder. Thyroid hormone are reported to have stimulatory effects on nucleic acids synthesis in mammals (Tata, 1964; Frieden and Just, 1970; Atkinson *et al.,* 1972; Kohl, 1972). Immersion of fishes in thyroxine solution for 30 days increased the protein and RNA content in the liver and muscle but no change was observed in their DNA content (Ray and Medda, 1977). Similar observation was made by Matty *et al.,* (1982) that a single injection of T_3 and T_4 induced an increase in the RNA content of the liver and muscle but no change of DNA content.

ATPases are a category of transport enzymes having a common characteristic function of catalyzing the hydrolysis of cytosolic ATP to ADP and inorganic phosphate and utilizing the free energy change of this reaction for the transport of ions and molecules against an electrochemical gradient across the membrane in which they are located. Thus ATPases are integral membrane protein. Their enzymatic activity leads to the transport of ions in a certain direction across a membrane.

AIM OF THE PRESENT STUDY:

The effect of thyroxine on metabolism in general and that of energy metabolism in particular, are widely studied. Some works has also been done on the effect of Na^+-K^+-ATPase in amphibians, especially frog. Amphibians differentiate themselves from other vertebrates in having a dual mode of life style. These organisms by combination of may unique morphological structures, physiological adaptation and behavioural responses have made themselves well suited to nearly all terrestrial habits. However the involvement of hormones in eliciting physiological and compensatory adaptation in amphibians is yet to be clearly understood. Thapliyal and his associates (Thapliyal and Gupta, 1984) predicted the involvement of hormone thyroxine, pituitary-thyroid-adrenal axis in mediating the endogenous heat production in reptiles.

Na^+-K^+-ATPase enhances substrates influx in plasma membrane of target tissue. Since normally about 40% of the oxygen consumption of the body is utilized for energy dependent ionic fluxes across plasma membrane mediated by Na^+-K^+-ATPase.

In view of the above, the present study aims to find out the effect of thyroxine on Na^+-K^+-ATPase activity in liver of Indian-toad; *Bufo melanostictus.*

MATERIALS AND METHODS

(A)ANIMAL:

Adult *Bufo melanostictus,* the common Indian toad of mixed sex (body wt. 18-76 gm and sv length range 4-8 cm) were collected in and around Berhampur locality (19° 19 30˝ N, 84° 47 45 E, H+ 12.8 meter from mean level, near Bay of Bengal) during February, March of 2013 to July 2013 for the present study. These animals belong to class-Amphibia, subclass-Anura, order-Procoela and family-Bufonidae.

PREPARATION OF HOMOGENATES:

A 2% homogenate was prepared from liver tissue in 0.25M Surcrose solution with a REMI Homogenizer (Type -15-6-3.1) at a speed of 2000rpm for one minute. This homogenate was used for assay of enzyme activity and also for estimation of inorganic phosphate content.

EXPERIMENTAL DESIGN:

After laboratory acclimation, two animals approximately of same sizes were taken at a time for experimental purpose. Then their body weights were measure. One of the animal was injected intra-muscularly with NaCl(0.65%) at a dose of 0.5µg/gm body weight and 2µg/gm body tissue weight marked as controlled animals.

The experimental set of animals were injected intramuscularly with the same dose of thyroxine at a dose of 0.5 µg/gm and 2µg/gm body tissue weight marked as the treated animals. The time of injection in both the cases were noted down. Both the control and treated animals were kept in laboratory condition for three hours and then sacrified for measurement of biochemical parameters and enzyme activity.

COLLECTION OF TISSUES:

The animals were pithed by piercing a pointed needle just posterior to occipital region. Immediately the liver was dissected out and transferred to a petridish containing ice cold amphibian ringer solution (KCl – 140mg, NaCl-6.5gm, $CaCl_2$ – 120 mg, $NahCo_3$ – 100 mg.litre) p^H 7.4. The adherent connective tissue, blood vessels and nerve fibres were removed. Then the liver was quickly soaked by Whatman filter paper No.40 and weighed. After that it was taken for biochemical estimation.

PREPARATION OF TISSUE EXTRACT

To obtain 2% homogenate:

The weighed liver was homogenized with required volume of 0.25M cold sucrose solution with a REMI Homogenizer (Type RQ – 127A) at a speed of 2000 rpm for 1-2 minutes keeping the homogenizing tube inside a beaker containing ice. The homogenate was immediately transferred to pre-cooled centrifuged tubes and centrifuged at 2000 rpm for 10 minutes in SORVALI RC-5B refrigerated super speed centrifuge maintained at 0°c - 4°c. The supernatant (tissue extract) was taken for the assay of Na^+-K^+-ATPase activity.

The estimation of Na^+-K^+-ATPase activity was done as follows.

The supernatant was taken for estimation of liberated ip following the method of sFiske and Subbarow (1925). The optical densities were taken at 730nm in a spectrophotometer (Elico-Model CL-27).

Estimation of total protein in homogenate:

The protein from 0.1ml of the homogenate was precipitated by addition of 1 ml of cold 10% TCA. The residue obtained after centrifugation was dissolved in 5ml of 0.1N NaOH (E Merck) from which 1ml solution was processed for estimation of total protein content following Lowry *et al*., (1951) and the total protein was expressed as mg protein/gm tissue wet weight.

RESULT

***In vivo* effect thyroxine on enzyme activity:**

In vivo effect of Thyroxine treatment for 3hrs at the dose of 0.5µg T_4/gm body weight did not induce any change in the activity of the enzyme Na^+-K^+-ATPase expressed in µg ip released/gm tissue wet wt./hr.

Further, the enzyme activities increased significantly ($P<0.001$) at the dose of 2µg T_4/gm body wt. in liver homogenate of toads (table-1).

The thyroxine did not show any appreciable change in the total protein content in liver homogenate of toads at the dose of 0.5µgm/gm body wt. However, the total protein content increased significantly ($P<0.01$) at the dose of 2µg/gm body wt. (Table-1).

Table – 1 :*In vivo effect* of thyroxine (0.5 µg.gm body wet weight) and (2µg.gm body wet weight) on Na^+-K^+-ATPase activity (µg ip/gm body weight) and total protein content in liver of Indian toads, *Bufo melanostictus.* Values for total protein content are mg protein /gm tissue wet wt; Mean ± SEM. Numbers in the parentheses indicate sample size NS not significant at 0.05 confidence level.

Experimental condition	Dose-I (0.5µgT4/gm body)		Dose-II (2µgT4/gm body wt)	
	Na+-K+-ATPase (µg ip released/gm tissue wet wt.)	mg protein/gm tissue wet wt.	Na+-K+-ATPase (µg ip released/gm tissue wet wt.)	mg protein/gm tissue wet wt.
Control	11047.85±499.912 (10)	62.16±2.18 (10)	11363.702±492.646 (10)	63.212±2.76 (10)
P	NS	NS	<0.001	<0.01
Thyroxine Treated	11048±494.035 (10)	64.58±3.08 (10)	16901.63±477.582 (10)	67.381±2.53
% Change	0.007	2.992	48.733	6.5

SUMMARY OF RESULTS

In vivo thyroxine treatment at different doses for 3 hrs showed the alteration in activity of enzyme Na+-K+-ATPase as follows.Their dose changed the body physiology and suitably alters precise biochemical pathways. The results obtained in the present investigation are summarized below:

1) 3 hr thyroxine treatment at the dose of 0.5µg/gm body wt./hr did not induce the enzyme activity in toad.
2) 3 hr thyroxine treatment at the dose of 2µg/gm body wt/hr induced a significant rise($p<0.001$) in the activity of Na+-K+ATPase in liver tissue.
3) The total protein content in liver homogenate of thyroxine treated toads did not show any significant change at the dose of 0.5µg/gm but increased significantly($P<0.01$) at the dose of 2 µg/gm body tissue wet wt/hr.

REFERENCES

1. Atkinson, BG.; Atkinson,K.H.; Just, J.J. and Frieden,E.(1972): DNA synthesis in Rana castesbiana tadpole liver during spontaneous and triiodothyronine-induced metamorphosis. Develop. Biol., Vol.29,p.162-175.
2. Barrington, E.J.W.;Barren,N.S. and Piggins,D.T.(1961): The influence of thyroid powder and thyroxine upon the growth of rainbow trout(Salmo gairdnerii). Gen. Comp.Endocrinol., Vol. 1, p. 170-178.
3. Chin, W.W. and Yen, P.M. (1997): Molecular mechanism of nuclear thyroid hormone action. In: Diseases of Thyroid,(Ed.L.C. Bravermann), Totowa, New Jercey: Humana Press,p.1-15.
4. Dauncey, M.J.(1990):Thyroid hormones and thermogenesis.Proceedings of the nutrition society., Vol.49,p.203-215.
5. Davis,P.J. and Davis, F.B.(1997): Nongenomic actions of thyroid hormone. InDiseses of thyroid, (Ed.L.Braverman), Totowa, New Jersey: Humana Press,p.17-34.
6. Dayton,S.; Dayton, J.; Drimmer, F. And Kendall, F.E. (1960):Am.J. Phisiol., Vol. 199, p.71.
7. De Leo, T.; Di Meo, S.; Barletta, A.; Martino, G. And Goglia, F. (1976): Modification of nucleic acid per mitochondria induced by thyroidectomy or triiodothronine administration. Pfluegers Arch. Eur. J.Physiol., Vol.366,p.73-78
8. Donati,K.M.; Warnecke, M.A. and Gallagher, N.I.(1964): Proc. Soc. Exp.Biol. Med.,p. 115-405.
9. Flaim, K.E.; Li, J.B. and Jafferson, L.S. (1978): Effect of thyroxine on hyperthyroidism and thyroxine on net synthesis of plasma proteins by isolated perfused rat liver.J.Biol.Chem.,Vol.248,p.4716.
10. Frieden, E. And Just, J.J.(1970): Hormonal responses in amphibian metamorphosis.In: Biochemical action of hormones.(Ed.G,Litwack), Academic press,New York, vol. I,p.2.
11. Gibson,K.D; Ben-Porath, E.; Dollaer, H.J. and Seger, B.J. (1978): Influence of thyroid hormones on on growth and growth related processes. In growth factors(ED. N. Kastrup), pergamon press, Oxford, p.101.
12. Green, S. And Chambon, P. (1986): A superfamily of potentially oncogenic hormone receptors. Nature., Vol. 234, p. 615-617.
13. Kochupillai, N. And Ramalingaswami, V. (1980): The thyroid gland. In: Textbook of Biochemistry and Human Biology(Ed. G.P. Talwar), Prentice Hall of India,New Delhi, p.724.
14. Kohl H.(1972): Depressed RNA synthesis in the brains and livers of thyroidectomised, normal and hormone injected rats. Brain res., vol.40,p.445-458.
15. Konecka,A.M. and Hanna,M.(1981): Effect of L-thyroxine on metabolism in Japanese quail(coturnix coturnix japonica). 1. Glycolytic enzymes activity in liver after multiple injections of L- thyroxine. Comp.Biochem. physiol.B comp. Biochem., vol.69,p.307-310.
16. Lowry, O.H., Rosebrough, N.J. Farr, A.L. and Randall, R.J.(1951): protein measurement with Folin-phenil reagent. J. Biol. Chem., Vol. 193, p.265-275.
17. Matty, A.J.; Choudhury, M.A. and Lone, K.P.(1982): The effect of thyroid hormones and temperature an protein and nucleic acid contents of liver and muscle of Sarotherodon mossambica. Gen.Comp. Endocrinol., Vol.47,p.497.
18. Munoz, A. And Bernal, J.(1997): Biological activities of thyroid hormone receptors. European J. Endocrinol., Vol. 137,p.433-445.
19. Muscat, G.E.; Downes, M. And Dow han, D.H.(1995): Regulation of vertebrate muscle differentiation by thyroid hormone: the role of the myoD gene family. Bio Essays., Vol. 17,p 211-218.
20. Oppenheimer, J.H.;Schwarthz, H.L.; Mariash, C.N.; Kinlaw, W.B.; Wong, N.C. and Freak, H.C.(1987):Advances in our understandingof thyroid hormone action at cellular level.Endocrine Rev., Vol. 8,p. 288-308.
21. Peschle, C.; Larjani, E.D.; Gidari, A.S.; Mc Lanvin, W. D. And Gordon, A.S.(1971): Endocrinology.,vol.89,p.609.
22. Ray, A.K. and Medda, A.K. (1977): changes in nitrogen and nuclic acid metabolism in lata fish(Ophicephalus punctatus) after thiourea and thyroxine treatment by immersion. Ind. J. Physiol. And Allied sci., Vol.31,p.58.
23. Rudland, P.S. and Jumenez, A.L.de(1976): Action of growth factor in the cell cycle.Biochem.Biophys.Acta., Vol.560,p.91.
24. Shalet, M.; coe,D. And Reissmann,K.R.(1966):Proc.Soc.Biol.Med., Vol.123,p.443.