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EFFECT OF THYROXINE ON FIBRINOLYTIC SYSTEM IN RAT

P. BUBBER[1]*, A. CHAUHAN[2], A. SHARMA[2], N. BUBBER[2] AND D. D. BANSAL[3]

Discipline of [1]Biochemistry,
School of Sciences, Indira Gandhi National Open University,
New Delhi – 110 068

and

Department of [2]Biotechnology,
Banasthali University,
Banasthali, Rajasthan

and

Department of [3]Biochemistry,
Panjab University,
Chandigarh – 160014

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Abstract : Thyroid hormones have many effects on the cardiovascular system. Thyroid dysfunction accelerates atherosclerosis not only through conventional risk factors (dyslipidemia) but they also show a very close relationship with hemodynamic parameters. Thyroxine is determinant of the several components of fibrinolytic system even though the exact relationship is far from clear. Present study was carried out to determine the effect of thyroxine on fibrinolytic parameters such as plasminogen activators (PA) in rat heart, levels of PA and plasminogen activator inhibitor (PAI), glucose in plasma and serum lipid profile. Rats were injected with 50 ug eltroxine/100 gm^{-1} body weight intraperitoneally for one week. Compared with controls, thyroxine treatment increased PA activity significantly in rat heart. No changes were seen in PA, PAI and glucose in plasma of two groups of rats. A significant decrease in total cholesterol and LDL-cholesterol levels was seen in serum of treated group resulting in the decrease of LDL/HDL and Total cholesterol/HDL-cholesterol ratios. These results suggest that thyroxine treatment may have considerable clinical significance. It raised PA activity in heart as well as reduced cholesterol content in blood. It is possible that thyroxine treatment may confer a beneficial effect on cardiovascular risk.

Key words : thyroxine plasminogen activator fibrin and cholesterol

INTRODUCTION

Disturbances of homeostasis are common in patients with thyroid dysfunction. These abnormalities may range from clinical significant coagulopathies and more rarely

*Corresponding Author : Dr. Parvesh Bubber, Associate Professor, Biochemistry, School of Sciences, Indira Gandhi National Open University, New Delhi – 110 068, India; Ph. : 011-29572839 (O); Fax : 011-2957216732 (O); Email: ignou.ac.in, parveshbubber@hotmail.com

major hemorrhagic or thromboembolic complications. Several studies corroborate the strong relationship between low plasma thyroid hormone levels and progression of cardiovascular disease (1). Mayer (2) reported that even mild changes in free thyroxine could influence the degree of heart failure. Both severe hypo and hyperthyroidism may alter hemodynamic parameters. The published data remained confounding and the different results observed are explained by severity of the disease. The mechanisms by which low levels of thyroid hormones may lead to atherosclerosis and its complications or alternatively to a bleeding tendency remain controversial. Indeed, these hormones have multiple effects on cardiovascular system including modification of lipoproteins, effect on myocardium and modification of circulating coagulation parameters and impaired fibrinolytic activity (3-4). Interaction between thyroid disorders and hemostasis seems to be more complex. Fibrinolytic parameters are one of the different factors focused in the pathogenesis of clinically relevant haemostatic abnormalities associated with thyroid dysfunctions.

A primary component of the fibrinolytic system is an inactive zymogen called plasminogen, which, after activation by plasminogen activators (PA) is able to remove fibrin clots (5). Fibrin deposition occurs possibly as a consequence of inefficient fibrinolysis. PA activity in heart has been reported before (6). Tissue plasminogen activator (tPA) is believed to play an important role in local regulation of fibrin deposition in the heart rather than urokinase plasminogen activator (uPA) (7). The aim of the present study is to assess the effect of thyroxine treatment on plasminogen activators in heart tissue and plasma as well as lipid metabolism in serum.

MATERIALS AND METHODS

All experiments involving animals were done in accordance with the guidelines laid down by Animal Ethics Committee Rules and Regulations of the Institute. Young male Wistar rats weighing 100-150 g were obtained from Central Animal House, Panjab University, Chandigarh, India. The animals were randomly distributed into two groups. During the acclimatization, rats were allowed with free access to pellet diet and water. Group 1 (number of animals, N1=5) served as control. Intraperitoneal injections of eltroxine (Glaxo India Ltd., Mumbai) 50 ug/100 gm body weights were given to second group of rats (group 2, number of animals, N2= 5) for one week (8). The rats were fasted overnight before being sacrificed by cervical dislocation. Blood and heart tissues were collected from five rats sampled in each group. All the experiments were replicated thrice.

From one sample of blood, plasma was prepared using sodium citrate as anti-coagulant and plasminogen activators (PA), plasminogen activator inhibitors (PAI) (9) and glucose (10) (standard kit of J. Mitra and Co. Ltd., New Delhi) estimations were carried out in replicate. Another part of blood sample was allowed to clot and used to obtain serum. Total cholesterol (11) in serum was measured by Zlatkis A, et al. 1953. Serum triglycerides (12) and high-density lipoprotein (HDL) cholesterol (13) were measured spectrophotometrically using standard kits of J. Mitra and Co. Ltd., and Ranbaxy

Laboratories Ltd., New Delhi respectively. However, very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol were calculated by Friedwald's (14) formula (VLDL = TG/5 and LDL = TC – (VLDL + HDL). Heart tissue was homogenized in 0.25 molar sucrose using a Potter-Elvejem homogenizer. PA activity was determined in heart homogenates and plasma euglobin fractions (15) immediately after sacrifice.

All assays for determination of plasminogen activator (PA) activity were performed in duplicate in a 96 microwell plate (16) in a total volume of 140 ul. The assay mixture contained plasmin substrate D-Val-Leu-Lys-p-nitoanilide (Chromogenex, Sweden, S-2251) at a final concentration of 37 ug/ml, plasminogen (American Diagnostica Inc. Greenwich, USA) 0.2 IU/ml, heat inactivated liver microsomes (7 ug protein) and 0.1 molar Tricine buffer, pH 8.4. The plate was read at 405 nm at 1 hour intervals using a Titertek Multiscan plate reader. Activity was expressed as international units of standard human tPA. Protein estimation in the test samples was also carried out (17). Statistical analysis of data was done using Student's t test, results were expressed as mean±SD, significance was chosen as $P<0.05$.

RESULTS

The physiological importance of thyroid hormone is well known. PA activity increased significantly ($P<0.01$) in rats on thyroxine treatment (7.98±0.64 IU/mg protein, specific activity) for one week as compared to control group (5.78±0.6 IU/mg protein) as shown in the Fig. 1. Thyroxine treatment did not induce any changes in

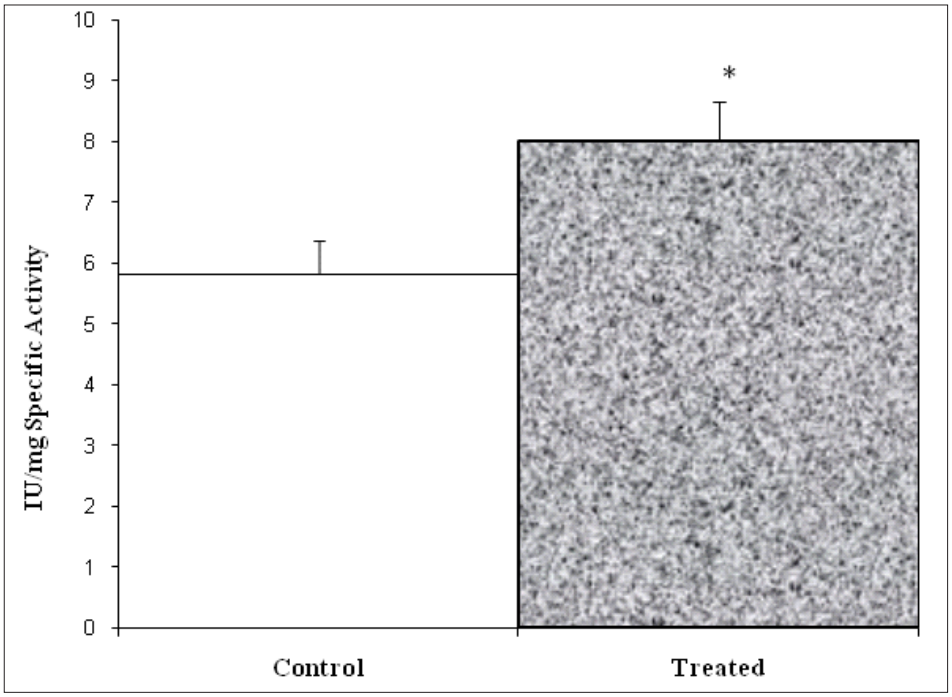

Fig. 1: Effect of thyroxine on plasminogen activator (PA) activity in rat heart. Number of animals in each group was five (replicated thrice). *$P<0.01$.

plasma glucose, PA and PAI in male Wistar rats (Table I). In our studies on lipid metabolism (Table II) a significant (P<0.02) decrease of 20.6% was seen in the total cholesterol content and 34.5% decrease was seen in LDL-Cholesterol content (P<0.05) of thyroxine treated group compared to controls. Triglyceride content was higher but the overall atherogenic index (LDL/HDL) ratio showed a decrease in the treated group.

TABLE I: Comparison of plasma concentrations of plasminogen activators (PA), plasminogen activator inhibitors (PAI), and glucose between control and thyroxine treated rats.

Parameter	*Control*	*Treated*
Glucose (mg/dl)	61.41±8.46	59.99±6.38
PA (IU/ml)	1.04±0.80	1.56±0.66
PAI (AU/ml)	12.02±1.40	12.29±4.04

All the values are Mean±SD. Number of animals N1(control) = N2 (treated) = 5.

TABLE II: Effect of thyroxine on lipid metabolism.

Parameter	*Control*	*Treated*	*%Change*
Total Cholesterol	124±09.79	98.4±11.2	–20.6**
HDL-Cholesterol	44.35±04.34	38.81±03.30	–12.5
Triglycerides	34.47±07.31	43.67±11.30	26.7
VLDL-Cholesterol	06.89±01.46	08.73±02.66	26.7
LDL-Cholesterol	73.21±14.88	47.96±09.62	–34.5*
LDL/HDL Ratio	1.65	1.23	–25.5
Total Cholesterol/ HDL Ratio	2.79	2.53	–9.3

*P<0.05; **P<0.02.

DISCUSSION

Kirichuk (18) experiments on animals also showed that thyroxine treatment promotes release of plasminogen activator activity from heart, kidney and liver. Tissues with highest level of tPA are brain and heart (18). tPA is associated with distinct subpopulation of endothelial cells and its levels can be raised when endothelial cells are exposed to pertinent stimuli (19). The specific stimulus for increased PA activity in heart on thyroxine treatment is not known but it may be due to the enhanced sensitivity of cardiac cells to catecholamines (20). However, a short term elevation of thyroid hormones may result in increase of myocardial contractility and cardiac output whereas long term elevation may again results in heart failure (2).

Many reports on PA and PAI levels in plasma are contradictory. Burgraaf and colleagues found elevated levels of most endothelium-associated proteins in hyperthyroid patients but no evidence of coagulation and fibrinolysis activation (21). However, Erem and his colleagues (22) found out lower levels of tPA and higher levels of PAI-I thereby suggesting reduced fibrinolytic capacity. Other studies found out that balance between tPA and PAI is disturbed in favour of PAI-1 in hyperthyroid patients resulting in impaired fibrinolysis (23-24). Similarly studies of hypothyroidism found low levels of tPA and PAI-1 activities (25) and increased fibrinolytic activity (26) whereas others found the opposite results i.e plasma PAI-activity increased (27-28). Circadian fluctuations and variations in the PAI and tPA levels may also be responsible for the contradictory results (29).

Untreated hypothyroidism is associated with hyperlipidemia specifically with higher levels of total and LDL cholesterol which could have serious cardiovascular consequences (30). Reduction in total cholesterol on thyroxine treatment could be

accounted due to decreased levels of HDL and LDL-cholesterol (31). An enhanced elimination of LDL particles via the LDL receptor pathway has been found to be the reason of lower levels of LDL-cholesterol in hyperthyroid patients (32). Both LDL/HDL ratio and Total Cholesterol/HDL ratio decreased on thyroxine treatment for one week. Our studies support the hypothesis that cardiovascular abnormalities can be corrected by thyroxine therapy in thyroid failure as its treatment affect PA activity and serum total, LDL cholesterol levels modestly.

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