

Effects of hypothyroidism induced by 6-n-propylthiouracil and its reversal by T₃ on rat heart superoxide dismutase, catalase and lipid peroxidation

S Chattopadhyay, G Zaidi, K Das & G B N Chainy*

Departments of Biotechnology & Zoology, Utkal University, Bhubaneswar 751004, India

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The present study critically evaluates the effects of hypothyroid and hyperthyroid states on lipid peroxidation and two enzymes of active oxygen metabolism, namely superoxide dismutase (SOD) and catalase (CAT) in the rat heart mitochondrial and post-mitochondrial fractions. Lipid peroxidation, an index of oxidative stress, was elevated in the heart tissue in hypothyroid state but reduced upon T₃ supplementation. Hyperthyroidism registered increased SOD activity in post-mitochondrial fraction. Mitochondrial SOD activity was reduced in hypothyroid state, which was further reduced by T₃ administration. In contrast, different thyroid states had no effect on catalase activity in the mitochondrial fraction. The hypothyroid state however, significantly augmented catalase activity in post-mitochondrial fraction. The results suggest that the antioxidant defence status of cardiac tissue is well modulated by thyroid hormone.

Keywords: Antioxidant enzymes, Heart, Lipid peroxidation, Mitochondria, 3,3',5-triiodothyronine, Rat

Thyroid disease states exert profound effects on heart and cardiovascular system. Several functional aspects of heart mitochondria are influenced by thyroid hormones e.g., NADH shuttle¹, cardiolipin content and cytochrome oxidase^{2,3}, mitochondrial protein import⁴, oxygen free radical production⁵ etc. In fact, the cardiac functional parameters are considerably an excellent index of the cellular action of thyroid hormones⁶. Since mitochondria plays a cardinal role in the physiology of heart contributing towards its highly active aerobic metabolism, it becomes imperative to elaborate the effects of thyroid hormone on cardiac mitochondrial antioxidant status in particular. There is little information available and mainly limited to quantitation of oxidative stress parameters and antioxidant enzymes primarily in whole tissue homogenates^{7,10} and sonicated supernatants⁹. With this perspective, the present investigation has been designed to study the effects of hypothyroid and hyperthyroid states on levels of lipid peroxidation and two important antioxidant enzymes, superoxide dismutase and catalase in rat heart mitochondrial fraction.

Superoxide dismutase (SOD; EC 1.15.1.1) is the primary antioxidant enzyme followed by glutathione

peroxidase (GPx; 1.11.1.9) and catalase (CAT; 1.11.1.6) for defence against free radicals. However, several reports reiterated the predominance of CAT over GPx in scavenging hydrogen peroxide (H₂O₂) and advocated a strong antioxidant status of rat heart mitochondrial CAT¹¹⁻¹³. This enzyme demands special emphasis since it has not yet been reported in mitochondria of any other tissue except heart¹⁴. To the best of our knowledge, the present work for the first time attempts to highlight the possible effects of thyroid hormones on peroxidative stress in mitochondrial milieu of rat heart with respect to its primary antioxidant capacity. For comparative purpose, the effect of thyroid hormone on antioxidant enzymes of post-mitochondrial fraction has also been investigated.

Materials and Methods

Animal treatment—Adult male Wistar rats obtained from National Institute of Nutrition, Hyderabad, India were used. Clearance was obtained from the Institutional Animal Ethics Committee of the Department for such experiments. Animals were rendered hypothyroid by oral administration of 0.05 % 6-n-propylthiouracil (PTU) in their drinking water for 30 days¹⁵. For thyroid hormone treatment of rats pretreated with PTU, a dose of 20 µg 3,3',5-triiodothyronine (T₃)/100 g body weight dissolved in 0.1 mM NaOH was injected, i.p for 3 consecutive

*Correspondent author

Phone: 91-0674-2587389 (O); 2581503 (R)

Fax: 91-0674-2509037

E-mail: chainyg@sify.com

days¹⁶. Control rats received only the vehicle for the same period. Animals were sacrificed 24 hr after the final administration. The hearts were promptly excised, cleaned in chilled normal saline solution and connective tissues including blood vessels were removed. The hearts were then pat dried, weighed and kept frozen at -80°C till further processing.

Homogenate preparation—A 10% (w/v) tissue homogenate was prepared in ice-cold homogenising buffer containing (mM) mannitol (23), sucrose (70), EDTA (1) and Tris-HCl (10), pH 7.3 as described by Poderoso *et al.*¹⁷. The homogenate was then subjected to differential centrifugation to obtain the mitochondrial fraction¹⁸.

Biochemical analyses—The mitochondrial fraction was freeze-thawed prior to the enzyme assays. Post-mitochondrial and mitochondrial samples (1 ml and 0.2 ml, respectively) were subjected to gel filtration through a 5 ml column of Sephadex G 25 and their respective elutes were used for the estimation of SOD activity¹⁹. The method involves generation of superoxide radical by photoreduction of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride using Greiss reagent at 543 nm. The post-mitochondrial fraction of the heart was used directly while freeze-thawed mitochondrial fraction was used for the assay of CAT according to the modified method of Aebi *et al.*²⁰. Lipid peroxidation level was quantitated in the mitochondrial fraction prepared in 1.15% KCl. The thiobarbituric acid assay was performed according to the method of Ohkawa *et al.*²¹. The concentration of thiobarbituric acid reactive substances (TBARS) was calculated from its extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ (ref. 22). Protein was estimated as per Lowry *et al.*, using bovine serum albumin as standard²³. Total serum T₃ and T₄ concentrations were determined by ELISA kits obtained from Monobind Inc., USA.

Statistics—The data are presented as mean \pm SD. Statistical significance between control and treatment means was determined by one-way analysis of variance followed by Duncan's New Multiple Range Test and a difference was considered significant at $P < 0.05$ level.

Results and Discussion

Serum T₃ concentration decreased significantly in hypothyroid rats in relation to control and increased significantly in the T₃-treated group. Serum T₄ concentration was lowered in hyperthyroid group than control as is typical after T₃ treatments due to negative feedback regulation through the hypothalamo-hypophysial-thyroid axis²⁴. Body weight gain was lowered by 6.45% in hypothyroid rats and by 11.92% in T₃-treated rats as against a 7.7% gain recorded in control animals. The relative heart weight was significantly lowered (18%) in hypothyroid rats in relation to both control and hyperthyroid rats (Table 1). All the changes recorded confirm the effectiveness of treatment. These changes are well consistent with earlier reports on heart^{5,8}.

Since protein content of both mitochondrial and post-mitochondrial fractions of heart was significantly influenced by thyroid state (Table 2), the enzyme activities were expressed both in terms of per mg protein (specific activity) and per g tissue weight (total antioxidant capacity of the tissue) for proper interpretation of the results. Interestingly enough, both in SOD and CAT, when results were expressed as per mg protein and per g tissue wt., the pattern of effect of thyroid hormone on mitochondrial and post-mitochondrial fractions was quite different.

Total dismutation capacity (SOD activity expressed/g tissue wt.) of post-mitochondrial fraction of heart tissue was reduced significantly in response to hypothyroidism but elevated following T₃

Table 1—Total serum T₃, T₄ concentrations, body weight gain and relative heart weight of rats differing in thyroid status

[Values are mean \pm SD of 5-6 animals per group. Figures in parentheses are % increase (+) or decrease (-) over control.]

Parameter	Control	Hypothyroid	Hyperthyroid	
Serum T ₃ (ng/ml)	1.69 \pm 0.19 ^a	0.29 \pm 0.09 ^b	7.55 \pm 0.008 ^c	
Serum T ₄ (ng/ml)	56.60 \pm 12.10 ^a	4.90 \pm 1.07 ^b	23.90 \pm 2.90 ^c	
Body weight (g)	Initial	347 \pm 29.50	387.50 \pm 8.30	293.75 \pm 9.0
	Final	373.75 \pm 27.7 (+7.70 ^a)	362.5 \pm 18.20 (-6.45 ^b)	258.75 \pm 23.80 (-11.92 ^b)
Relative heart weight (mg/100 g bw)	3.41 \pm 0.10 ^a	2.75 \pm 0.09 ^b	3.32 \pm 0.66 ^c	

Data having superscripts of different letters differ significantly ($P < 0.05$) from each other.

administration. On the other hand, hypothyroidism failed to alter the total dismutation capacity of mitochondrial fraction of heart but reduced significantly when challenged by T_3 . When results were expressed as specific activity, one finds no significant change in SOD activity of post-mitochondrial fraction of PTU-treated rats that elevated significantly following T_3 treatment. In contrast, specific activity of mitochondrial SOD reduced significantly following hypothyroidism and reduced further by T_3 treatment (Table 3). Augmentation of SOD in post-mitochondrial fraction by T_3 treatment may be an adaptive response to high T_3 titre. Reduction of mitochondrial SOD following T_3 treatment to hypothyroid rats reflects a negative regulation of the enzyme by the hormone.

Total deperoxidation capacity of post-mitochondrial fraction of the heart of rat did not change in response to either hypo- or hyperthyroid state but elevated significantly in mitochondrial fraction following T_3 administration. On the other hand, specific activity of CAT elevated following hypothyroidism in post-mitochondrial fraction but remained unaltered in mitochondrial fraction. No change was recorded in its specific activity in both the fractions following T_3 treatment (Table 3). Thus, it is apparent that mitochondrial CAT is less sensitive to altered thyroid state of the body than its cytosolic counter-

part. It has been reported that hyperthyroidism induced mitochondrial SOD and lipid peroxide level and decreased cytosolic SOD, GPx and CAT in heart tissue of rats⁹. Similarly, Venditti *et al.*⁷ observed no change in lipid peroxidation level of hypothyroid rats but an elevated level of lipid peroxidation in hyperthyroid rats. The discrepancy in present work and results obtained thus far by other laboratories regarding hypo- and hyperthyroid state on heart LPx and antioxidant enzymes such as SOD and CAT may be due to differences in the experimental design adopted in the present study and that by the earlier workers for measurement of LPx and antioxidant enzymes. While in the present study, LPx was measured in mitochondrial fraction and enzymes in both mitochondrial and post-mitochondrial fractions of the heart, Asayama *et al.*⁹ have estimated LPx and antioxidant enzymes in homogenate and sonicated 13,000 g supernatant, respectively, whereas Venditti *et al.*^{7,8} used homogenate of heart of rats directly for their assay. However, following the same experimental procedure, Das and Chainy¹⁸ reported that hypothyroidism did not influence LPx level in MF of rat liver while T_3 treatment recorded a significant increase. This may be attributed to a tissue-specific response of mitochondria to thyroid status.

The results suggest that any deviation in thyroid state of the body may cause subtle changes in the ratio

Table 2 — Effect of thyroid hormone on protein content of post-mitochondrial fraction (PMF) and mitochondrial fraction (MF) and lipid peroxidation level in mitochondrial fraction of heart of rats differing in thyroid status

[Values are mean \pm SD of 5-6 animals per group]				
Fraction	Parameter	Control	Hypothyroid	Hyperthyroid
PMF	Protein (mg/g tissue)	28.61 \pm 4.31 ^a	20.72 \pm 3.78 ^b	22.16 \pm 2.77 ^b
MF	Protein (mg/g tissue)	3.65 \pm 0.47 ^a	5.68 \pm 1.76 ^b	5.16 \pm 0.68 ^b
MF	nmol TBARS formed/mg protein	1.18 \pm 0.07 ^a	1.88 \pm 0.42 ^b	1.36 \pm 0.33 ^a

Data having superscripts of different letters differ significantly ($P < 0.05$) from each other.

Table 3 — Effect of thyroid hormone on antioxidant enzymes, SOD and CAT activities in the post-mitochondrial fraction (PMF) and mitochondrial fraction (MF) of heart of rats differing in thyroid status.

[Values are mean \pm SD of 5-6 animals per group]					
Fraction	Parameter	Control	Hypothyroid	Hyperthyroid	
PMF	SOD	Units/mg protein	22.29 \pm 2.69 ^a	18.03 \pm 2.71 ^a	28.76 \pm 8.80 ^b
		Units/g tissue	673.25 \pm 153.77 ^a	386.45 \pm 107.45 ^b	626.21 \pm 278.48 ^a
	CAT	pkat/mg protein	153.30 \pm 35.92 ^a	215.22 \pm 26.92 ^b	156.94 \pm 69.41 ^{ab}
		pkat/g tissue	4414.47 \pm 1356.22 ^a	4417.29 \pm 735.47 ^a	3371.75 \pm 1328.78 ^a
MF	SOD	Units/mg protein	23.58 \pm 3.58 ^a	18.32 \pm 3.35 ^b	8.03 \pm 2.37 ^c
		Units/g tissue	83.65 \pm 19.57 ^a	113.69 \pm 48.73 ^a	41.48 \pm 12.84 ^b
	CAT	pkat/mg protein	47.49 \pm 13.35 ^a	48.59 \pm 17.73 ^a	59.15 \pm 13.17 ^a
		pkat/g tissue	170.90 \pm 42.44 ^a	264.09 \pm 94.11 ^{ab}	311.87 \pm 102.58 ^b

Data having superscripts of different letters differ significantly ($P < 0.05$) from each other.

of SOD and CAT in mitochondria of heart thus causing imbalance in clearance of reactive oxygen species that may push the tissue from normooxidant to prooxidant state.

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