 ISSN 0172–780X **www.nel.edu**

ORIGINAL ARTICLE

Pinealectomy inhibits antioxidant system in rats with hyperthyroidism

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Submitted: March 16, 2004 *Accepted: May 24 , 2004*

Key words: **hyperthyroidism; pinealectomy; brain; liver; heart; rat.**

Neuroendocrinol Lett 2005; **26**(6):795–798 **PMID:** 16380688 NEL260605A24 © Neuroendocrinology Letters **www.nel.edu**

Abstract

OBJECTIVE: Thyroid hormones regulate energy metabolism and act on the mitochondria, which is an important source of free radicals in the cell. Reactive oxygen types play a significant role in physiological mechanisms, but in excessive amounts they can cause oxidative damage in molecules. The aim of the present study was to determine levels of lipid peroxidation caused by induced hyperthyroidism in cerebral, hepatic and cardiac tissues of pinealectomized rats. **METHODS:** Experimental animals used in the study were allocated to three groups as general control group, hyperthyroidism-sham pinealectomy group and hyperthyroidism-pinealectomy group. GSH and MDA levels in cerebral, hepatic and cardiac tissues were evaluated at the end of the 3-week study period. **RESULTS:** It was found that MDA levels in cerebral, hepatic and cardiac tissues were the highest in hyperthyroidism and pinealectomy group and that these values were higher in hyperthyroidism-sham pinealectomy group than in the control group ($p<0.001$). It was seen that tissue GSH levels significantly increased in hyperthyroidism-sham pinealectomy group ($p<0.001$) and that the increase in hyperthyroidism and pinealectomy group was higher than the increase in the control group only ($p<0.001$). **CONCLUSION:** Results of our study show that MDA and GSH levels in cerebral, hepatic and cardiac tissues increased due to hyperthyroidism and that the increase in MDA levels became more evident and GSH levels were significantly suppressed after pinealectomy.

Introduction

Thyroid hormones act on mitochondria by regulating the energy metabolism and mitochondrion is a major source of free radicals in the cell [1]. High concentrations of thyroid hormones affect oxygen metabolism and stimulate formation of free radicals in mitochondria [2]. Reactive oxygen types play an important role in physiological mechanisms, but extremely reactive types also cause oxidative damage in molecules [3]. Reactive oxygen types formed in this manner are toxic for biomembranes and can lead to lipid peroxidation if not swept away from the setting by eliminator mechanisms. Oxygen radicals are destructive for all lipid structures, but cellular destruction is more evident in membranes [4]. It was shown that hyperthyroidism stimulated by L-thyroxin increased sensitivity to lipid peroxidation in female rats and enhanced mitochondrial damage in the heart [5]. It was reported that PTU treatment did not influence oxidative damage in lipids, but reduced mitochondrial DNA damage. Another change brought about by thyroid hormones is renal hypertrophy observed in renal hyperthyroidism [6]. Similar changes are observed in renal cortex and medulla in hyperthyroidism stimulated by PTU

and induced by thyroidectomy [7]. It was reported that renal hypertrophy stimulated by in vivo thyroxin was associated with an increase in mitotic index and similar changes were seen simultaneously in the cardiac tissue [8]. It was stated in previous studies that not only hyperthyroidism, but also hypothyroidism caused alterations in oxidant and antioxidant systems [2, 9]. Besides, it was reported that thyroid hormones reduced low-density lipoprotein oxidation stimulated by in vitro $Cu^{(2+)}$, depending on the differences in molecular structures [10]. It was demonstrated that the hypermetabolic condition in hyperthyroidism was associated with an increase in formation of free radicals and lipid peroxide levels [11–13]. Lipid peroxidation occurs in polyunsaturated lipids and takes part in the direct reaction of oxygen and lipids that are going to constitute forms associated with stable peroxides and free radicals. The resulting lipid peroxidation influences functions of the cardiac muscle in rats with hyperthyroid or hypothyroid [14].

It was demonstrated in various studies that melatonin, which is secreted mainly by the pineal gland, had an antioxidant effect as a free radical eliminator [15–18]. It was reported in a similar study that melatonin decreased lipid peroxidation caused by phenton in thyroid gland [4]. Number of studies investigating how pineal gland and thyroid gland together affect free radical formation is very few. It is seen that studies about the relation between melatonin and thyroid hormone examine the suppression in thyroid hormones due to melatonin administration or the changes in hormone production in hypophysis-thyroid axis due to pinealectomy [19, 20]. Surgical removal of the pineal gland can reduce endogenous melatonin and make cellular damage caused by oxidative changes more marked [21].

The aim of the present study was to determine levels of lipid peroxidation caused by hyperthyroidism in cerebral, hepatic and cardiac tissues of pinealectomized rats.

Materials and Methods

The study was carried out at Selcuk University Experimental Medicine Research and Application Center. Ethics committee of the concerned center approved the study protocol. The study included 30 male rats of Spraque-Dawley species weighing 240–280 g. The experimental animals were fed with standard rat pellet and tap water and kept under 12h/12h dark/light conditions. The rats were divided into three groups as follows:

1-**Intact Control Group (n=10):** The group fed with normal rat pellet and not subjected to any procedure.
2-**Sham Pinealectomy-Hyperthyroidism Group (n=10):** Sham-pinealectomy was performed under general anesthesia and hyperthyroidism was induced with intraperitoneal 0.3 mg/kg/day L-thyroxin administration for 3 weeks.
3-**Pinealectomy-Hyperthyroidism Group (n=10):** Pinealectomy was performed under general anesthesia, as described earlier [22], and hyperthyroidism was induced with intraperitoneal 0.3 mg/kg/day L-thyroxin (Sigma) administration for 3 weeks.

The animals were decapitated at the end of the 3-week study period and brain, liver and heart tissues were collected. The tissues were kept at –80°C until analyses.

Tissue MDA Analysis. The wet weight of the tissue samples at pH 7.4 were measured, then divided into pieces and transferred into tubes and homogenized to about 10% in 150 mM KCl at 4°C using a Misonix's Microscam ultrasonic cell disruptor. The homogenate was added to 2 ml of 8% HClO4 and centrifuged at 3000 rpm for 25 min. To 0.5 ml supernatant, 3 ml of 1% H3PO4 and 1 ml of 0.675% TBA were added and incubated in a 90°C water bath for 45 min. After cooling of mixture, the MDA-TBA complex was extracted with 4 ml of n-butanol and its absorbance was read against an n-butanol blank, as described earlier. The concentration was obtained as c=108.9. The above conversion factor included the extinction coefficient, cell length, and dilution factor and was finally expressed in nmol/g protein [23].

Tissue GSH Analysis. The tissue was homogenized in 150 mM KCl at 4°C as described for MDA, centrifuged at 3000 rpm for 15 min. In the samples, the level of GSH was measured by Ellman's method, as described earlier. Tissue protein (μmol/ g protein) was obtained by Biuret's method [24]. To 200 μL of the supernatant, 8 ml of pH 6.8 phosphate buffer, 78 ml of 1 N NaOH, and 100 μL Ellman's solution were added and read at 412 nm after standing for 5 min. The activity (a) was calculated from a= (A standard/ A samples) X C Standard where C Standard=5.34 g/100 ml.

Statistical analysis. The statistical analysis was performed using SPSS statistical program. The results were expressed as mean ± standard deviation. Kruskal-Wallis analysis of variance was used for comparison between groups and Mann-Whitney U-test was applied to those with $P<0.05$.

Results

MDA levels in the cerebral tissue were 56.20 ± 2.58 in the control group (Group I), 99.93 ± 4.50 in sham pinealectomy-hyperthyroidism group (Group 2) and 121.00 ± 4.80 in pinealectomy-hyperthyroidism group (Group 3). The comparison among groups showed that MDA levels in Group 3 were higher than those in the other two groups and MDA levels in Group 2 were higher than those in Group 1 ($p<0.001$, Table 1). GSH levels in the same tissue were 16.27 ± 0.80 in the control group, 54.27 ± 2.83 in sham-pinealectomy-hyperthyroidism group and 32.38 ± 2.48 in pinealectomy-hyperthyroidism group. It was seen that this parameter was statistically higher in Group 2 than in other groups and was statistically higher in Group 3 than in Group 1 ($p<0.001$, Table 2).

Comparison of MDA levels in hepatic tissue revealed that MDA levels in the control group (58.11 ± 3.91) were lower than those in sham pinealectomy-hyperthyroidism (104.66 ± 4.94) and pinealectomy-hyperthyroidism (122.49 ± 5.65) groups ($p<0.001$, Table 1) and that levels in Group 2 were lower than those in Group 3 ($p<0.001$, Table 1). GSH levels of that tissue were higher in Group 2 (54.86 ± 2.53) than in Groups 1 and 3 (23.22 ± 2.83 and 32.86 ± 3.86, respectively) and were higher in Group 3 than in Group 1 ($p<0.001$, Table 2).

Table 1: MDA Levels in Control and Experimental Groups

Groups	Brain	Liver	Heart
Intact Control Group (Group 1)	56.20 ± 2.58 [c]	58.11 ± 3.91 [c]	61.32 ± 3.12 [c]
Sham Pinealectomy-Hyperthyroidism Group (Group 2)	99.93 ± 4.50 [b]	104.66 ± 4.94 [b]	120.00 ± 4.10 [b]
Pinealectomy-Hyperthyroidism Group (Group 3)	121.00 ± 4.80 [a]	122.49 ± 5.65 [a]	186.60 ± 4.10 [a]

Different letter in same column are significant as statistic (P<0.001), (n=10, each group)
a>b>c

Table 2: GSH Levels in Control and Experimental Groups

Groups	Brain	Liver	Heart
Intact Control Group (Group 1)	16.27 ± 0.80 [c]	23.22 ± 2.83 [c]	8.35 ± 1.86 [c]
Sham Pinealectomy-Hyperthyroidism Group (Group 2)	54.27 ± 2.83 [a]	54.86 ± 2.53 [a]	30.21 ± 1.89 [a]
Pinealectomy-Hyperthyroidism Group (Group 3)	32.38 ± 2.48 [b]	32.86 ± 3.86 [b]	14.34 ± 0.92 [b]

Different letter in same column are significant as statistic (P<0.001), (n=10, each group)
a>b>c

As for cardiac tissue, it was determined that MDA levels in Group 3 (186.60 ± 4.10) were significantly higher than those in Group 1 (61.32 ± 3.12) and Group 2 (120.00 ± 4.10) (p<0.001, Table 1). Similarly, this parameter was found higher in Group 2 than in Group 1 (p<0.001, Table 1). GSH levels in cardiac tissue were 8.35 ± 1.86 in the control group, 30.21 ± 1.89 in sham pinealectomy-hyperthyroidism group and 14.34 ± 0.92 in pinealectomy-hyperthyroidism group. Statistical comparison among groups showed that GSH levels were higher in Group 2 than in the other two groups and in Group 3 than in Group 1 (p<0.001, Table 2).

Discussion

Lipid peroxidation is caused by oxidant changes at tissue and cell levels due to endocrine and pathological conditions of the organism. Two of these endocrine disorders are thyrotoxicosis and hypothyroidism. Oxygen radicals formed because of malfunctioning of the thyroid gland lead to lipid peroxidation by affecting cellular membranes [4]. Various methods are used to determine the level of lipid peroxidation in the organism and the most frequently used method is determination of MDA levels [25]. In this study, changes in MDA levels were found to determine oxidative damage. An overall examination of our study results shows that there were increases in MDA levels of cerebral, hepatic and cardiac tissues due to hyperthyroidism. Pinealectomy in addition to hyperthyroidism made the increase in MDA levels more evident. Parallel to this, while there was an increase in the activity of antioxidant system in hyperthyroidism group, this increase was suppressed by pinealectomy.

When the part of our study about cerebral tissue is examined, it is seen that the increase in MDA levels caused by hyperthyroidism was intensified by pinealectomy. Galkina et al. [26] examined the effect of different thyroxin isomers on the development of free radical oxidation in rat cerebral cortex and reported that the effect of the antioxidant activity of D-thyroxin was about two and a half times more than that of L-thyroxin. Oziol et al. [10] who reported similar findings determined increases in oxidant and antioxidant system activities at varying degrees, depending on the structural differences in thyroid hormones. In our study we used only in vivo L-thyroxin and found increases in both MDA and GSH levels in the cerebral tissue due to hyperthyroidism. Therefore, the increase we observed in oxidant and antioxidant system activities due to hyperthyroidism is parallel to the findings of other researchers [26, 27]. These researchers reported that their results were dependent on dose under in vitro conditions and were obtained with micromolar, not nanomolar doses. Mano et al. [28] studied lipid peroxidation and free radical eliminating mechanisms in cerebral tissues of rats with hyperthyroidism and hypothyroidism and stated that the free radicals formed were compensated for by the eliminating systems. However, in our study, the increase in free radicals caused by hyperthyroidism could not be eliminated by the antioxidant system and additionally, the changes observed in MDA and GSH levels due to hyperthyroidism were affected by pinealectomy. Performing pinealectomy before thyroxin administration increased the level of oxidative damage, while levels of GSH, a marker of antioxidant system activity, were inhibited because of the operation. Other researchers reported a similar inhibition of this system due to pinealectomy, as well [21,29,30]. Our findings are consistent with the findings of other researchers. The decrease in the free radical eliminating effect of melatonin, which is reduced after pinealectomy, brings with it an increase in the oxidative damage caused by hyperthyroidism.

When the part of our study about hepatic tissue is considered, it is observed that the oxidant damage in the hepatic tissue increased due to hyperthyroidism and this increase became more marked after pinealectomy. Previous studies also reported an increase in oxidative damage in hepatic tissue because of hyperthyroidism [31–33]. Thyroid hormones lead to oxidative damage as they increase reactive oxygen types when activating metabolic systems of the body in general [11,13,34]. Similarly, we established an increase in oxidative damage in not only hepatic, but also cerebral and cardiac tissues due to hyperthyroidism. It is possible that the hypermetabolic condition brought about by thyroid hormones increase the amount of free radicals by increasing mitochondrial electron transport. Our study demonstrates that there were augmentations in the oxidant and antioxidant systems at the end of the 3-week study period, but the

oxidative damage caused could not be eliminated by the increase in antioxidant system. It is also seen that although GSH levels in hepatic tissue increased due to hyperthyroidism, pinealectomy inhibited GSH levels and oxidative damage caused in hyperthyroidism became more evident as a result. This finding is parallel to the results obtained by some researchers [17, 30].

Another result of our study is that the oxidant damage in cardiac tissue, which increased in hyperthyroidism, was further aggravated by pinealectomy and that the activity of antioxidant system declined due to the decrease in endogenous melatonin caused by pinealectomy operation. The finding that damage caused by hyperthyroidism led to increases in various tissues like muscle, liver and heart is parallel to the results of other researchers [13,32]. Shinohara et al. [14] established an increase in lipid peroxidation in cardiac muscle due to induced hyperthyroidism. Likewise, Mano T et al. [28] reported that GSH-PX levels decreased and SOD levels increased in hyperthyroidism induced by 4-week L-thyroxin administration. The increase in SOD activity, which has an antioxidant effect, is similar to the increase we obtained in GSH, which is a marker of antioxidant response. When endogenous melatonin decreases due to pinealectomy, antioxidant system activities are inevitably inhibited and as result oxidative damage occurs in tissues. Thus, it seems possible that when thyroid hormones increase in pinealectomy, the increase in lipid peroxidation triggers oxidative damage.

Acknowledgement: This study was supported by Scientific Research Project Council of Selcuk University (SUBAP). Project number is 2002/089.

REFERENCES

1 Goglia F, Silvetri E, Lanni A. Thyroid hormones and mitocondria. Biosci Rep 2002; **22**17–32.
2 Sewerynek E, Wiktorska J, Lewinski A. Effects of melatonin on the oxidative stress induced by thyrotoxicosis in rats. Neuroendocrinol Lett 1999; **20:**157–161.
3 Karbownik M, Lewinski A. The role of oxidative stress in physiological and pathological processes in the thyroid gland; possible involvement in pineal-thyroid interactions. Neuroendocrinol Lett 2003; **24:**293–303.
4 Karbownik M, Lewinski A. Melatonin reduces fenton reaction-induced lipid peroxidation in porcine thyroid tissue. J Cell Biochem 2003; **90:**806–811.
5 Gredilla R, Lopez Torres M, Portero-Otin M, Pamplona R, Barja G. Influence of hyper- and hypothyroidism on lipid peroxidation, unsaturation of phospholipids, glutathione system and oxidative damage to nuclear and mitochondrial DNA in mice skeletal muscle. Mol Cell Biochem 2001; **221:**41–48.
6 Kobori H, Ichihara A, Miyashita Y, Hayashi M, Saruta T. Mechanism of hyperthyroidism-induced renal hypertrophy in rats. J Endocrinol 1998; **159:**9–14.
7 Mogulkoc R, Canpolat L, Baltacı AK, Yılmaz B and Keleştimur H. Histological examination of various tissues in propylthiouracil and thyroidectomy-induced hypothyroidism in the rat. Medical Science Research 1999; **27:**801–805.
8 Lopez-Torres M, Romero M, Barja G. Effect of thyroid hormones on mitochondrial oxygen freee radical production and DNA oxidative damage in the rat heart. Mol Cell Endocrinol 2000; **168:**127–134.
9 Berzinska-Slebodzinska E. Fever induced oxidative stress: the effect of thyroid status and the 5'-monodeiodinase activity, portective role of selenium and vitamin E. J Physiol Pharmacol 2001; **52**275–284.
10 Oziol L, Faure P, Vergely C, et al. In vitro free radical scavenging capacity of thyroid hormones and structural analogues. J Endocrinol 2001; **170:**197–206.
11 Fernandez V, Barrientos X, Kipreos K, Valenzuela A, Videla LA. Superoxide radical generation, NADPH oxidase activity, and cytochrome P-450 content of rat liver microsomal fractions in an experimental hyperthyroid state: relation to lipid peroxidation. Endocrinology 1985; **117:**496–501.
12 Asayama K, Dobashi K, Hayashibe H, Megata Y, Kato K. Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. Endocrinology 1987; **121:**2112–2118.
13 Venditti P, Balestrieri M, Di Meo S, De Leo T. Effect of thyroid state on lipid peroxidation, antioxidant defences, and susceptibility to oxidative stress in rat tissues. J Endocrinol 1997; **155:**151–157.
14 Shinohara R, Mano T, Nagasaka A, et al. Lipid peroxidation levels in rat cardiac muscle are affected by age and thyroid status. J Endocrinol 2000; **164:**97–102.
15 Rapozzi E, Comelli M, Mavelli I, et al. Melatonin and oxidative damage in mice liver induced by the prooxidant antitumor grug, adriamycin. In Vivo 1999; **13:**45–50.
16 Akbulut GK, Gonul B, Akbulut H. Differential effects of pharmacological doses of melatonin on malondialdehyde and glutathione levels in young and old rats. Gerontology 1999; **45:**67–71.
17 Reiter RJ, Tan D, Kim SJ, et al. Augmentation of indices of oxidative damage in life-long melatonin-deficient rats. Mech Ageing Dev 1999; **110:**157–173.
18 Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Czarnocki Z. Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta Biochim Pol 2003; **50:**1129–1146.
19 Baltaci AK, Mogulkoc R,, Bediz CS, Kul A, Uğur A. Pinealectomy and Zinc Deficiency Have Opposite Effects on Thyroid Hormones in Rats. Endocrine Research 2003; **29:**473 – 481.
20 Baltaci AK, Mogulkoc R, Bediz CS, Kul A, Uğur A. Opposite Effects of Zinc and Melatonin on Thyroid Hormones in Rats. Toxicology 2004; **195:**69–75.
21 Reiter RJ, Tan DX, Qi W, Manchester LC, Karbownik M, Calvo JR. Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. Biol Signals Recept 2000; **9:**160–171.
22 Kuszack J, Rodin MA. New technique of pinealectomy for adult rats. Pro Experimentis 1977; **32**283–284.
23 Uchiyama M and Mihara M. Determination of malondyaldehyde precurser in tissues by thiobarbituric acid test. Anal Biochem 1977; **86:**271–278.
24 Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; **82:**70–77.
25 Tsai LY, Lee KT, Tsai SM, Lee SC, Yu HS. Changes of lipid peroxide levels in blood and liver tissue of patients with obstructive jaundice. Clin Chim Acta 1993; **215:**41–50.
26 Galkina V, Prokopenko VM, Putilina FE, Eshchenko ND, Arutyunyan AV. The effects of thyroxine isomers on free-radical oxidation processes in subcellular fractions of rat cerebral cortex. Neurosci Behav Physiol 2001; **31:**463–465.
27 Faure M, Lissi EA, Videla LA. Evaluation of the antioxidant properties of thyroid hormones and propylthiouracil in the brain-homogenate autoxidation system and in the free radical-mediated oxidation of erythrocyte membranes. Chem Biol Interact 1991; **77:**173–185.
28 Mano T, Sinohara R, Sawai Y, et al. Changes in lipid peroxidation and free radical scavengers in the brain of hyper- and hypothyroid aged rats. J Endocrinol. 1995; **147:**361–365.
29 Skaper SD, Floreani M, Ceccon M, Facci L, Giusti P. Excitotoxicity, oxidative stress, and the neuroprotective potential of melatonin. Ann N Y Acad Sci 1999; **890:**107–118.
30 Wajs E, Lewinski A. Inhibitory influence of late afternoon melatonin injections and the counter-inhibitory action of melatonin-containing pellets on thyroid growth process in male Wistar rats: comparison with effects of other indole substances. J Pineal Res 1992; **13:**158–166.
31 Civelek S, Seymen O, Seven A, Yigit G, Hatemi H, Burcak G. Oxidative stress in heart tissue of hyperthyroid and iron supplemented rats. J Toxicol Environ Health A 2001; **64**499–506.
32 Venditti P, De Rosa R, Di Meo S. Effect of thyroid state on susceptibility to oxidants and swelling of mitochondria from rat tissues. Free Radic Biol Med 2003; **35:**485–494.
33 Andican G, Gelisgen R, Civelek S, et al. Oxidative damage to nuclear DNA in hyperthyroid rat liver: inability of vitamin C to prevent the damage. J Toxicol Environ Health A 2004; **67**413–420.
34 Asayama K, Kato K. Oxidative muscular injury and its relevance to hyperthyroidism. Free Radic Biol Med 1990; **8:**293–303.