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ORIGINAL ARTICLE

Hyperthyroidism causes lipid peroxidation in kidney and testis tissues of rats: Protective role of melatonin

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Abstract

OBJECTIVE: The present study aimed at determining how 3-weeks intraperitoneal melatonin administration affected oxidative stress caused by experimental hyperthyroidism. **MATERIALS AND METHODS**: The study was conducted on 30 male rats of Spraque-Dawley species. The experimental animals were divided to 3 groups (control, hyperthyroidism and hyperthyroidism+melatonin). The supplementation was continued for 3 weeks after which the animals were sacrified and tissue malondyaldehyde (MDA) and glutathione (GSH) levels were determined. **RESULTS**: MDA levels in kidney and testis tissues in hyperthyroidism group were higher than those in control and hyperthyroidism+melatonin administered groups ($p<0.001$) and levels in hyperthyroidism + melatonin administered group were higher than those in the control group ($p<0.001$). The highest GSH levels were obtained in hyperthyroidism + melatonin-administered group ($p<0.001$) and GSH levels in hyperthyroidism group were higher than those in the control group ($p<0.001$). **CONCLUSION**: Results of the study demonstrate that hyperthyroidism induced by 3-weeks L-thyroxine administration increased oxidative stress in kidney and testis tissues and that although melatonin administration inhibited this stress to a certain extent, it could not bring the stress down to the level in controls.

Introduction

High concentrations of thyroid hormones act on the oxygen metabolism and stimulate free radical formation in the mitochondria [1]. Reactive oxygen species play an important role in physiological mechanisms, but extremely reactive species can lead to oxidative stress in molecules [2]. Reactive oxygen species formed in that way are toxic for biomembranes and lead to lipid peroxidation if they are not swept away from the medium by necessary eliminating mechanisms. Previous studies reported that the hypermetabolic condition observed in hyperthyroidism was associated with an increase in free radical formation and lipid peroxide levels [3–5]. Lipid peroxidation occurs in polyunsaturated lipids and involved in the direct reaction of oxygen and lipids that are going to produce the forms associated with stable peroxides and free radicals. It was stated in the previous studies that not only hyperthyroidism, but also hypothyroidism led to changes in oxidant and antioxidant systems [1,6]. Pathological disorders in thyroid gland bring about functional changes in different organs of the body. Findings

obtained in both in vivo and in vitro studies point out that thyroid hormones have a strong impact on oxidative stress [2]. By regulating the energy metabolism, thyroid hormones act upon the mitochondria, which comprise the major source of intracellular free radicals [7]. It was shown that hyperthyroidism stimulated by L-thyroxine increased susceptibility to lipid peroxidation in female rats and that mitochondrial stress in cardiac tissue could not be corrected despite increased GSH levels [8]. It was demonstrated in the same study that propylthiouracil (PTU) administration to rats reduced mitochondrial DNA stress. Another change caused by thyroid hormones is renal hypertrophy observed in hyperthyroidism [9]. It was reported that structural impairments were found in renal tissue in case of hypothyroidism stimulated by PTU or induced by thyroidectomy [10]. It was determined that renal hypertrophy was associated with an increase in mitotic index [11]. In another study, it was shown that lipid peroxidation that developed due to either hypothyroidism or hyperthyroidism influenced cardiac tissue functions of rats [12].

It was established in numerous studies that melatonin, which is mainly secreted from the pineal gland in the body, reduced oxidative stress by its free radical eliminating and direct antioxidant effects [13–15]. Previous studies reported that melatonin administration inhibited lipid peroxidation stimulated by fenton in the thyroid gland [2], as well as ischemia-reperfusion injury stimulated by ochratoxin in the liver and kidney [16], by amicacine in the kidney [17] by methotrexate in the hepatorenal tissue [18], experimentally induced ischemia-reperfusion in the testes [19] and testis damage experimentally formed in varicocele [20]. Studies examining the relation between melatonin and thyroid hormones generally focus on the inhibition that melatonin administration causes in thyroid hormones or changes in hormone production in hypophysis-thyroid axis due to pinealectomy [21,22].

Number of studies investigating the stress caused by hyperthyroidism in kidney and testis tissues of rats is fairly limited. The aim of the present study was to determine how experimental hyperthyroidism and melatonin administration affected the level of lipid peroxidation and antioxidant system activity in kidney and testis tissues of 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. This study was carried out on 30 Spraque-Dawley species male rats, aged 8 weeks, weighing 250–300g. The experimental animals were fed with standard rat pellet and tap water and kept under 12h/12h dark/light conditions. The temperature and humidity were controlled at 21°C and 50 ± 5% respectively.

The rats were didived into 3 groups of 10 animals each, as follows:

1 **General Control Group (n=10)**: The group that was fed with standard rat pellet and not subjected to any procedure.
2 **Hyperthyroidism Group (n=10)**: Hyperthyroidism was induced in this group by 0.3 mg/kg/day intraperitoneal L-thyroxine administration for 3 weeks [12].
3 **Hyperthyroidism and Melatonin-Administered Group (n=10)**: The rats were given 0.3 mg/kg/day intraperitoneal L-thyroxine (Sigma) to induce hyperthyroidism and 3 mg/kg/day melatonin (Sigma) for 3 weeks.

At the end of the 3-week study period, animals were sacrificed by decapitation and kidney and testis tissues were collected. The tissues were kept at –80°C until analyses. MDA and GSH levels in the tissues were determined.

Tissue Analyses

Tissue Malondyaldehyde (MDA) Analysis: The wet weight of the tissue samples at pH 7.4 were measured. Tissues were then divided into pieces, 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% $HClO_4$ and centrifuged at 3000 rpm for 25 min. To 0.5 ml supernatant 3 ml of 1% H_3PO_4 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 A. The above conversion factor included the extinction coefficient, cell length and dilution factor and was finally expressed in nmol/g protein_[23].

Tissue Glutathione (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 GSH level was measured by Ellman's method [24], as described earlier. Tissue protein (μmol/g protein) was obtained by Biuret's method. 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) × C Standard, where C Standard =15.34 g/100 ml.

Statistics

Statistical analysis was performed using SPSS statistical program. The results were expressed as mean ± SD. 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

Findings obtained in the study are presented in Tables 1 and 2. Kidney MDA levels were 59.92 ± 2.55 in the control group, 103.70 ± 2.01 in hyperthyroidism group and 75.45 ± 0.85 in hyperthyroidism + melatonin-administered group. Comparison of groups revealed that the highest MDA levels were in hyperthyroidism group ($p<0.001$) and that MDA levels in the control group were higher than those in hyperthyroidism+melatonin-administered group ($p<0.001$) (Table 1). GSH levels in the kidney tissue were found 16.50 ± 0.84 in the control group, 33.36 ±

0.79 in hyperthyroidism group and 45.25 ± 1.47 in hyperthyroidism + melatonin-administered group. When the groups were compared, it was seen that the highest GSH levels were in hyperthyroidism + melatonin group, while the lowest levels were in the control group. GSH levels in hyperthyroidism group were higher than those in the control group ($p<0.001$) (Table 2).

MDA levels in testis tissue were 59.88 ± 2.04 in the control group, 97.37 ± 1.47 in hyperthyroidism group and 67.51 ± 02.83 in hyperthyroidism+melatonin-administered group. It was found that MDA levels were statistically higher in hyperthyroidism group than in the control and hyperthyroidism + melatonin-administered groups and the levels in the control group were higher than those in hyperthyroidism + melatonin-administered group ($p<0.001$) (Table 1). GSH values in the testis tissue were 25.29 ± 2.18, 54.48 ± 2.26 and 72.27 ± 1.29 in the control, hyperthyroidism and hyperthyroidism+melatonin administered groups, respectively. Comparison of groups showed that GSH levels in hyperthyroidism + melatonin-administered group were higher than those in the other two groups and the levels in hyperthyroidism group were higher than those in the control group ($p<0.001$) (Table 2).

Discussion

Results we obtained in this study show that lipid peroxidation increased in both testis and kidney tissues of the studied rats due to hyperthyroidism induced by 3-weeks L-thyroxine administration and that there was a parallel increase in antioxidant system activity, but lipid peroxidation could not be prevented despite the increase in antioxidant system activity. Besides, it was seen that additional melatonin administration for 3-weeks together with hyperthyroidism inhibited lipid peroxidation to a certain extent by activating the antioxidant system. Various circumstances may lead to various degrees of oxidative stress in the organism. One of the methods used to determine lipid peroxidation is to find MDA levels [25]. In the present study we determined MDA levels in kidney and testis tissues in order to identify the degree of oxidative stress caused by hyperthyroidism.

Since thyroid hormones in general activate all the systems in the body, hyperthyroidism inevitably causes lipid peroxidation in different tissues depending on its severity. It was reported that different thyroid hormone isomers used in induced hyperthyroidism led to different degrees of oxidative stress. For example, Galkina et al. [26] investigated the effect of different thyroxine isomers on free radical formation in rat cerebral cortex and stated that antioxidant activity of D-thyroxine was about two and a half times greater than that of L-thyroxine. Oziol et al. [27] found increases in oxidant and antioxidant system activities depending upon structural differences in thyroid hormones also reported similar findings. In this study we used only L-thyroxine derivative under in vivo conditions and established an increase in MDA and GSH levels in both kidney and testis tissues due to hyperthyroidism at the end of the 3-weeks administration period. Our findings are parallel to those of other researchers in this respect [26,28]. These researchers reported that their results were dose-dependent under in vitro conditions and were obtained in micromolar doses, not nanomolar doses. In the present study we administered intraperitoneal 0.3 mg/kg/day L-thyroxine for 3 weeks and found increases in oxidant and antioxidant system activities. Melatonin administration in addition to hyperthyroidism prevented cellular stress to a great extent and led to a significant increase in GSH levels, an indicator of antioxidant system activity. Likewise, it was put forward in previous studies that melatonin administration at different doses and for different periods hindered oxidative stress formed due to various procedures and aging [17,29,30]. Although there are not any similar experimental models with which we can compare the results we obtained from our experimental model, Venditti et al. [31] reported in their study that antioxidant capacity of mitochondria did not change in hypothyroidism and euthyroidism, while it decreased in hyperthyroidism, increasing lipid peroxidation in the mitochondria. These findings are similar to our finding that oxidative stress increased in hyperthyroidism. However, as opposed to the study by Venditti et al. [31], increased antioxidant capacity could not prevent oxidative stress in our study. These results may be explained by the increase in mitochondrial free

Table 1 : Tissue MDA Levels in Control and Experimental Groups

Groups	Kidney MDA(nmol/g protein)	Testis MDA(nmol/g protein)
Control (n=10)	59.92 ± 2.55 [c]	59.88±2.04 [c]
Hyperthyroidism (n=10)	103.70±2.01 [a]	97.37±1.47 [a]
Hyperthyroidism + Melatonin (n=10)	75.45±0.85 [b]	67.51±2.83 [b]

* Different letters on each column indicate significance ($P<0.001$) (a>b>c).

Table 2 : Tissue GSH Levels in Control and Experimental Groups

Groups	Kidney GSH (µmol/g protein)	Testis GSH (µmol/g protein)
Control (n=10)	16.50 ± 0.84 [c]	25.29±2.18 [c]
Hyperthyroidism (n=10)	33.36± 0.79 [b]	54.48±2.26 [b]
Hyperthyroidism + Melatonin (n=10)	45.25±1.47 [a]	72.27±1.29 [a]

* Different letters on each column indicate significance ($P<0.001$) (a>b>c).

radical formation stimulated by thyroid hormones and associated changes observed in mitochondrial hemoprotein and antioxidants [32]. It was reported in similar studies that hyperthyroidism increased oxidant stress and reduced GSH levels [32–34].

As far as the part of our study about testis tissue was concerned, it was seen that oxidative stress in testis tissue increased due to hyperthyroidism and was inhibited by melatonin administration. Findings we obtained in this study are parallel to the results of the studies reporting that hyperthyroidism caused oxidative stress in various tissues like muscle, liver and heart [5,35]. This result suggests that the hypermetabolic condition brought about by hyperthyroidism could lead to oxidative stress in more than one tissue in the body. It was shown that renal hypertrophy stimulated by in vitro thyroxine was associated with an increase in mitotic index and these changes were observed in the cardiac tissue as well [11]. The increase in mitotic index activity in hyperthyroidism might also have caused oxidative stress. Results of our study indicated that lipid peroxidation in testis tissue increased in hyperthyroidism induced by 3-week L-thyroxine administration and that melatonin administration together with hyperthyroidism prevented the increase in lipid peroxidation. It was reported that biochemical changes stimulated by hyperthyroidism increased disposition towards lipid peroxidation in different tissues of the body [5,35] and that melatonin administration at various doses and for various periods prevented oxidant stress caused by various drug administrations, if not by hyperthyroidism [36,37].

Thyroid hormones increase oxygen consumption via a thermogenetic effect. In hyperthyroidism caused by thyroxine or triiodothyronine administration, the increase in metabolic rate together with the increase in oxygen consumption enhances microsomal oxidative capacity and free radical formation. Chouldhury et al.[38] reported that such oxidative stress parameters as hydrogen peroxide and protein carbonyl increased in testis homogenates of rats due to hypothyroidism and that T_3 administration to rats with hypothyroidism did not cause a significant change in hydrogen peroxide level, but further increased protein carbonyl content. In our study there was an increase of about 60% in MDA levels in testis tissue due to 3-weeks L-thyroxine administration.

It was reported the hypermetabolic condition in hyperthyroidism was associated with an increase in free radical formation and lipid peroxidation levels [3–5]. In our study the increase in free radical formation due to hyperthyroidism could not compensated for by the increase in antioxidant system activity. However, 3-weeks melatonin administration in addition to hyperthyroidism significantly inhibited lipid peroxidation stimulated by L-thyroxine and reduced it almost to the level in the control group. Direct antioxidant and free radical eliminating effects of melatonin, which is released from the pineal gland, have been shown in different experimental models. For example, it was reported that melatonin administration significantly inhibited testis stress stimulated by indomethacin [39], experimental testis ischemia-reperfusion injury [19], experimental stress caused in varicocele [20] and testis stress stimulated by iron in hamsters [40]. In our study, significant increases in levels of MDA, an indicator of oxidative stress, in testis tissue due to hyperthyroidism were inhibited to a large extent by the increase in levels of GSH, an indicator of antioxidant system activity, as a result of melatonin administration.These results demonstrate that the oxidative stress brought about by hyperthyroidism was hindered to a great degree by melatonin's increasing antioxidant system activities. Exogenous melatonin administration in addition to endogenous melatonin secretion makes antioxidant defense system of the body more marked. Similarly, Sainz et al. [41] reported that pinealectomy eliminated the antioxidant effect brought about by melatonin and these data are consistent with our findings. It was found in another study by Baydas et al. [42] that oxidative stress increased and antioxidant system activity was inhibited due to pinealectomy. Thyroid hormones cause oxidative stress as they increase reactive oxygen species, while activating metabolic systems of the body in general [4,5]. That is how we found an increase in oxidative stress due to hyperthyroidism not only in the kidney tissue, but also in the testis tissue, too. The hypermetabolic condition caused by thyroid hormones leads to an increase in the amount of free radicals by enhancing mitochondrial electron transport. It was seen in our study that there were increases in the oxidant and antioxidant systems at the end of the 3-weeks study period, but oxidative stress could not be removed despite the increase in the antioxidant system. It was demonstrated that oxidative stress caused in the testis due to various applications varied not only with age [43], but also with period of application [44]. In our study we used 3-months-old male rats and established that hyperthyroidism triggered oxidative stress in both kidney and testis tissues. However, melatonin administration together with hyperthyroidism suppressed oxidative stress in both tissues to a considerable extent. One explanation of our findings may be the inhibition of thyroid hormones by melatonin administration. Likewise, it was reported in previous studies that melatonin administration inhibited thyroid hormone levels [22, 45].

Results of our study demonstrate that oxidative stress in kidney and testis tissues increased in hyperthyroidism and that the parallel increase in levels of GSH, an antioxidant system marker, could not prevent the oxidative stress. Besides, it is seen that melatonin administration together with hyperthyroidism reduced MDA levels by its free radical eliminating effect and increased GSH levels by its antioxidant effect, thereby preventing, to a great extent, the lipid peroxidation caused by hyperthyroidism in kidney and testis tissues.

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