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ORIGINAL ARTICLE

Effects of different antioxidants on lipid peroxidation in brain homogenates induced by thyrotoxicosis in rats

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Abstract

OBJECTIVE: It is known that thyrotoxicosis induces lipid peroxidation (LPO). In contrast, propylthiouracil (PTU), a thyrostatic drug is a well-known antioxidant. Also melatonin has been shown to protect against free radical-induced neuronal destruction. At the same time, it is generally accepted that the brain is the most vulnerable tissue to oxidative stress.

METHODS: The goal of the study was to examine the components of LPO, i.e., conjugated dienes (CD), malondialdehyde (MDA) and Schiff's bases (SB), in the brain of male Wistar rats. Two experiments were performed, with two control groups created for each experiment: Group 1 – intact animals and Group 2 – animals injected with 0.9% NaCl. In Experiment I, the animals received L-thyroxine (L-T_4) in a dose of 100 μg/kg BW, i.p., daily, for two weeks (Groups 3–5). After one week of L-T_4 treatment, the following agents were added during a subsequent week: Group 4 – PTU in drinking water (45 mg/kg BW/day); Group 5 – melatonin (5 mg/kg BW, daily). In Experiment II, lasting 7 days, the animals were divided into the following groups: Group 1 – intact animals; Group 2 – animals injected with 0.9% NaCl; Group 3 – PTU in drinking water (45 mg/kg BW/day); Group 4 – melatonin (5 mg/kg BW, daily).

RESULTS: In Experiment I, we observed a significantly higher SB level in saline treated animals and a significant increase in both CD and SB levels in rats treated with L-T_4, compared to those in non treated control. CD levels were also elevated in rats treated with L-T_4, compared to values in the saline only treated animals. Melatonin and PTU reduced CD levels and melatonin diminished SB levels, as compared to those in L-T_4-treated rats. In Experiment II, we observed significantly higher CD, SB and MDA levels in saline treated rats, when compared to respective values in non treated control. Melatonin decreased CD levels, when compared to CD levels in both the non treated and saline injected controls. Additionally, melatonin reduced SB levels relative to change in the brains of saline treated rats. Furthermore, PTU decreased CD levels in brain homogenates compared to non-treated animals.

CONCLUSIONS: (1) Thyrotoxicosis stimulates LPO in the rat brain; (2) All the examined antioxidants decrease LPO in L-T_4-administered animals; (3) All the examined antioxidants reduce the basal LPO; 4) Stress, when induced by handling, intensifies oxidative processes in the organism.

Introduction

The central nervous system is highly susceptible to damage by oxidative stress induced by a variety of biological agents [1, 2]. Because of its high metabolic activity, the brain utilizes large amounts of molecular oxygen, followed by high free radical generation; it is also contains very large amounts of polyunsaturated fatty acids which are easily oxidized [2]. Cerebrospinal fluid is highly enriched with iron and ascorbic acid which can generate the highly toxic hydroxyl radical (•OH) [1]. On the other hand, the brain is poorly equipped with an endogenous antioxidative defense system [3]. This organ exhibits a higher degree of tissue peroxidation than does either the liver or the lung [4].

It is well established that thyroid hormones accelerate the basal metabolic rate and, particularly, oxidative metabolism, as evidenced by the induction of certain mitochondrial enzymes in target tissues. Thyroid hormone administration is associated with a higher electron flow through the mitochondrial electron-transport systems, as indicated by an enhanced production of superoxide radicals ($O_2^{\bullet -}$). In the course of thyrotoxicosis, the oxidative stress and lipid peroxidation (LPO) are generated, which are not compensated by changes in the endogenous antioxidative system [5].

Propylthiouracil (PTU) is widely used in the therapy of hyperthyroidism. There is also some evidence that thyrostatic agents *per se* may reduce the oxidative stress [6]. It has been shown that PTU can directly scavenge hydrogen peroxide (H2O2) and •OH; but it is, however, less reactive towards $O_2^{\bullet -}$. Moreover, it protects lipids from peroxidation [6]. PTU has been demonstrated to reduce LPO in patients with hyperthyroidism [5, 7].

Melatonin, the main secretory product of the pineal gland, is an efficient free radical scavenger and antioxidant. It directly neutralizes •OH, $O_2^{\bullet -}$, hypochlorus acid, nitric oxide, peroxynitrite anion, H_2O_2, and singlet oxygen (8–10). The efficacy of melatonin in scavenging the peroxyl radical is controversial [11]. It has well been documented that melatonin is capable of inhibiting LPO [12,13]. Also, melatonin has been reported to stimulate activities of several antioxidative enzymes, for example, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd); also it is known to inhibit nitric oxide synthase, a prooxidative enzyme [14–16]. At the same time, melatonin can synergistically interact with other antioxidants [17]. Additionally, this indoleamine stabilizes cell membranes [18].

The goals of this study were :

1) to evaluate the levels of the three LPO parameters, i.e, conjugated dienes (CD), malondialdehyde (MDA) and Schiff's bases (SB) in the brain of male Wistar rats treated with L-thyroxine (L-T_4);
2) to examine the effectiveness of both PTU and melatonin in inhibiting oxidative stress;
3) to evaluate the action of antioxidants on basal LPO levels.

Materials and Methods

In the study, 70 male Wistar rats, weighing 150–175 g each, were used. The animals were fed with standard diet, having a free access to food and water. The rats were kept in controlled lighting conditions (12 h light; 12 h darkness).

Two experiments were performed (Fig. 1). The following control groups were created in each experiment: Group 1 – intact animals and Group 2 – animals injected with 0.9% NaCl. In Experiment I, the animals were intraperitoneally (i.p.) injected with L-thyroxine (L-T_4) in a dose of 100 μg/kg BW, daily for two weeks (Groups 3–5). After one week of L-T_4 treatment, the rats received the following substances for subsequent 7 days: Group 4 – PTU in drinking water (45 mg/kg BW/day); Group 5 – melatonin (5 mg/kg BW, i.p.; at 16.00 p.m.). In Experiment II, which lasted 7 days, the animals were divided into the following groups: Groups 1 – intact animals, Group 2 – animals injected with 0.9% NaCl, Group 3 – PTU in drinking water (45 mg/kg BW/day), Group 4 – melatonin (5 mg/kg BW, daily). At the end of the study, the animals were decapitated, the brains were removed and stored at –80°C.

In the present study, the following products of LPO were examined in the homogenates of the brain: MDA concentrations [19], the levels of CD and SB [20]. The results are expressed as MDA, CD, or SB per gram of wet tissue. A one-way analysis of variance (ANOVA) and the Newman-Keuls' test were applied to evaluate statistical significance. All the calculations were performed with the use of the Statistica `99 computer software.

Results

In Experiment I, we observed a significantly higher SB levels in saline treated animals, compared to those in non treated controls (Fig. 2). Moreover, we showed significantly higher CD (Fig. 3) and SB (Fig. 2) levels in rats treated with L-T_4, compared to those in non treated controls; CD level was also elevated, compared to values in saline injected animals (Fig. 3). Melatonin and PTU reduced CD levels (Fig. 3) and melatonin diminished SB levels (Fig. 2), as compared to those in L-T_4-treated rats.

In Experiment II, we noticed significantly higher CD (Fig. 4) and SB levels (Fig. 5) and MDA concentrations (Fig. 6) in saline treated rats, when compared to respective values in non treated animals. Melatonin decreased CD levels, when compared to CD levels in both the saline trated animals and non treated controls (Fig. 4). Melatonin reduced also SB levels, when compared to those in saline injected controls (Fig. 5). On the other side, concentrations of MDA after melatonin were higher, when compared to those in untreated controls (Fig. 6). Propylthiouracil decreased CD levels in brain homogenates compared to those in respective values in non treated animals (Fig. 4).

Discussion

In the reported study, we have established that thyrotoxicosis induces LPO in the brain, what is consistent with other previously published data. Chehade et al. [21] demonstrated an increased MDA concentration in the brain of rats injected with triiodothyronine (T_3), while no changes in SB level were noticed. Many authors emphasize excessive LPO in other organs of thyrotoxic rats after T_3 administration [22]. In another investigation, thyrotoxicosis, induced by L-T_4, was accompanied by excessive LPO [23]. Moreover, numerous publications have reported an intensification of oxidative metabolism and, as a consequence, an acceleration of LPO in hyper-

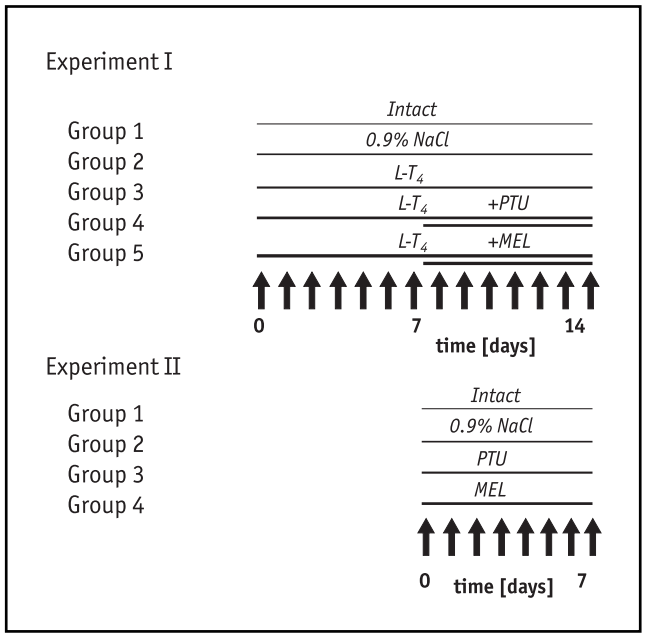

Figure 1. Scheme of Experiment I and Experiment II: L-thyroxin (L-T_4); Melatonin (MEL); propylothiouracil (PTU).

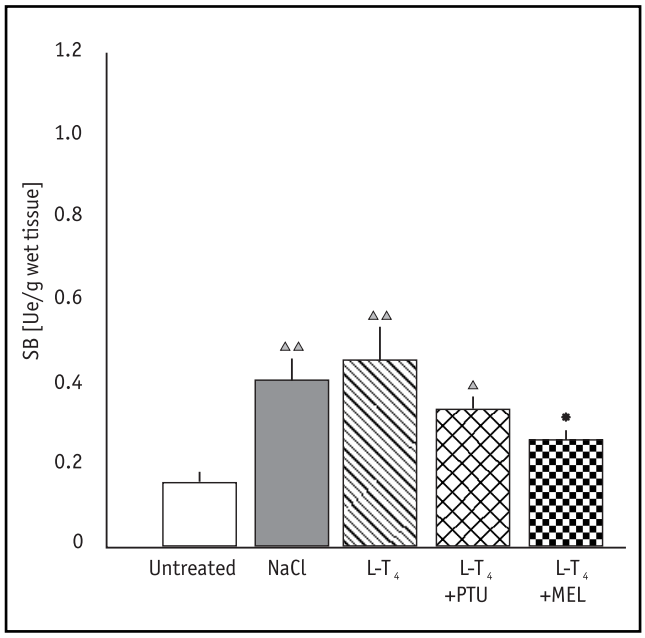

Figure 2. Levels of Schiff's bases (SB) in the brains of rats in the five treated groups. Data represent means ± SEM. Level of significance:
△△ – vs. untreated controls, p<0,005;
△ – vs. untreated controls, p<0,05;
✱ – vs. L-T_4-treated group, p<0,05;

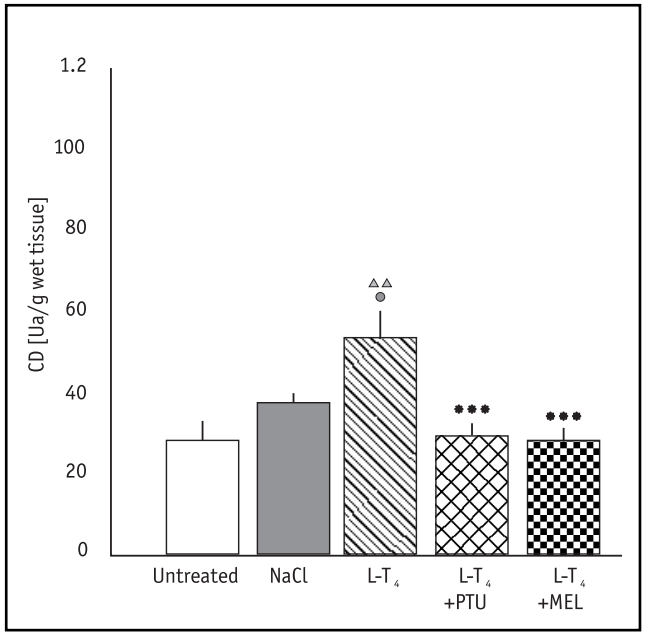

Figure 3. Levels of conjugated dienes (CD) in the brains of rats in the five treated groups.
Data represent means ± SEM. Level of significance:
△△ – vs. untreated controls, p<0,005;
● – vs. saline treated group, p<0,05;
✱✱✱ – vs. L-T_4-treated group, p<0,0005;

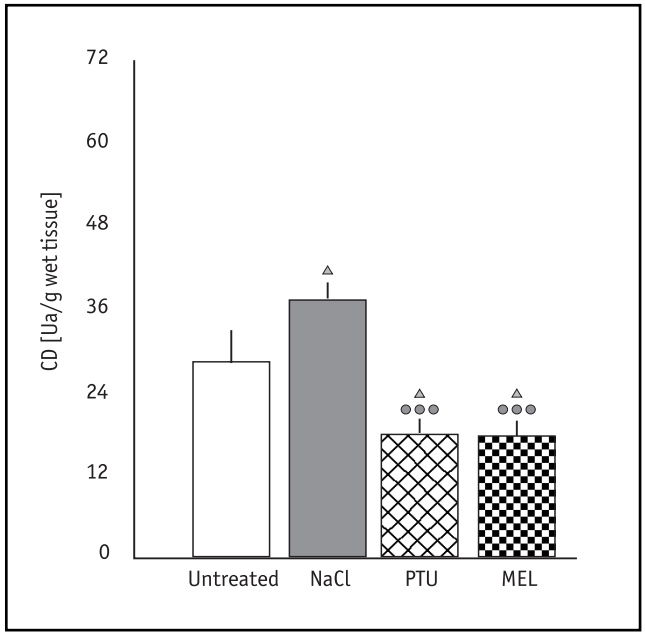

Figure 4. Levels of conjugated dienes (CD) in the brains of rats in the four treated groups.
Data represent means ± SEM. Level of significance:
△ – vs. untreated controls, p<0,05;
●●● – vs. saline treated group, p<0,0005;

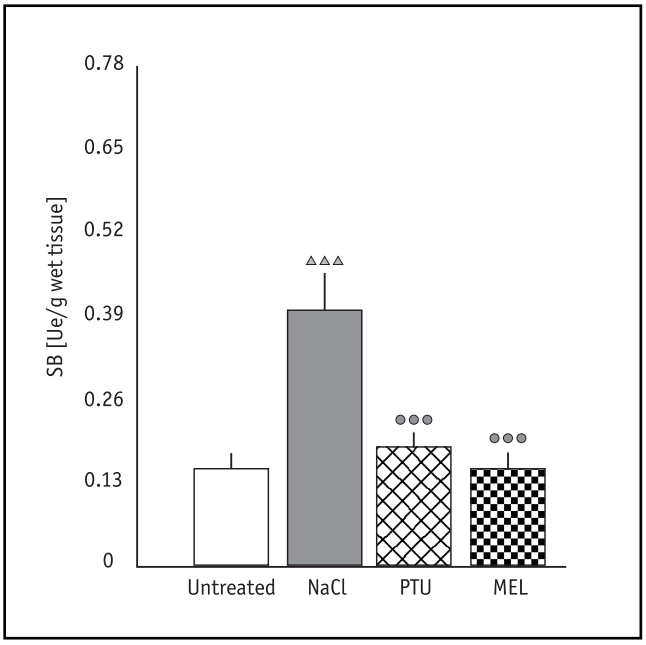

Figure 5. Levels of Schiff's bases (SB) in the brains of rats in the four treated groups.
Data represent means ± SEM. Level of significance:
△△△ – vs. untreated controls, p<0,0005;
○○○ – vs. saline treated group, p<0,0005;

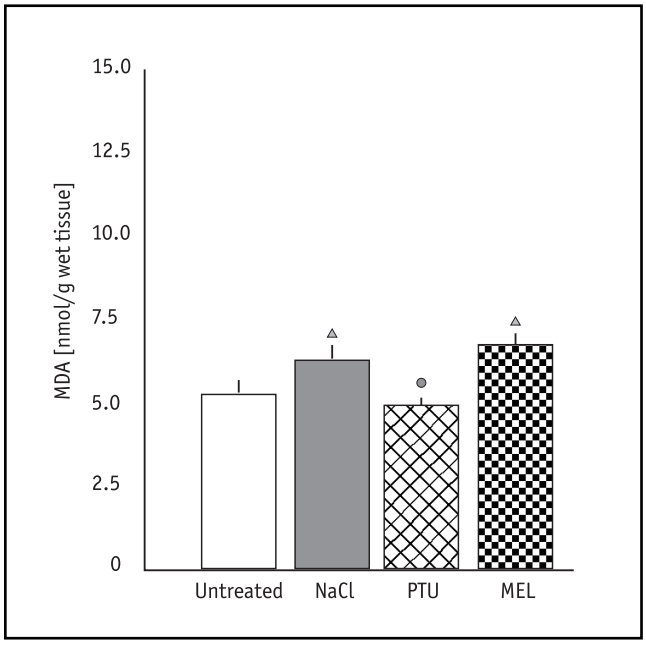

Figure 6. Concentrations of malondialdehyde (MDA) in the brains of rats in the four treated groups.
Data represent means ± SEM. Level of significance:
△ – vs. untreated controls, p<0,05;
○ – vs. saline treated group, p<0,05

thyroid men and their normalization after therapy [5, 7, 24]. Also, other authors have shown increased LPO both, in hyper- and hypothyroidism [25].

The effects of PTU on oxidative processes in the brain during thyrotoxicosis have not been carefully examined to date. We observed in the study that PTU reduced CD levels, both in hyperthyroid rats and under basal conditions. However, it has been reported in numerous publications that PTU therapy inhibits LPO and normalizes the activity of antioxidative enzymes in blood of hyperthyroid patients [5, 7]. At the same time, it has not yet been possible to ascertain whether PTU directly scavenges free radicals [6] or whether its effect is a consequence of euthyreoidism restoration [5]. Recent data from animal studies have demonstrated that PTU protects from acetaminophen and thioacetamide hepatotoxicity in rats; this thyrostatic drug loweres MDA concentration, as well as diminishes liver necrosis and inflammation [26,27]. The authors believe that the advantageous action of PTU is exerted at the metabolic level, depending on induced hypothyroid state.

Recently, melatonin has been shown to be highly effective in abating oxidative damage in the central nervous system [2, 28]. It has been reported that this indolamine easily crosses through the blood-brain barrier and is rapidly taken up by the brain. Skinner and Malpaux [29] have recently found that melatonin concentrations in the cerebro-spinal fluid of the third ventricle is 20-fold higher than its nocturnal plasma levels. In our study, we observed that melatonin reduced LPO in rats injected with L-T_4. There has been no other published work, concerning the role of melatonin in oxidative processes in the course of thyrotoxicosis but numerous authors have described a neuroprotective action of this indoloamine. Experiments under both in vivo [30] and in vitro [31] conditions have shown that melatonin effectively inhibits LPO in the brain, induced by kainic acid, a neuroexitotogic agent. In our experiments, melatonin reduced the basal LPO in the brain. The results are in agreement with those published in other papers, showing that melatonin diminishes MDA and 4-hydroxyalkenals (4-HDA) in the brain during ischemia-reperfusion, another high oxidative stress condition [32, 33]. Additionally, many authors have noticed that this indoleamine limits the basal LPO in rat [15, 31] and mouse brains [34]. Accordingly, Tan et al. [35] have found that melatonin reduces the autooxidation of lipids in homogenates of monkey brain cortex.

Unexpectedly, in the present study, a difference in LPO was noted between the two control groups, i.e., untreated and saline injected. Our results suggest that the stress, associated with handling and injections, intensifies oxidative processes in the organism and, as a consequence, exaggerates LPO. In numerous publications, the authors have reported that surgical stress intensifies free radical production [36]. Well-known stressful procedures, such as immobilization or excessive physical exercise, may also result in LPO acceleration [37].

In summary, our results demonstrate that thyrotoxicosis, as well as stress, induced by handling, stimulate LPO in rat brains and that the examined antioxidants (PTU, melatonin) exert protective effects, i.e., decrease LPO in the rat brain under both basal conditions and in animals treated with L-T_4. These results are consistent with a large number of studies, documenting the ability of melatonin to limit peroxidation of lipids under various conditions [38–40].

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