Oxidative Status and Citrate Concentration in Rat Tissues during Experimental Hyperthyroidism and Melatonin Treatment

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Biochemiluminescence increased, while aconitate hydratase activity and citrate accumulation in tissues of the liver and heart and blood decreased in rats with experimental hyperthyroidism. These changes reflect activation of free radical oxidation, damage to enzyme molecules with reactive oxygen species, and impaired utilization of citrate under pathological conditions. Melatonin treatment during hyperthyroidism normalized aconitate hydratase activity and citrate concentration. Biochemiluminescence study showed that the effect of melatonin is related to antioxidant activity of this hormone, inhibition of free radical oxidation, and suppression of reactive oxygen species generation.

Key Words: *experimental hyperthyroidism; melatonin; oxidative status*

Hyperthyroidism, a syndrome related to increased concentration of thyroid hormones in the blood, is observed under various pathological conditions or under the effect of exogenous thyroid hormones. Dysfunction of various systems of the organism during hyperthyroidism is related to the effect of excess thyroid hormones on various metabolic processes, organs, and tissues. These changes are accompanied by damage to the cardiovascular (thyrotoxic heart), digestive (thyrotoxic hepatitis), and central nervous systems, visual organs, gonads, and other organs [3].

There are conflicting data on the role of thyroid hormones in the regulation of free radical oxidation (FRO). Some authors showed that these metabolites have antioxidant properties and neutralize reactive oxygen species. Other investigators reported that these compounds intensify FRO [10]. An imbalance between FRO and functional activity of the antioxidant system is the major pathogenetic factor of various diseases. Multilevel antioxidant system consists of the enzymatic and nonenzymatic compartments. The nonenzymatic compartment includes a variety of substances chelating ions of transition metals (*e.g.*, citrate). The reaction of citrate conversion into isocitrate is catalyzed by aconitate hydratase (AH, EC 4.2.1.3). Its molecule is easily disintegrated by reactive oxygen species. Hence, this enzyme can serve as the target for free radicals. Studying the antioxidant effect of citrate is an urgent problem, because citrate eliminates Fe^{2+} ions involved in the formation of hydroxyl radical (one of the most reactive oxygen species) from H_2O_2 in the Fenton reaction [11].

Bioactive substances activating the antioxidant system and exhibiting antioxidant properties are of considerable interest. Recent studies were devoted to antioxidant activity of hormone melatonin [4]. Melatonin, an amino acid derivative synthesized primarily in the pineal gland, synchronizes diurnal and seasonal rhythms in the organism, plays a role in neuroendocrine regulation of the gastrointestinal

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tract and reproductive and immune systems, inhibits several functions of the hypothalamic-pituitary complex [1], and produces antitumor and antistress effects [4]. However, the use of melatonin in medical practice is confined to regulation of the sleep—wake cycle.

Our previous studies showed that melatonin normalizes oxidative status of the liver during toxic hepatitis [6]. The effect of melatonin during other diseases accompanied by oxidative stress remains unknown.

Here we studied parameters of biochemiluminescence (BCL) and measured citrate concentration and AH activity in blood plasma and tissues of the liver and heart in rats during experimental hyperthyroidism and treatment with exogenous melatonin.

MATERIALS AND METHODS

Male albino rats (*Rattus rattus* L.) weighing 150-200 g were divided into 3 groups. Group 1 animals were maintained under standard vivarium conditions (control, n=8). Group 2 animals (n=9) received 3 intraperitoneal injections of triiodothyronine (BioChemika, 100 μg/100 g in 0.9% NaCl) over 6 days for induction of experimental thyrotoxicosis [8]. Group 3 animals (n=8) received intraperitoneal injections of melatonin (2 mg/kg) starting from day 1 after the induction of experimental thyrotoxicosis. Melatonin was administered in the morning for 3 days.

The plasma was obtained from venous blood. Weighted tissue samples of rat liver and heart were separately homogenized in a 4-fold volume of cold isolation medium containing 0.1 M Tris-HCl buffer (pH 7.8), 1 mM EDTA (Reanal), and 1% β-mercaptoethanol and centrifuged at 10,000g for 12 min to obtain tissue homogenates. Parameters of BCL were estimated on a BKhL-06M biochemiluminometer equipped by special software. The kinetic curve of BCL was recorded over 30 sec. This period most adequately reflects the intensity of FRO. We estimated the following parameters: total chemiluminescence (S), flash intensity (I_{max}), and slope ratio of the kinetic curve ($tg\alpha_2$). AH activity was measured spectrophotometrically (SF-56) at 235 nm in a medium containing 0.05 mM Tris-HCl buffer (pH 7.8) and 4 mM citrate. The amount of AH that catalyzed transformation of 1 μmol substrate at 25°C for 1 min was taken as a unit of enzyme activity. Citrate concentration (Sigma) was estimated by the method of Natelson [5]. Total protein content was measured by the method of Lowry.

The results were analyzed by Student's t test.

RESULTS

S and I_{max} are parameters of BCL that reflect the intensity of FRO. Experimental hyperthyroidism was accompanied by an increase in these parameters in blood plasma (by 1.5 and 1.2 times, respectively), liver (by 1.3 and 1.4 times, respectively), and heart (by 1.4 and 1.8 times, respectively; Table 1). Our results are consistent with the data that thyroid hormones in high concentration may induce oxidative stress. It is probably related to a decrease in the membrane potential and increase in the rate of O_2 consumption during thyrotoxicosis [7]. During thyrotoxicosis the value of $tg\alpha_2$ characterizing total antioxidant activity of blood plasma and liver was higher compared to normal (by 2.2 and 1.2 times, respectively; Table 1). However, small changes in $tg\alpha_2$ were revealed in the heart (10% increase). These data illustrate mobilization of the antioxidant system during thyrotoxicosis.

TABLE 1. BCL Parameters in Rat Tissues under Normal Conditions, Experimental Thyrotoxicosis, and Treatment with Exogenous Melatonin ($M\pm m$)

Group	Study object	S, mV×sec	I_{max}, mV	$tg\alpha_2$
1	Blood	31.71±1.42	5.99±0.27	0.99±0.03
	Liver	23.70±1.05	4.30±0.19	1.50±0.06
	Heart	14.54±0.62	2.43±0.08	1.80±0.08
2	Blood	47.34±1.55*	7.37±0.30*	2.21±0.08*
	Liver	30.79±1.22*	6.13±0.25*	1.82±0.07*
	Heart	20.70±0.98*	4.38±0.17*	1.96±0.05*
3	Blood	36.73±1.67**	6.33±0.20**	1.45±0.06**
	Liver	25.78±0.59**	4.87±0.19**	1.60±0.06**
	Heart	17.43±0.85**	3.93±0.18**	1.75±0.07**

Note. p<0.05: *compared to group 1; **compared to group 2.

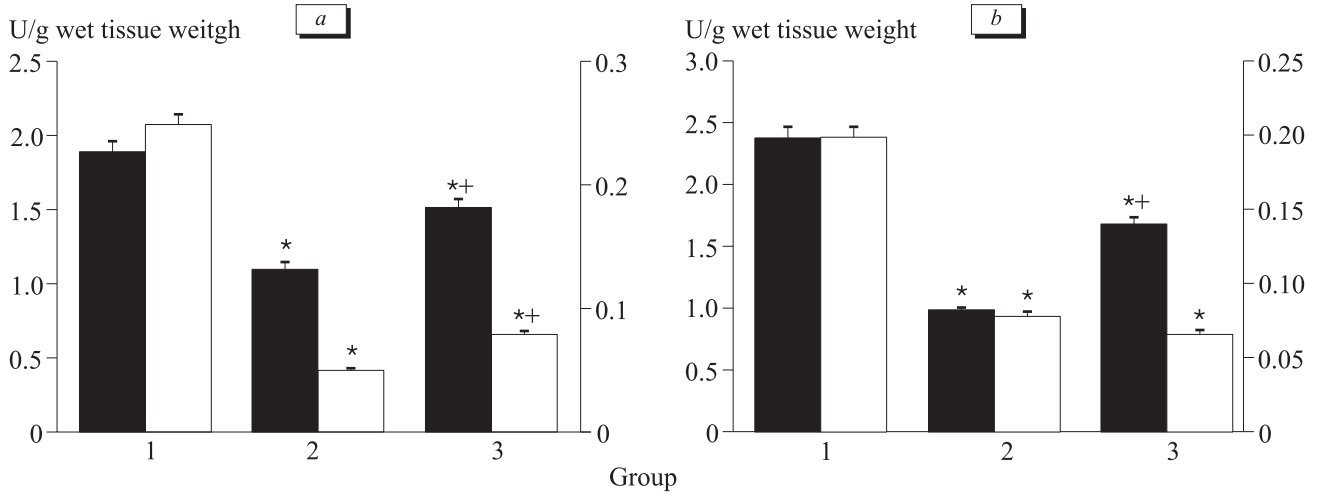

Fig. 1. AH activity in rat liver (*a*) and heart (*b*). Here and in Fig. 2: dark bars, enzyme activity (left ordinate); light bars, specific enzyme activity (right ordinate). Here and in Figs. 2 and 3: $p<0.05$: *compared to group 1; +compared to group 2.

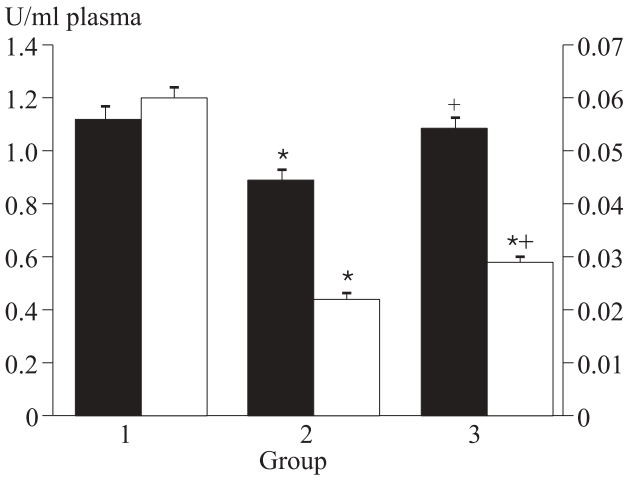

Fig. 2. AH activity in rat plasma.

Administration of melatonin to animals with experimental hyperthyroidism was followed by a decrease in S and I_{max} in blood plasma, liver (by 1.2 and 1.3 times, respectively), and heart (by 1.2 and 1.1 times, respectively). These changes were accompanied by a decrease in $tg\alpha_2$ in blood plasma, liver, and heart (by 34, 12, and 11%, respectively; Table 1). The inhibition of FRO is probably related to the fact that melatonin acts as a radical trap in various tissues and organs.

Experimental hyperthyroidism was accompanied by a decrease in AH activity in rat liver, heart, and blood plasma (by 1.7, 2.4, and 1.3 times, respectively, compared to normal; Figs. 1 and 2). Under these conditions we revealed a greater decrease in specific enzyme activity in the liver, blood plasma, and heart (by 5, 2.7, and 2.5 times, respectively, compared to the control). The observed changes in enzyme activity are probably associated with the fact that thyroid hormones in high concentration induce oxidative stress due to activation of NADPH-cytochrome P-450 reductase and NADPH oxidase. These changes contribute to intensification of reactive oxygen species generation. Hyperthyroidism is accompanied by acceleration of cell respiration and increased formation of superoxide radical (ubiquinone). The increase in the content of reactive oxygen species during thyrotoxicosis is probably followed by disintegration of iron-sulfur clusters in AH and transformation of the enzyme into an inactive form [9]. Administration of exogenous melatonin to animals with hyperthyroidism increased AH activity in the liver and heart by 1.4 and 1.7 times, respectively (Fig. 1). AH activity in blood plasma increased by 1.2 times (Fig. 2). Specific activity of AH in rat tissues underwent similar changes. The antioxidant compound melatonin probably inhibited FRO, which protected the enzyme molecule from free radicals. The antioxidant effect of melatonin was most pronounced in the liver. Melatonin is transformed into 6-hydroxymelatonin, which is characterized by higher antioxidant activity [2].

Citrate concentration in the liver, heart, and blood plasma of rats with hyperthyroidism increased by 1.5, 1.9, and 1.9 times, respectively, compared to intact animals (Fig. 3). Changes in citrate concentration were probably associated with the decrease in AH activity. Citrate accumulation during oxidative stress can be considered as the adaptive process. It is related to chelating activity of the citric acid anion to Fe^{2+} ions that catalyze chain processes of FRO. The increase in citrate concentration during thyrotoxicosis is a protective response of the organism directed toward mobilization of

the antioxidant system, which decreases the risk of hydroxyl radical formation.

Exogenous melatonin decreased citrate concentration in animals with experimental hyperthyroidism. We revealed a decrease in citrate concentration in the liver, heart (by 1.6 times), and blood plasma (by 2 times) compared to group 2 animals (Fig. 3). Melatonin administration is probably followed by an increase in the antioxidant potential and normalization of citrate concentration.

Our results suggest that the increase in BCL in tissues of the liver and heart and blood plasma from rats with experimental hyperthyroidism reflects FRO activation. AH activity in tissues decreases in rats with thyrotoxicosis, which is probably associated with damage to the enzyme molecule with reactive oxygen species. Citrate concentration increases, which probably resulted from its impaired utilization. Melatonin normalizes AH activity and citrate concentration in animals with this disorder. BCL study showed that the effect of melatonin is related to antioxidant activity of this hormone, inhibition of FRO, and suppression of reactive oxygen species generation.

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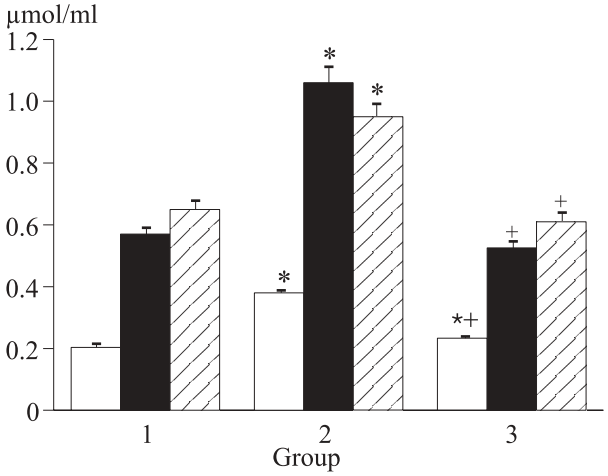

Fig. 3. Citrate concentration in rat heart (light bars), blood plasma (dark bars), and liver (shaded bars).

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