# Effect of Melatonin on Antioxidant Activity and Free Radical Lipid Oxidation in Traumatic Shock

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Effect of melatonin on antioxidant activity and lipid peroxidation in blood, heart, liver, and brain was studied in rats exposed to traumatic shock. Melatonin exerted a potent modulatory effect on antioxidant enzyme activity. Its efficacy depended on organ sensitivity to oxygen deficiency under conditions of traumatic shock.

**Key Words:** *traumatic shock; antioxidant system; lipid peroxidation; melatonin*

Traumatic shock (TSh) can be considered as a regulatory disease. Investigation of these diseases led to the discovery of a high-level melatonin-opioid regulatory system directly modulating the functional state of the hypothalamic-pituitary-adrenal system in shock [10,14]. Under critical conditions, pineal hormones, including melatonin, restore the water-salt balance by improving partial kidney functions and accelerate the onset of the anabolic phase of posttraumatic reaction [9]. Under conditions of hypovolemic shock melatonin exerts potent immunostimulatory [14] and antioxidant effects [12,13]. The properties of melatonin as the endogenous adaptogen necessitate the study of its effects on cell metabolism under extreme conditions.

The purpose of this work was to investigate the effects of melatonin on activity of the antioxidant system (AOS) and the content of lipid peroxidation (LPO) products in vital organs of rats exposed to TSh.

## MATERIALS AND METHODS

Experiments were carried out on 90 outbred male rats weighing 180-200 g. Traumatic shock was caused by a 6-h compression of femoral muscles. The rats were divided into 5 groups: 1) control rats; 2 and 3) rats exposed to trauma; 4 and 5) rats treated with melatonin (Springboard) immediately after decompression (buccally, 5 mg/kg). Groups 2 and 4 rats were decapitated 1 h after decompression and groups 3 and 5 rats — 24 h after decompression. The heart, liver and brain were frozen in liquid nitrogen (the brain was removed after freezing the head), homogenized in cold Tris-HCl (pH 7.4) at a 1:4 weight/volume ratio, and centrifuged at 8000 rpm for 15 min. The activity of LPO was assayed by measuring the content of primary and secondary LPO products in the plasma and supernatants [3]. The function of AOS was assessed by measuring activities of SOD [2], glutathione reductase [8], and total peroxidase [7] in erythrocytes and tissue, and by the plasma level of ceruloplasmin [4]. The data were analyzed statistically by Student's and Fisher's tests.

## RESULTS

Severe compression trauma accompanied by shock initiates free radical oxidation of various intensity in diverse body systems. No accumulation of LPO products was found in the plasma 1 h after reperfusion, while after 24 h their concentration 3-fold surpassed the control. This elevation can be explained by washout of LPO products from the organs, where a 2-fold activation of free radical peroxidation was observed 1 h after decompression and a high level of LPO products was maintained for 24 h. Melatonin exerted a potent and fast effect on the myocardial and brain tissues, where no accumulation of LPO products was observed 1 h after decompression. The increase in the liver content of LPO products in the melatonin-treated

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rats (group 4), although statistically significant, was 1.3 times lower than in the untreated rats (group 2). A single injection of melatonin at the early stage of TSh had a positive effect on its dynamics. Despite a considerable increase in blood and tissue content of LPO products in group 5 rats compared to control it was approximately 1.5 times lower than in untreated rats (group 3, Table 1).

Intensification of LPO after TSh implies metabolic disturbances in blood, myocardial, cerebral, and liver cells due not only to peroxidation of membrane lipids, but also to AOS dysfunction. As evidenced by the 1.6-fold increase in glutathione reductase activity 1 h after reperfusion (Fig. 1), the antioxidant protection under conditions of TSh was predominantly effected via the glutathione system. The pentose cycle in erythrocytes supplies NADH for reduction of oxidized glutathione. This reaction is catalyzed by glutathione reductase. Then the reduced glutathione undergoes peroxidation with gluthathione peroxidase, thus protecting membrane lipids and erythrocyte hemoglobin from oxidation with peroxides. This defensive reaction is very important, since accumulating hydroperoxides shorten the erythrocyte lifetime by promoting hemoglobin oxidation to methemoglobin. Activation of this reaction is confirmed by a 2-fold increase in total peroxidase activity. It is noteworthy that at the early stages of TSh the changes in activities of gluthathione reducing and antiradical enzymes are oppositely directed. Ceruloplasmin content decreased more than 1.5 times, while SOD activity did not differ from the control (Fig. 1). It explains why the plasma level of LPO products remained unchanged 1 h after reperfusion: plasma is sufficiently protected by AOS. Twenty-four hours after the trauma activities of all plasma antiradical enzymes significantly decreased, and the content of LPO products increased not only due to reduced AOS activity, but also to the release of LPO products from organs and tissues.

Despite significant elevation of LPO products, the brain was the most protected organ at the early stage of TSh. One hour after reperfusion SOD activity in brain tissue decreased less markedly than in the heart and liver (1.1-fold *vs.* 3- and 3.5-fold, respectively), where it remained low for at least 24 h (Fig. 2). Brain activity of glutathione reductase did not significantly differ from the control, and tended to increase after 24 h. In the heart and liver, activity of this enzyme significantly increased at the early period of TSh. Interestingly, in the liver high activity of glutathione reductase was observed throughout the experiment, which is probably due to a high concentration of glutathione in this organ (up to 5-10 mM *vs.* 3 mM in the brain) [5]. Thus, reduced activity of SOD, the principal cell generator of hydroperoxides, with paral-

**TABLE 1.** Effects of Melatonin on LPO during Traumatic Shock (*M*±*m*)

| Group | Plasma | | | Heart | | | Brain | | | Liver | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | optical density units/ml | | | optical density units/100 g | | | | | | | | |
| | IDC | CD | CT | IDC | CD | CT | IDC | CD | CT | IDC | CD | CT |
| 1 | 1.7±0.1 | 1.1±0.1 | 0.20±0.02 | 1.40±0.07 | 0.90±0.05 | 0.70±0.07 | 1.8±0.2 | 1.0±0.1 | 0.50±0.06 | 2.7±0.1 | 2.0±0.1 | 1.2±0.1 |
| 2 | 1.7±0.1 | 0.80±0.04 | 0.20±0.01 | 3.0±0.1* | 1.7±0.1* | 1.20±0.01* | 3.0±0.2* | 1.7±0.1* | 1.00±0.05* | 5.0±0.3* | 3.7±0.3* | 2.2±0.1* |
| 3 | 4.0±0.4* | 2.9±0.3* | 0.60±0.01* | 3.60±0.06* | 2.40±0.05* | 1.80±0.05* | 3.0±0.3* | 1.40±0.08* | 0.90±0.01* | 5.7±0.2* | 4.3±0.1* | 1.7±0.1* |
| 4 | 2.5±0.4* | 1.1±0.1 | 0.30±0.04 | 1.6±0.2 | 1.20±0.02 | 1.00±0.02 | 2.7±0.2* | 1.3±0.1 | 0.40±0.06 | 3.9±0.1* | 2.6±0.1* | 2.1±0.1* |
| 5 | 2.90±0.03* | 1.90±0.02* | 0.30±0.02 | 2.20±0.08 | 1.30±0.02* | 1.40±0.01* | 2.5±0.4* | 1.3±0.1 | 0.80±0.05* | 4.8±0.3* | 3.5±0.3* | 1.7±0.1* |

**Note.** IDC — isolated double bonds; CD — conjugated dienes; CT — conjugated trienes. *$p<0.05$ in comparison with the control group.

lel increase in glutathione reductase activity (the peroxide-utilizing enzyme), that were observed in the blood and vital organs, suggest imbalance in the AOS function resulting from disturbed regulation of the glutathione/SOD redox system. Similar changes observed in the lungs after infection/intoxication-induced shock and hypothetically resulted from SOD release from "shocked" lung cells [6]. In this situation, glutathione reductase activity should decrease, which was not the case in the abovementioned and our study. We believe that the reduced activity of SOD is associated with hypoxia, since this enzyme is activated by oxygen [11]. The imbalance in the AOS functioning leads to accumulation of LPO products and cell destruction [1], which eventually deteriorates the structure and function of organs, inducing polyorganic deficiency and death of the majority of animals exposed to traumatic shock within the 24-h period (mortality in group 3 was 65%).

Trauma-induced imbalance of the highly organized multicomponent antioxidant defense system is triggered by hemodynamic disturbances, which, in turn, are due to the improper neurohumoral response to critical factors. This response is modulated by melatonin, which regulates activity of the AOS enzymes in different organs. One hour after hormone administration (group 4) SOD activity in erythrocytes increased 1.5-fold. This increase was coupled to the glutathione system, as evidenced by a 2-fold increase in glutathione reductase and total peroxidase activities (Fig. 1). Similar elevation of antiradical and glutathione reductase activities was observed in the brain and liver. Melatonin exerted a delayed effect on the myocardium: SOD activity in the myocardium returned to normal only after 24 h, while activity of glutathione reductase elevated at the early stage of TSh became 1.2 times lower than the control. At the later stages of TSh the distant antioxidant effect of melatonin paral-

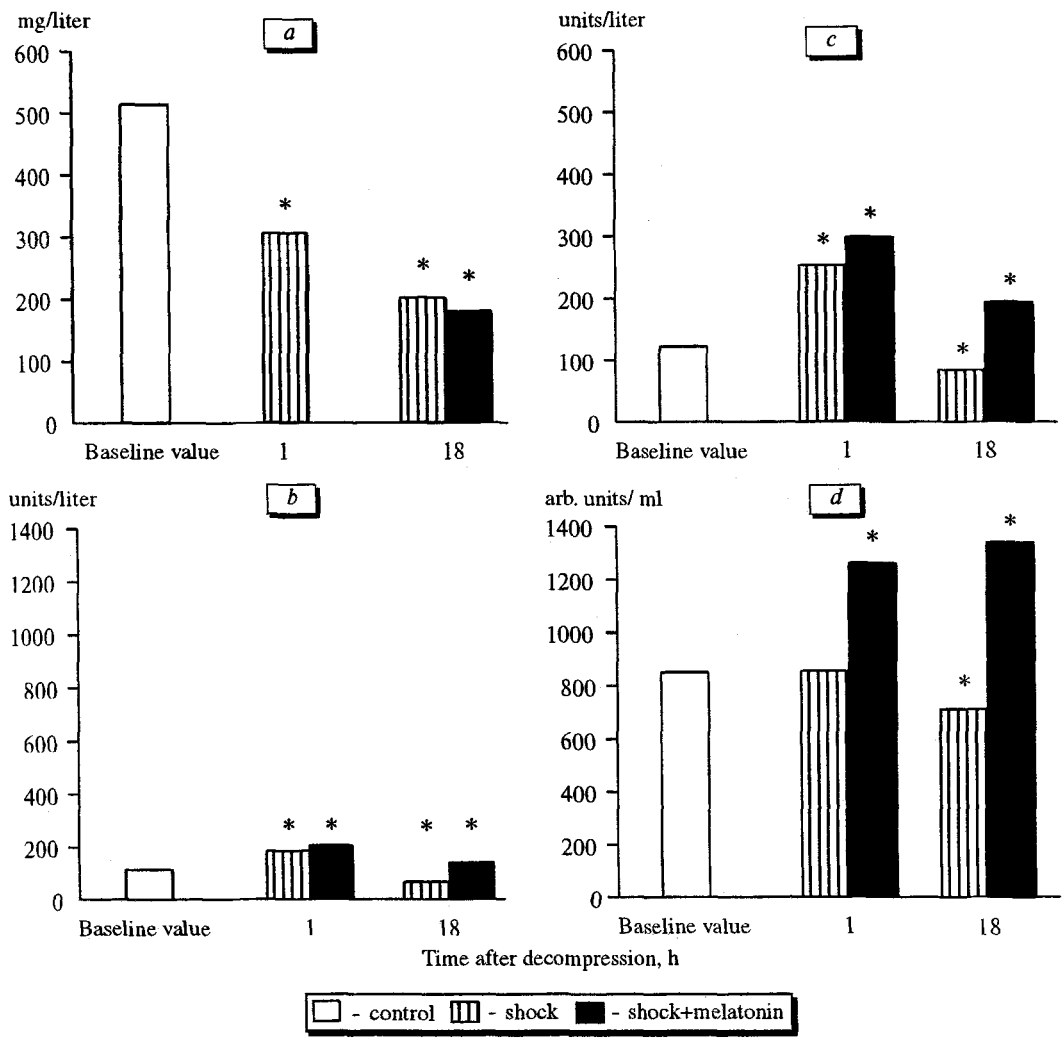


**Fig. 1.** Effects of melatonin on activity of blood antioxidant enzymes in rats with traumatic shock. *a*) ceruloplasin; *b*) glutathione reductase; *c*) total peroxidase activity; *d*) SOD. Here and in Fig. 2 *$p<0.05$ in comparison with baseline value (control group).

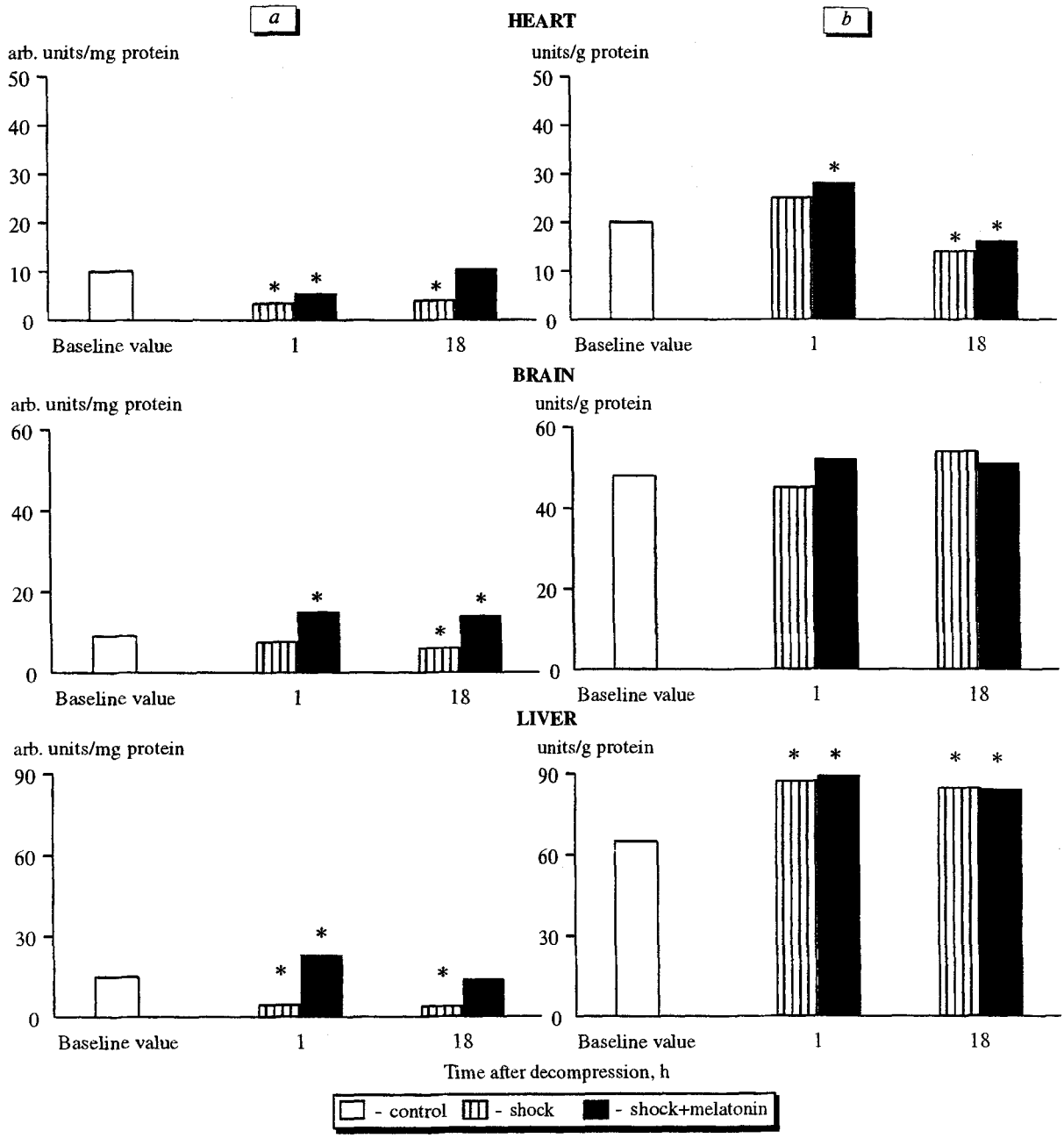


**Fig. 2.** Effects of melatonin on SOD (*a*) and glutathione reductase (*b*) activities.

leled by a decrease in LPO products was observed in the blood and myocardium. An immediate defensive reaction in the brain (1 h after melatonin administration) was manifested in LPO inhibition due to coordinated activity of the SOD/glutathione redox system. It implies that the brain has several defensive systems operating under critical conditions.

Our data suggest that the pineal hormone melatonin belongs to a system that triggers the adaptive mechanisms after TSh. The high survival rate (75%) after single melatonin injection allows us to recommend it for complex antishock therapy as an activator of the organisms reserve capacities under critical conditions.

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