BIOPHYSICS AND BIOCHEMISTRY

Protective Effect of Carnosine on Cu,Zn-Superoxide Dismutase during Impaired Oxidative Metabolism in the Brain *in Vivo*

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Natural hydrophilic antioxidant carnosine protects cerebral cytosolic Cu,Zn-superoxide dismutase (SOD) under conditions of oxidative stress in various *in vivo* models: short-term hypobaric hypoxia in rats and accumulation of age-related changes in senescence-accelerated mice (SAMP). Administration of carnosine preventing Cu,Zn-SOD inactivation reduced mortality in rats and prolonged average life span in SAMP-mice.

Key Words: *hypoxia*; *senescence accelerated mice (SAMP)*; *Cu,Zn-SOD*; *brain*; *carnosine*; *antioxidants*

Activity of cytosolic Cu/Zn-SOD characterizes organism's resistance to oxidative stress [9]. Cu/Zn-SOD is a target for reactive oxygen species (ROS). ROS inhibit this enzyme both *in vitro* [7] and during the neurodegenerative diseases associated with ROS generation [8,13]. It was demonstrated that inactivation of Cu,Zn-SOD induced by ROS *in vitro* is determined by fragmentation of the enzyme molecules attacked by free radicals. Antioxidants, *e.g.* carnosine, can prevent Cu,Zn-SOD fragmentation and inactivation [7]. Carnosine also prevents modification of Cu,Zn-SOD in reactions with fructose and glycolaldehyde *in vitro* [15].

The antioxidant properties carnosine at both the cellular and organism levels were demonstrated [1]. In order to answer the question whether *in vivo* antioxidant activity of carnosine is related to improvement on the antioxidant defense system, we studied the protective effects of carnosine on Cu,Zn-SOD activity in two various models of oxidative stress. In this work, we evaluated changes in Cu,Zn-SOD activity in carnosine-treated rats exposed to hypobaric hypoxia and in SAMP feeding a carnosine-containing diet.

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MATERIALS AND METHODS

Hypobaric hypoxia in adult male Wistar rats was modeled in a flow altitude chamber at 0.18 atm for 15 min. The animals were decapitated 1 h after restoration of normal pressure. Freshly prepared brain homogenates were used for the analysis of lipid peroxidation by chemiluminescent method and for isolation of cytosolic fraction containing Cu/Zn-SOD.

SAMP1 (Prone) mice were provided by Prof. T.Takeda (Kioto University, Japan). These mice are characterized by shortened life span and accelerated accumulation of senile features starting from the age of 4 month [10].

These peculiarities are associated with changes in free radical metabolism and disorganization of the tissue antioxidant defense systems [2,5]. SAMR1 (Resistant) mice of the corresponding age served as the control.

The experimental rats (n=12) received carnosine (150 mg/kg) with drinking water one day before hyp-

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oxic exposure. The rats were deprived of water for 24 h before administration of carnosine. The control group consisted of 15 rats. Experimental SAMP mice (n=36) received carnosine (100 mg/kg per day) in drinking water for 12 months starting from the age of 2 month. The control group included 36 SAMR1 (Resistant) mice aged 14-16 months. After the end of the experiment the mice were decapitated and the brain homogenates were processed as described above.

Cytosolic fraction was obtained by centrifugation of brain homogenates at 100,000g. Cu/Zn-SOD activity in this fraction was measured by the rate of inhibition of nitroblue tetrazolium reduction in the presence of xanthine and xanthine oxidase [12].

The resistance of cell membranes to oxidative stress, the kinetics of $FeSO_4$-induced lipid oxidation in brain homogenates was studied. The parameters of chemiluminescence flash reflected the state of lipid peroxidation in the studied sample. The fast chemiluminescence flash (parameter h) observed immediately after addition of $FeSO_4$ correlates with the initial content of lipid hydroperoxides and other products of lipid peroxidation, whereas the latency (parameter τ) between the fast flash and the major burst of chemiluminescence depends on the relative content of pro- and antioxidants and reflects the endogenous antioxidant potential of the tissue [4]. The intensity of chemiluminescence was measured with an Luminometer-1251 (LKB) in a medium containing 60 mM KH_2PO_4 (pH 7.45) and 105 mM KCl; $FeSO_4$ was added in a final concentration of 2.5 mM; sample volume was 1 ml.

RESULTS

It is well known that tissues subjected to hypoxic stress are characterized by enhanced production of free radicals [3]. Chemiluminescence analysis of lipid peroxidation in brain homogenates from rats subjected to hypoxia revealed increased initial content of lipid peroxidation products (h) and significant decrease in endogenous antioxidant potential (parameter τ, Table 1). In rats receiving carnosine before hypoxic exposure, the initial level of lipid peroxidation products and τ did not differ from the corresponding parameters in intact rats (Table 1).

Even short-term hypoxic exposure decreased activity of cytosolic Cu/Zn-SOD in rat brain (by 18% compared to intact animals, p<0.05). Pretreatment with carnosine not only prevented the hypoxia-induced decrease in Cu/Zn-SOD activity, but even increased it by 25% (p<0.05, Fig. 1). Carnosine improved rat survival of after hypoxia: 9 rats died from hypoxia in the control group, while only 1 of 12 rats died in the test group. Convulsions during hypoxia occurred

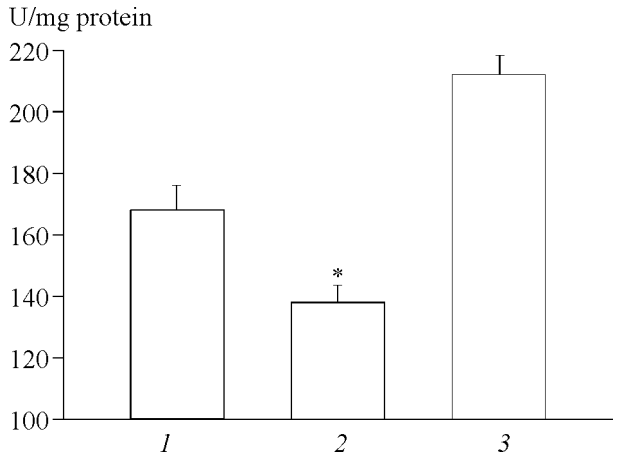

Fig. 1. Activity of cytosolic Cu,Zn-SOD in the brain of intact rats (*1*), rats after hypoxia (*2*), and carnosine treated-rats after hypoxia (*3*). *p<0.05 compared to intact rats.

in 12 rats of the control group and only in 3 rats treated with carnosine. Our findings suggest that carnosine can mobilize antioxidant system of the organism and improve resistance to hypoxia. In other words, it is a potent preconditioning factor. The fact that Cu/Zn-SOD activity in carnosine-treated rats even after hypoxia was higher than in intact rats indicates that carnosine can protect this enzyme (and, probably, other proteins) against ROS attack not only under extreme conditions, but also under normal conditions.

Accelerated aging in SAMP1 mice is determined by accumulation of ROS and deficiency of the antioxidant defense system [2,5]. In fact, the amplitude of the fast chemiluminescence flash increased and the latency of Fe^{2+}-induced chemiluminescence was shorte-

TABLE 1. Chemiluminescent Analysis of Lipid Peroxidation in the Brain of Carnosine-Treated and Untreated Rats Exposed to Hypoxia (M±m)

Conditions	n	h, mV	τ, sec
Hypoxia	6	234±18*	131±42*
Hypoxia+carnosine	7	203±26	195±17
Intact animals	7	190±19	202±17

Note. *p<0.05 compared to intact rats.

TABLE 2. Chemiluminescent Analysis of Lipid Peroxidation in the Brain of Carnosine-Treated and Untreated SAMP1 Mice and SAMR1 Mice (M±m)

Conditions	n	h, mV	τ, sec
SAMP1	36	256±34*	10.0±0.1*
SAMP1+carnosine	36	148±12*	16.7±2.9*
SAMR1	36	188±24	12.5±3.5

Note. *p<0.05 compared to SAMR1 mice.

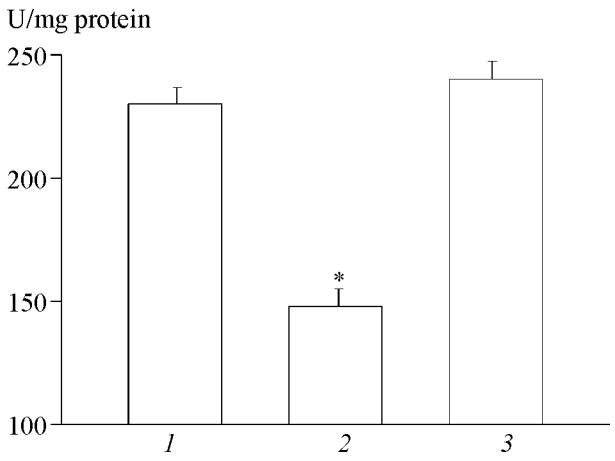

Fig. 2. Activity of cytosolic Cu,Zn-SOD in the brain of SAMR mice (*1*), SAMP mice (*2*), and SAMP mice received carnosine (*3*). The mice aged 14-16 months. *$p<0.05$ compared to SAMR mice.

ned in SAMP1 mice (Table 2), which suggests higher initial level of lipid hydroperoxides and exhaustion of the brain antioxidant system in SAMP1 mice compared to SAMR1 mice. The use of carnosine as a dietary supplement significantly decreased the content of lipid hydroperoxides in the brain of SAMP1 mice below the control level. The latency of slow chemiluminescence flash increased in carnosine-treated mice, which attested to enhanced efficiency of the endogenous antioxidant system in these mice (Table 2).

SAMP1 mice were characterized by low activity of Cu/Zn-SOD in the brain cytosolic fraction (by 35%, $p<0.05$), which agrees with the conclusion on senescence-related impairment of the antioxidant function in these mice. At the same time, SAMP1 mice receiving carnosine had normal SOD activity (Fig. 2). This correlated with longer life span and better physiological and morphological parameters of mice kept on the carnosine-enriched diet [16].

The correlation between low cytosolic activity of SOD in the brain, on the one hand, and high mortality of rats during hypobaric hypoxia and shorter life span of SAMP mice, on the other, attests to a critical role of ROS in these phenomena. On the other hand, the possibility of improving animal resistance to oxidative stress by increasing SOD levels attests to the key role of this enzyme in tissue protection against ROS.

Cu/Zn-SOD plays a dual role in ROS metabolism. In parallel to its antioxidant effect, it catalyzes generation of $OH^{\bullet}$ from H_2O_2 (which, in turn, is a product of Cu/Zn-SOD-catalyzed reaction) [9]. The latter reaction can lead to Cu/Zn-SOD degradation similar to that induced by other free radicals.

Numerous *in vitro* studies showed that carnosine is a potent scavenger of various ROS, including O_2^{-} [14], $OH^{\bullet}$, and OCl^{-} [6]. The data obtained *in vitro* [7] suggest that carnosine prevents both ROS generation and oxidative damage to SOD leading to copper release from the enzyme molecule, its destabilization, and fragmentation. Our experiments demonstrated a protective effect of carnosine on SOD *in vivo* under conditions of two independent experimental models of oxidative stress. Other favorable effects of carnosine cannot be excluded. It was recently found that dipeptide carnosine can protect copper-transporting protein ceruloplasmin against oxidative damage [11]. Nevertheless, our work provides the first evidence that antioxidant effect of carnosine *in vivo* is due to the maintenance of high activity of Cu/Zn-SOD, the major endogenous antioxidant in the brain.

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