Protective Effect of Carnosine in Hyperthermia

L. I. Deev, E. N. Goncharenko, A. A. Baizhumanov, M. Ya. Akhalaya, S. V. Antonova, and S. V. Shestakova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 7, pp. 50-52, July, 1997
Original article submitted March 6, 1996

Under the conditions of hyperthermia, carnosine (β-alanine-L-histidine) normalizes tissue contents of lipid peroxidation products, cytochrome P-450, serotonin, and histamine in rats and increases their survival after extreme hyperthermia.

Key Words: *hyperthermia; lipid peroxides; biogenic amines; cytochrome P-450; carnosine*

It has been suggested that some manifestations of the lipoperoxidation syndrome and changes in the biogenic amine metabolism developing in stress reflect the severity of homeostasis impairments and can be employed to evaluate the effects of various preparations on the resistance of an organism to extreme influences [1,2]. The ability of carnosine to correct lipid peroxidation (LPO) and tissue contents of serotonin and histamine in rats subjected to short-term hypothermia has been recently demonstrated [4]. In the present study we explored the possibility of using this naturally occurring dipeptide to increase the resistance of an organism to hyperthermia.

MATERIALS AND METHODS

Adult outbred male rats weighing 180-200 g were used. The rats were maintained under standard vivarium conditions and received standard chow. Hyperthermia (40-45°C, 1 h) was produced using a ventilated chamber. Rectal temperature was measured with a Term-9 electrothermometer. Carnosine (purity >97%) was isolated from bovine muscles and administered *per os* via gastric tube in a single dose of 250 mg/kg 2.5 h before hyperthermia. The intensity of LPO in blood serum was estimated as described [10]. The content of cytochrome P-450 in liver microsomes was measured by the method [13]. The contents of free histamine [8] and the activity diamino-oxidase (DO) in the myocardium [9] were determined. Serotonin content of the spleen was measured by the method [12]. The significance of differences between experimental and control values was evaluated using Student's *t* test.

Faculty of Biophysics, Laboratory of Radiation Biophysics, Biological Faculty, M. V. Lomonosov Moscow State University

RESULTS

After 10 min in thermal chamber (40°C), rectal temperature of rats increased from 37.3±0.1 to 40.1±0.2°C and practically did not change during 50 min of the observation period. Under these conditions (1-h hyperthermia, rectal temperature about 40°C for at least 50 min) the intensity of LPO increased considerably. Serum content of LPO rose during hyperthermia, reached the maximum (almost 7-fold above the norm) 30 min after termination of hyperthermia, and then rapidly decreased (Table 1).

A similar dynamics of serum content of LPO products, which points to an LPO "flash" in the body, was observed after other extreme influences, for example, in rats after hypothermia [1,4]. It was hypothesized that LPO products play a dual role in stress: they act as mediators of stress and stress pathology [1]. A decrease in activity of liver cytochrome P-450 system after immobilization, large physical load, and other stressful influences is mediated by LPO products [5,6]. In this study, the activity of liver cytochrome P-450 system was modified by hyperthermia: the cytochrome P-450 content in cytoplasmic reticulum of hepatocytes remained at the level of 65-70% of the norm for at least 60 h (Fig.

0007-4888/97/0007-0665$18.00

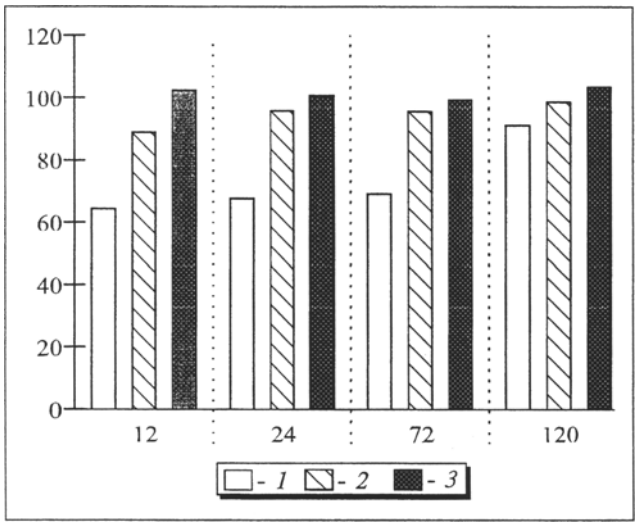

Fig. 1. Effect of carnosine (250 mg/kg) on the dynamics of the cytochrome P-450 content in rat liver microsomes after hyperthermia (40°C, 60 min). Abscissa: time after hyperthermia, hours; ordinate: cytochrome P-450 content, % of the norm (cytochrome P-450 content in liver microsomes of control rats varied from 0.60 to 0.64 nmol/mg protein). At least 8 animals were used for each point. *1*) hyperthermia; *2*) carnosine+hyperthermia; *3*) carnosine.

1). A similar amplitude of the of effect (30-35% decrease in the cytochrome P-450 concentration) was observed with other stressful factors, which may be due to abnormal sensitivity of 30-35% of the cytochrome P-450 pool to the damaging effect of lipid peroxides [3,11].

TABLE 1. Serum Content of LPO Products in Rats After Hyperthermia (40°C, 60 min)

Experimental condition	Time after hyperthermia, min	Number of animals in group	LPO products, %
Control	—	10	100±13
Hyperthermia	1	8	233±26**
	15	10	453±53*
	30	10	687±13*
	40	8	260±57***

Note. Here and in Table 2: *$p<0.001$, **$p<0.01$, ***$p<0.02$ compared with the control.

In addition to the LPO syndrome, we also observed the effects indicating activation of serotoninergic system and modification of histamine metabolism. During the maximum increase in blood and tissue contents of LPO products after hyperthermia, the serotonin and histamine contents increased and DO activity changed (Table 2). A similar phenomenon was observed after hypothermia [4]. However, an increase in myocardial histamine content after hypothermia coincided with a decrease in DO activity, while after hyperthermia it was accompanied by a 1.5-fold increase in DO activity.

Previously, it was shown that carnosine markedly reduces the intensification of LPO caused by hypobaric hypoxia and short-term hypothermia [4].

Carnosine also elicits a protective effect in hyperthermia after *per os* administration, which provides its maximum antioxidant activity *in vivo* (as estimated by reduction of basal LPO in the organism) at the moment of extreme influence [4,7].

It was found that in rats subjected to hyperthermia 2.5 h after administration of carnosine in a dose of 250 mg/kg the increase in blood content of LPO products was 2-fold lower than in rats which did no receive carnosine (Table 2). In carnosine-treated rats, the decrease in liver content of cytochrome P-450 was less pronounced and occurred during a shorter time period than in control rats (Fig. 1), which confirms the hypothesis that LPO is the primary factor of impaired cytochrome P-450 metabolism in stress [5].

Carnosine modifiers tissue contents of biogenic amines after hyperthermia as well as in hypothermia [4]. However, unlike in hypothermia, carnosine did not prevent alterations in DO activity in the myocardium, indicating that the processes leading to modifications in the histamine/DO system are different in hypo- and hyperthermia.

It has been hypothesized that there is a direct relationship between the survival of animals under extreme conditions and the level of toxic peroxides

TABLE 2. Changes in Tissue Contents of LPO Products, Serotonin, and Histamine and DO Activity After Hyperthermia (40°C, 60 min) and Administration of Carnosine (250 mg/kg)

Parameter	Tissue	Control	Carnosine	Hyperthermia	Carnosine+ hyperthermia
LPO products, ng/mg/ml	Blood serum	0.55±0.02	0.24±0.09**	3.74±0.11*	2.06±0.31*+
Serotonin, μg/g	Spleen	4.0±0.1	4.1±0.3	4.9±0.2***	4.0±0.3*+
Histamine, μg/g	Myocardium	3.6±0.2	4.1±0.3	4.3±0.2***	3.7±0.4
DO activity μg/g/h	Myocardium	32.4±1.9	32.7±1.3	49.9±1.6*	46.3±3.2**

Note. Each group consisted of 8-12 animals; ***$p<0.05$; *$p<0.001$, *+$p<0.05$ compared with rats subjected to hyperthermia.

in the body [1]. Since carnosine inhibits LPO in hyperthermia, it was analyzed for the effectiveness against severe hyperthermia leading to high mortality. Experiments were performed on 24 rats: 12 rats served as the control and 12 rats were given carnosine. Survival rate after a 1-h hyperthermia (45°C) in the control group was 25%, while in carnosine-treated rats (*per os*, 250 mg/kg, 2.5 h before hyperthermia) in was 67%.

Our results confirm the hypothesis that LPO plays a pathogenic role in the mechanisms of stress-induced damage to the body and indicate that carnosine increases the resistance of the organism not only to hypo- but also to hyperthermia.

REFERENCES

1. V. A. Baraboi, I. I. Brekhman, V. G. Golotin, and Yu. B. Kudryashov, *Peroxidation and Stress* [in Russian], St. Petersburg (1992).
2. I. L. Vaisfel'd and G. N. Kassil', *Histamine in Biochemistry and Physiology* [in Russian], Moscow (1981).
3. O. V. Vasilenko, L. I. Deev, and O. V. Lyubovtseva, *Proceedings of the Moscow Society of Scientists. Biological Systems Under Different Conditions* [in Russian], Moscow (1982), pp. 71-72.
4. E. N. Goncharenko, L. I. Deev, M. Ya. Akhalaya, *et al.*, *Vestn. Mosk. Universiteta. Ser. Biologiya*, No. 2, 37-41 (1995).
5. L. I. Deev, M. Ya. Akhalaya, E. A. Illarionova, and Yu. B. Kudryashov, *Byull. Eksp. Biol. Med.*, **95**, No. 5, 51-53 (1983).
6. L. I. Deev, M. Ya. Akhalaya, and Yu. B. Kudryashov, *Ibid.*, **92**, No. 8, 28-30 (1981).
7. O. V. Naumova, L. I. Deev, and E. N. Goncharenko, *Biokhimiya*, **57**, No. 9, 1373-1377 (1992).
8. G. A. Sivoraksha and Yu. N. Sidel'nikov, *Lab. Delo*, No. 2, 51-54 (1991).
9. S. Yu. Yudenfrend, *Fluorescence Analysis in Biology and Medicine* [in Russian], Moscow (1965).
10. T. Asakava and S. Matususita, *Lipids*, **15**, No. 1, 137-140 (1980).
11. E. N. Jeffery, A. Kotake, R. El-Arhary, and G. J. Mannering, *Mol. Pharmacol.*, **13**, No. 3, 415-425 (1977).
12. E. P. Miller and R. P. Maikel, *Life Sci.*, **9**, No. 3, 747-752 (1970).
13. B. Schoene, R. A. Fleischmann, and H. Remmer, *Eur. J. Clin. Pharmacol.*, **4**, No. 2, 65-73 (1972).