BIOPHYSICS AND BIOCHEMISTRY

Modulatory Effect of Carnosine and Related Compounds on the Oxidative Burst in Barium Sulfate-Activated Leukocytes

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The effect of carnosine and its natural derivatives on the generation of reactive oxygen species by leukocytes and on the myeloperoxidase reaction was studied. The chemiluminescence of samples in the course of cell activation was measured in the presence of luminol or lucigenin. Carnosine was found to produce a two-way effect, i.e., neutralization of singlet oxygen and hypochlorite anion simultaneously with activation of the system of superoxide-anion generation.

Key Words: *carnosine and related compounds; leukocyte oxidative burst; myeloperoxidase*

After the direct antioxidant action of carnosine had been described [3], it was subjected to a thorough investigation. The compound was demonstrated to be able to bind singlet oxygen [9], peroxyl radical [13], hydroxyl radical [7], and hypochlorite anion [6]. However, even the unequivocally proven antioxidant properties of carnosine [8] failed to give the whole picture of its biological activity. Its immunostimulatory activity remained unexplained [4], and reports appeared concerning the ability of carnosine and its derivatives to provoke the reactive oxygen species (ROS) formation under certain conditions [10,14]. All this led to the need to compare the effects of carnosine and its natural derivatives on ROS generation by leukocytes under various conditions.

MATERIALS AND METHODS

The total preparation of leukocytes was obtained from 20 ml of heparinized rabbit blood following 10-min hemolysis with 5 volumes of 0.83% NH_4Cl followed by washing with phosphate-buffered saline (2.7 mM KCl, 136.7 mM NaCl, 1.5 mM KH_2PO_4, and 8.1 mM Na_2HPO_4, pH 7.4 [hereafter designated as Ph-Sal], at 4°C). The cell sediment was resuspended in Hanks solution with a final cell concentration of 3-5 mln/ml, and stored cooled for not more than 2 hours before the start of an experiment [5].

Chemiluminescence (CL) of the samples in the course of cell activation was recorded in the presence of luminol or lucigenin (both in a concentration of 10^{-5} M) at 37°C, pH 7.4, on a PKhL-1 recording chemiluminometer, the cells being activated with barium sulfate (3 mg per sample) [1].

Detection of the myeloperoxidase (MP) reaction was performed by two methods. In one variant, using the same chemiluminometer, we measured CL during the enzymatic reaction in the Ph-Sal with the addition of 40 μM H_2O_2, 2 nM MP, and 10^{-5} M luminol. In the other method, the catalytic activity of MP was measured by spectrophotometry

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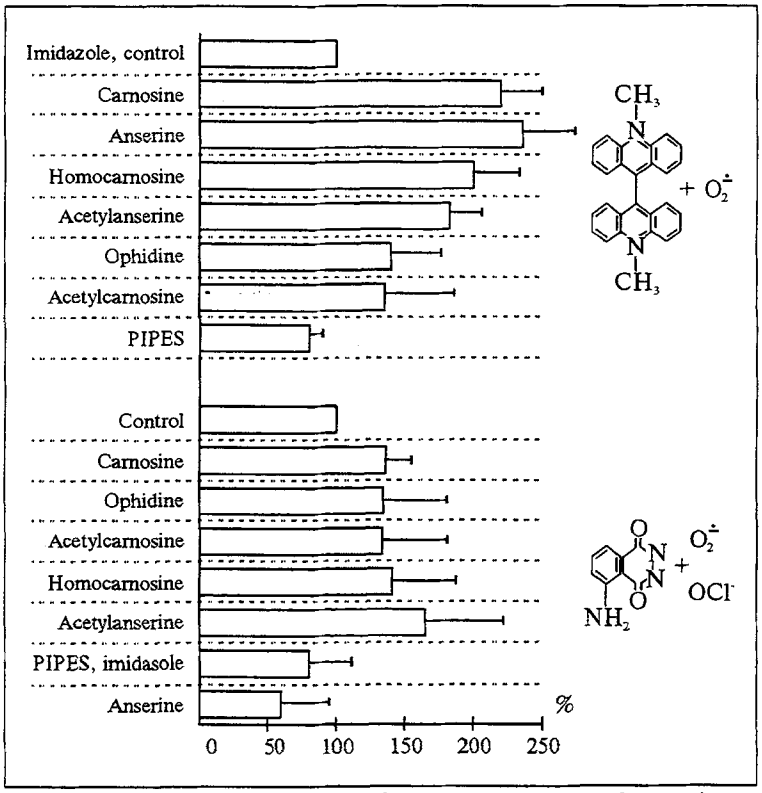

Fig. 1. Effect of carnosine and related compounds on the maximum CL signal of leukocytes stimulated by BaSO4, expressed in percentage of control (without addition of compounds) in the presence of lucigenin (top part) and luminol. The test compounds were used in a concentration of 10 mM; the results of 5 independent experiments and values of mean square deviations are presented.

in a medium containing o-dianisidine instead of hydrogen peroxide. A unit of enzyme activity was defined here as an increment of optical density in the sample by 0.001 unit for 1 min at wavelength 460 nm [2]. The first method made it possible to characterize simultaneously the effect of the test substances on the MP reaction and its product -hypochlorite anion. By the second method only the rate of the enzymatic process could be evaluated, due to exclusion of the interactions of the substances with hypochlorite anion.

TABLE 1. Effect of Carnosine and Related Compounds on the Level of Maximum CL Signal Induced by the Course of the MP Reaction (the Concentrations Inducing 50% and 90% Inhibition of CL are Presented; the Results of a Typical Experiment Are Given)

Compound	Signal inhibition, %	
	50	90
Anserine (nitrate)	0.05	0.5
Carnosine	0.20	1.5
Homocarnosine	0.50	2.6
Histidine + β-alanine	0.50	3.2
Histidine	0.55	3.7
β-alanine	5.00	Not detected up to 10 mM
Taurine	0.10	2.6

Carnosine, anserine, and homocarnosine used in most of the experiments were synthesized by Sigma (anserine is produced in the form of nitrate). In the experiments with cells we also used preparatively isolated carnosine (99.5% purity) and anserine (99% purity), whose effects were the same as those of commercial preparations. Ophidine, preparatively isolated from whale muscle (99.9% purity), was a generous gift of Dr. X. Abe (Kyoritzu Women's University, Japan). Acetylated dipeptide derivatives synthesized by B. Kh. Shavratskii, were of the following purity: acetylcarnosine 93% (7% free carnosine); acetylanserine 100%. The purity of carnosine and its derivatives was controlled by high-performance liquid chromatography. Luminol and Hanks solution were of Russian manufacture; the other reagents were purchased from Sigma.

The H_2O_2 concentration was estimated by spectrophotometry at wavelength 230 nm, ε=72.4 $M^{-1}\times cm^{-1}$.

RESULTS

The leukocyte cell activation with barium sulfate induces a flash of luminescence, and the shape of the curve is identical regardless of the use of lucigenin or luminol, although in the case of luminol ten times fewer of cells are needed to attain the same level of response. The "enhancers" of the cell response used in our experiments interact with different ROS: lucigenin interacts mostly with the oxygen superoxide anion, while luminol reacts also with singlet oxygen and other ROS, the most abundant of which under the given conditions is represented by hypochlorite anion [11]. It was shown accordingly, that the addition of superoxide dismutase totally inhibits the cell response in the presence of lucigenin but only by 70% in the presence of luminol.

In the presence of luminol the addition of imidazole (10 mM) weakly inhibits the cell response, while carnosine produces an effect that is dependent on the state of the cells, i.e., 10% activation in freshly prepared samples, and 20-30% inhibition in cells prestored for more than 3 h. In the presence of lucigenin, imidazole did not produce any visible effect, whereas carnosine markedly enhanced the reaction when introduced into each sample individually and interfered with total

inhibition of the cell response with superoxide dismutase.

These experiments show that carnosine manifests a two-way effect. Neutralizing singlet oxygen and hypochlorite anion, as was shown earlier [6,9], it simultaneously activates the system of superoxide-anion generation. The equivocal effect of carnosine on cell activation by barium sulfate in the presence of luminol may be due to a change in the ratio between different leukocyte-generated ROS in the course of cell aging.

We compared the ability of carnosine-related compounds to affect the $BaSO_4$-induced cell activation recorded in the presence of luminol or lucigenin (Fig. 1). Freshly isolated cells were used in these experiments. It can be seen that in the presence of luminol carnosine, ophidine, and acetylcarnosine produce no reliable effect on the reaction (slight activation); homocarnosine and acetylanserine both reliably enhance CL ($p<0.05$), and anserine acts as a potent inhibitor of CL.

In the presence of lucigenin all tested compounds (excluding imidazole) enhanced CL, though to different degrees. The most active were carnosine and homocarnosine, and the least active, ophidine and acetylcarnosine. These data point to a certain correlation between the structure and activity of the compounds in question. In order to obtain direct results, we studied the MP reaction using both methods of recording.

The CL method revealed marked inhibition of the MP reaction by various carnosine derivatives (Table 1). Among the inhibitors, anserine proved the most potent, histidine the least potent, and homocarnosine and taurine equally potent. Additional experiments were set up using KNO_3, which had no appreciable effect upon the sample CL when applied in a concentration of less than 1 mM. Anserine used in the same concentration exhibited its inhibitory effect in full.

The results obtained could reflect both an interaction of carnosine and its derivatives with OCl^-, a product of the MP-mediated reaction, and their direct effect on the enzyme. Therefore, we measured the MP reaction according to the oxidation of o-dianisidine, performing it in the presence of the compounds tested (Table 2). Almost all of them, with the exception of anserine and acetylcarnosine, inhibited the reaction rather weakly, even when given in a concentration of 10 mM. It should be pointed out that acetylcarnosine, while unable to bind the reaction product, still significantly reduced its rate. Even more potent inhibitory activity was exhibited by anserine; this product also inhibits leukocyte activation measured in the presence of luminol.

TABLE 2. Effect of Carnosine and Related Compounds on MP Activity

Compound, 10 mM	Activity, % of control
Histidine + β−alanine	81±19
Homocarnosine	77±13
Histidine	75±17
Carnosine	66±12
Acetylcarnosine	50±8
Anserine	0

Thus, obviously, the effect of anserine includes both the removal of the reaction product and direct inhibition of the enzyme. In the case of carnosine, homocarnosine, and histidine (alone or mixed with β-alanine), the inhibition of the reaction is due mostly to the neutralization of its product.

The findings show that the substitution of β-alanine for the γ-aminobutyric acid residue in the carnosine molecule leads to a reduced function of hypochlorite neutralization, acetylation leads to the loss of this function, while methylation considerably enhances it, imparting additional properties to the dipeptide. These facts explain the importance of carnosine metabolism in the organs and tissues of vertebrates.

REFERENCES

1. Yu. A. Vladimirov, M. P. Sherstnev, and A. P. Piryazev, *Biofizika*, **34**, № 6, 1051-1055 (1989).
2. N. Yu. Govorova, S. N. Lyzlova, B. P. Sharonov, and O. Yu. Yankovskii, *Biokhimiya*, **52**, 1670-1676 (1987).
3. A. M. Dupin, A. A. Boldyrev, Yu. V. Arkhipenko, and V. E. Kagan, *Byull. Eksp. Biol. Med.*, **97**, № 5, 186-188 (1984).
4. E. G. Kurella, V. V. Mal'tseva, L. S. Seslavina, and S. L. Stvolinskii, *Ibid.*, **110**, № 7, 52-53 (1990).
5. A. A. Totolyan, N. V. Shamkova, and O. Yu. Danilevskii, Lab. Delo, № 4, 215 (1986).
6. V. E. Formazyuk, T. Yu. Gorshkova, A. A. Boldyrev, and V. I. Sergienko, *Biokhimiya*, **57**, № 9, 1324-1329 (1992).
7. O. I. Aruoma, M. J. Lauchton, and B. Halliwell, *Biochem. J.*, **264**, 863-869 (1989).
8. F. F. Boldyrev, *Int. J. Biochem.*, **25**, 1101-1107 (1993).
9. T. Dahl, R. Midden, and P. Hartmann, *Photochem. Photobiol.*, **47**, 357-362 (1988).
10. Z. Hartman and P. Hartman, *Chem. Biol. Interact.*, **84**, 153-168 (1992).
11. M. E. Holt, *Brit. J. Exp. Path.*, **65**, № 2, 231-241 (1984).
12. Y. Gyllenhammar, *J. Immunol. Methods*, **97**, № 2, 209-213 (1987).
13. R. Kohen, Y. Yamamoto, K. Condy, and B. Ames, *Proc. Nat. Acad. Sci. USA*, **85**, 3175-3179 (1988).
14. X. Shi, N. Dalal, and K. Kasprzak, *Arch. Biochem. Biophys.*, **299**, 154-162 (1992).