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Comparison of the Antioxidant Activity of Carnosine in Different Chemical and Biological Models

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The antioxidant activity of carnosine has been demonstrated in several laboratories by different methods [2,3,9-11]. However various authors have obtained diverse values of carnosine efficiency as an antioxidant [4,8,10,11], which could be due to various sources of carnosine preparations differing in purity, as well as to differences in methodology. For this reason we compared the efficacy of carnosine manufactured by Sigma and Serva with that obtained by column chromatography from meat extract at a Russian drug factory. The comparison was performed with the use of different chemical and biological models.

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MATERIALS AND METHODS

Different methods of inducing free-radical reactions were used to compare the interaction of carnosine with different active forms of oxygen: chemical reactions (interactions with H_2O_2, ClO^-, and OH formed in the Fenton reaction); model processes in which superoxide anion formation in the biological structures was induced by iron ions; and free-radical generation in leukocyte suspension provoked by $BaSO_4$ (mainly O^-_2 and ClO^- and, to a lesser extent, $OH^\bullet$).

The products of carnosine interaction with active forms of oxygen in chemical reactions were measured spectrophotometrically (Hitachi-557); in model processes malonic dialdehyde was measured as the major thiobarbituric acid reaction product whose formation was induced by the addition of Fe^{2+} ions to rat

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serum lipoproteins [7] or to rabbit skeletal muscle sarcoplasmic reticulum fragments [3]. The rabbit serum leukocyte reaction to the addition of $BaSO_4$ suspension was assessed in the presence of luminol [5] with a PKhL-1 luminometer (Russia).

RESULTS

All of the carnosine preparations used were characterized by a relatively high purity but were contaminated with optically active compounds (Fig. 1). In the absorption spectra of the Sigma and Serva carnosines additional peaks (shoulders of absorption) were found at 260-320 nm which were absent in carnosine prepared from meat extract (Fig. 1). The content of these optically active contaminants was not more than 0.5%, but they could be responsible for the diversity in the effects of different carnosine preparations. We thus compared the efficacies of carnosine preparations obtained from different sources with the use of several models of free-radical generation.

The nature of impurities was not specially examined, but it is noteworthy that after carnosine interaction with active oxygen forms obtained during water radiolysis, two optically active products were registered with absorption maxima at 265 and 320 nm [4]. One of them, identified as a charge transfer complex, could be similar to the stable chloramine

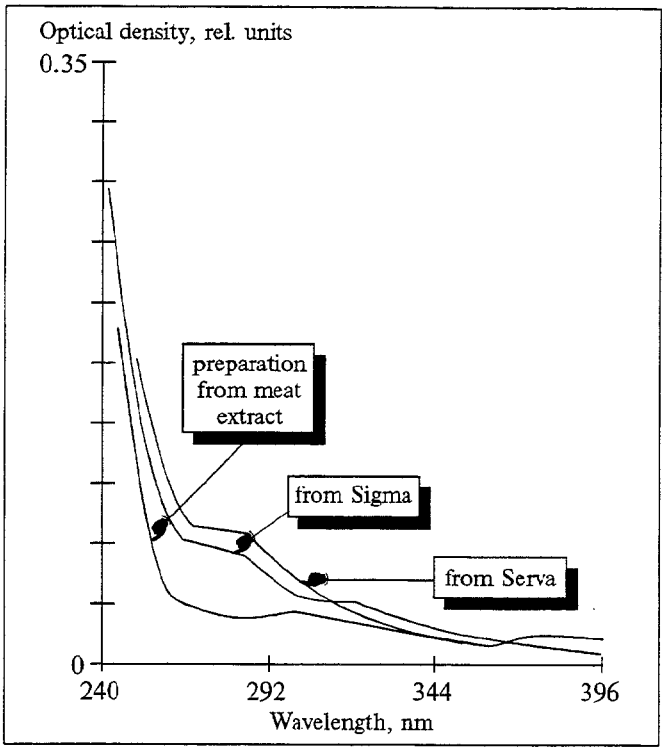

Fig. 1. Absorption spectra of 5 mM aqueous solutions of carnosine.

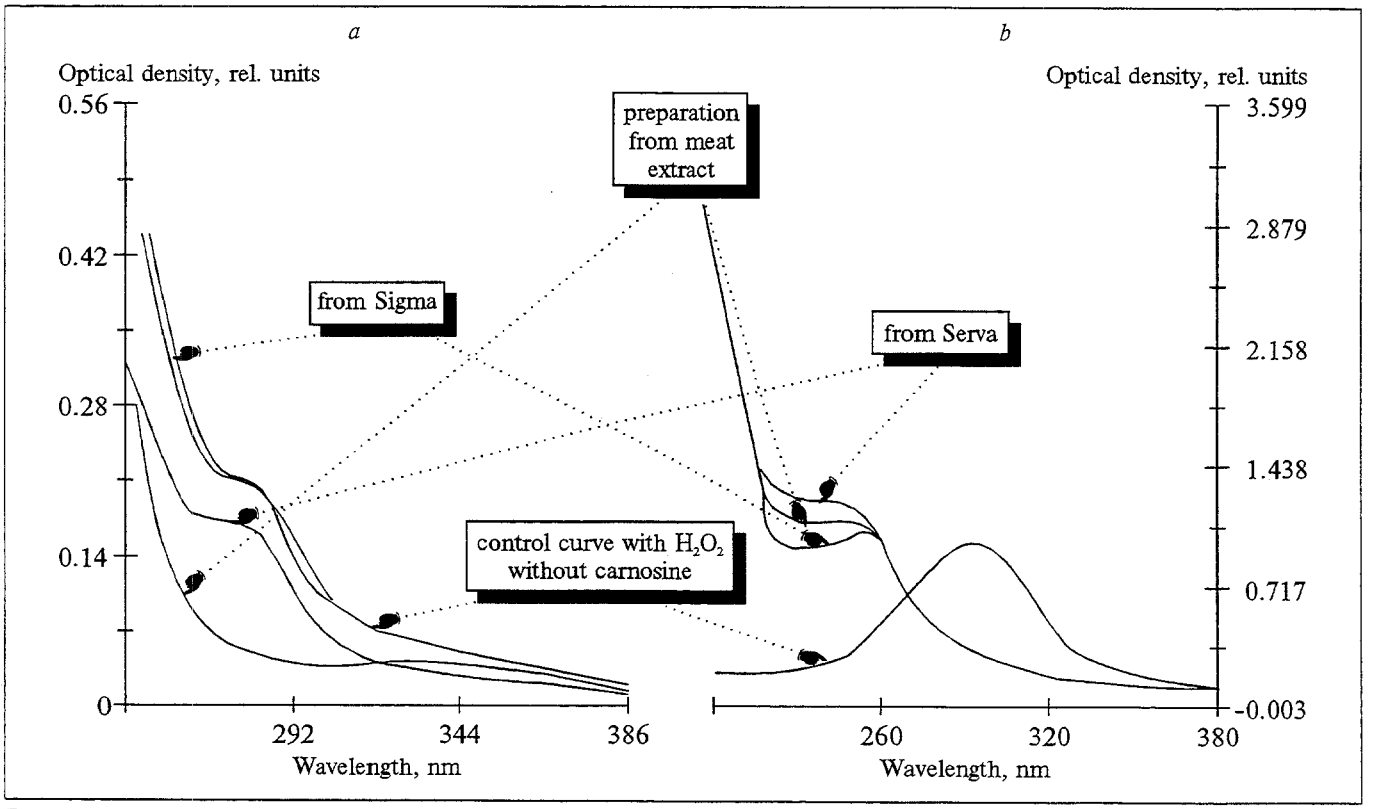

Fig. 2. Absorption spectra of aqueous carnosine solutions after interaction with H_2O_2 (*a*) or hypochlorous anion (*b*). Registration 48 h (*a*) or 1 h (*b*) after start of reaction. Temperature 25°C. Concentrations of carnosine 5 mM, of H_2O_2 1%, and of sodium hypochlorite 4 mM.

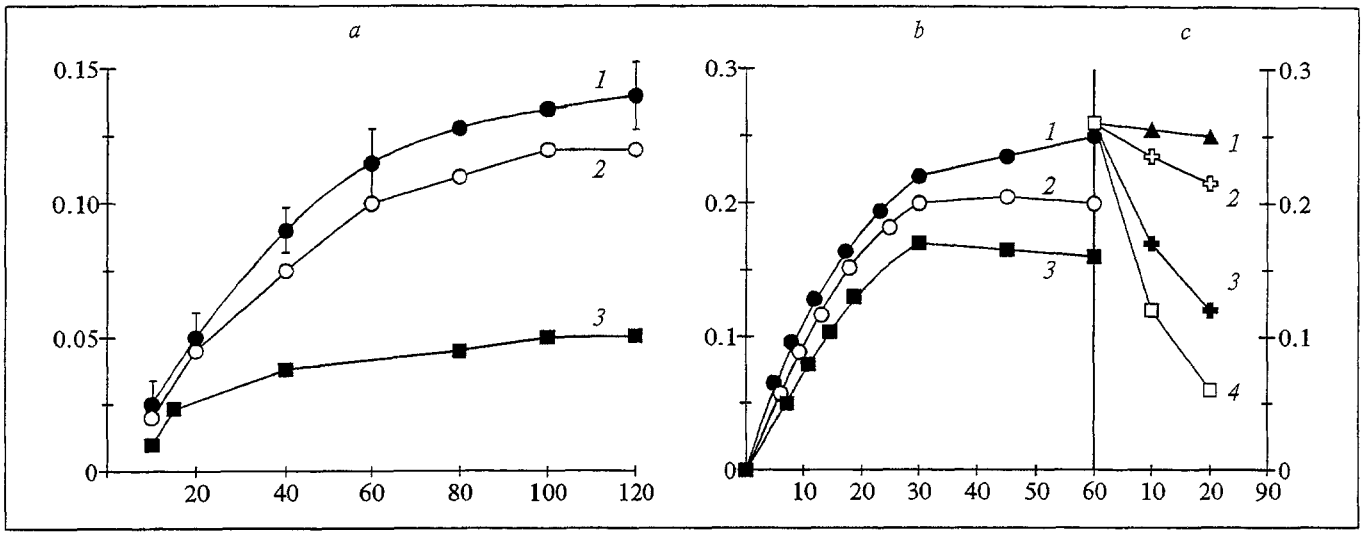

Fig. 3. Accumulation of LPO products induced by 200 μM Fe^{2+} added to suspension of rat serum lipoproteins (*a*, [7]) and rabbit sarcoplasmic reticulum (*b*, [3]). Ordinate: optical density at 535 nm (thiobarbituric acid reaction products); abscissa: time of reaction or concentration of reagents, as shown. 1) control; 2) native carnosine, 5 mM; 3) carnosine from Serva, 1 mM. *b*: 1) control; 2, 3) carnosine from Sigma: original (3) and after recrystallization (2), both in 50 mM concentration. c: concentration dependence for TBA reaction products measured 30 min after addition to sample (at 60 min of reaction) of different carnosine preparations: 1) native preparation; 2, 3) the same as in b; 4) mother liquor after recrystallization of 50 mM Sigma sample.

complex obtained in the reaction of carnosine with ClO^- (absorption maximum 265 nm) [6].

The interaction of carnosine with H_2O_2 in aqueous solution led to an increase of the absorption shoulder at 265 nm. The reaction proceeded very slowly: the product appeared clearly only after 24 h, the differences in the spectra of various carnosine preparations being negligible (Fig. 2, *a*).

No differences were found in the efficacies of the carnosine preparations used in reaction with hypochlorous anion. This reaction was an extremely rapid one [6]. Ten minutes after 5 mM carnosine were mixed with 4 mM hypochlorite, the ClO^- peak disappeared and the shoulder on the carnosine spectrum was registered in the range of 255-270 nm, corresponding to the chloramine complex described earlier. It is seen from Fig. 2, *b* that the nature of the spectral curves obtained does not depend on the source of carnosine.

It was also impossible to discriminate between different carnosines during Fenton reaction registration under previously described conditions [1]. After 2.5 min from the start of the experiment the ESR signal used to estimate the number of $OH^\cdot$ radicals interacting with the spin trap was suppressed by 70-80% in the presence of 40 mM carnosine, whatever its source. Therefore, we concluded that in chemical reactions with direct inductors of free-radical reactions the efficiency of carnosine is determined by its own properties and by the nature of the reaction, while contaminating admixtures do not modify its activity.

Another picture was observed in biological models when lipid peroxidation of rat serum lipoproteins or rabbit sarcoplasmic reticulum was induced by Fe ions alone (Fig. 3, *a*) or together with ascorbic acid (Fig. 3, *b*). In this case the effective increase in LPO products (registered as the amount of malonic dialdehyde) was noticeably inhibited only by synthetic carnosine. Native preparation free of contaminations was either much less effective (in the case where rat serum lipoproteins were used) or ineffective altogether (with rabbit sarcoplasmic reticulum). To elucidate the reasons for such a diversity between synthetic and native preparations, we recrystallized the Sigma compound and found that its efficiency was considerably decreased and the shoulder in the spectrum between 260 and 320 nm largely removed; at the same time, the mother liquor after recrystallization was found to possess a strong inhibitory activity (Fig. 3, *c*). Thus, we found that commercial carnosine preparations contain negligible but active contaminants characterized by antioxidant activity and tentatively referred to as intermediate products of carnosine interaction with free-radical forms of oxygen.

This conclusion is in good agreement with the hypothesis on the antioxidant action of carnosine [9]; at the same time, it raises the problem of the compatibility of data obtained with carnosines from different sources. As we know, some authors have used native preparations [1,4,6,7], others commercial ones [3,4,10-11]. One may wonder, therefore, whether the antioxidant activity of carnosine is an intrinsic feature or is caused by impurities in the preparation used. In the latter case the biological action of carnosine should be explained by another mechanism.

To solve this problem, we used a cellular model of active forms of oxygen generation in which a burst

of free-radical formation in rabbit leukocytes was induced by $BaSO_4$ and registered in the presence of luminol as a chemiluminescent response [5]. We could reliably measure this reaction even though the amount of malonic dialdehyde formed was at least 10 times less than in previous experiments (Fig. 3). In these experiments carnosine effectively suppressed the oxidizing response of leukocytes, the native preparation being even more effective than the synthetic one.

The data presented show that the antioxidant activity of carnosine is governed by carnosine itself, the result of this action being not only local suppression of the chain LPO process, but also more intrinsic cell reactions, such as immunomodulating activity, stimulation of wound healing, stress adaptation, etc., - effects recently described [9]. At the same time, it is necessary to reinvestigate some effects of carnosine obtained with the use of commercial preparations in order to elucidate the contribution of the contaminations to the phenomena described.

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Effects of Steroid Hormones and Anti-Migraine Drugs on Serotonin Transport in Platelets of Patients Suffering From Migraine and in Those of Healthy Subjects

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Serotonin appears to play an important role in the pathogenesis of migraine. It has been established that during an attack of migraine the secretion of 5-hydroxyindoleacetic acid (the main serotonin metabolite) rises [9], while the serotonin level falls in platelets [2,4]. It is significant that migraine attacks are most often preceded by states characterized by elevated blood levels of steroid hormones (stress, emotional tension, use of contraceptives, etc.). Normally, steroid hormones probably do not participate in the regulation of serotonin transport in nerve endings or platelets [7,8]. Since increased levels of steroid hormones figure prominently among the factors triggering migraine attacks, the serotonin-transporting system of

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