error. The plasma emoxipine clearance under stress conditions took place more slowly than normally, but faster than after an infarct. The results are thus evidence of a marked effect of the experimental pathological states on the pharmacokinetics of emoxipine. The possibility cannot be ruled out that similar changes in kinetics will also be observed during its therapeutic use, and this must be taken into account when an adequate system of dosage and optimal schedules of treatment of postinfarct states are worked out.

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INTERACTION OF CARNOSINE WITH SUPEROXIDE RADICALS IN AQUEOUS SOLUTIONS

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UDC 612.015.3:577.112.382.3].014.482

KEY WORDS: carnosine; superoxide radicals.

The study of the biological role of the natural skeletal muscle dipeptide carnosine (β-alanyl-L-histidine) has led to the discovery of its marked antioxidative activity [3, 7]. Carnosine was found not only to inhibit ascorbate-dependent membrane lipid peroxidation (LPO) and to interact with lipid conversion products [1], but also to quench singlet oxygen [10] and, in the form of a complex with Zn^{2+} and Cu^{2+}, to exhibit superoxide dismutase activity [2]. Investigation of the antiradical activity of carnosine by pulsed radiolysis may help to shed light on the mechanism of its biological action. There is information in the literature on interaction of carnosine with the primary products of radiolysis of water, but these results were obtained under anaerobic conditions [11, 14]. In the present investigation, eth method of pulsed radiolysis of water under conditions of synchronous spectrophotometric recording of optical absorption of short-living particles was used to study the kinetics of formation and destruction of intermediate radiolysis products in the presence of carnosine under aerobic conditions.

It was concluded from the results that carnosine interacts with the superoxide anion and the OH-radical and that it can form a complex with the superoxide anion with charge transfer with a maximum of absorption at 265 nm.

EXPERIMENTAL METHOD

Pulsed radiolysis of aqueous solutions of carnosine was carried out on the apparatus of the A. N. Frumkin Institute of Electrochemistry, Academy of Sciences of the USSR, using the U12 accelerator (E = 4.5 MeV, τ = 2.2 μsec, dose per pulse 30-40 Gy) and with spectrophotometric recording of the intermediate products thus formed, by the method described pre-

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 391-393, October, 1990. Original article submitted March 19, 1990.

0007-4888/90/0010-1374$12.50

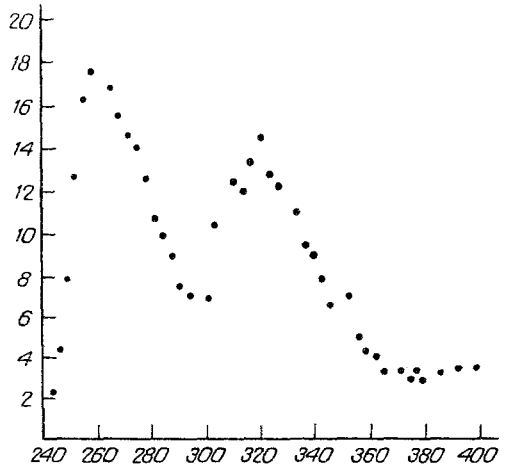

Fig. 1. Difference absorption spectrum of carnosine solution incubated in the cold under aerobic conditions, and of freshly prepared (original) solution. Concentration of carnosine 20 mM, K-phosphate buffer, pH 7.4. Abscissa, wavelength (in nm); ordinate, optical density (in relative units $\times 10^2$).

viously [4], within the spectral band 245-670 nm and within the time interval from 2 μsec to 20 sec after the end of the electron pulse. The continuous flow system enabled the specimens to be continually changed and saturated with various gases (He, O_2, N_2O). Dosimetry of the absorbed radiation was carried out by ferrocyanide and thiocyanate methods [5].

The radiation stability of carnosine was estimated in doses of 0.4 to 40 kGy. The radiolysis products were analyzed by high-performance liquid chromatography under isocratic conditions, in a medium of 0.1% trifluoroacetic acid (Beckman-332, Austria) on a 3.3 × 300 mm column with C18 "Separon" carrier (Czechoslovakia). All the experiments were carried out in 10 M phosphate buffer (pH 5.5-9.7).

EXPERIMENTAL RESULTS

When aqueous solutions of carnosine were kept under aerobic conditions, an increase in optical density of the solution was found in the 260-350 nm region. Two maxima, caused by absorption of chromophore groups at 265 and 320 nm were found on the difference spectrum of "oxidized" and freshly prepared solutions in air (Fig. 1). After pulsed radiolysis of the aqueous solutions of carnosine under aerobic conditions absorption also appeared at λ_{max} = 265 nm; the increase in optical density, moreover, was a linear function of radiation dose within the range 0.02-2 kGy. This indicated that the product formed (P_{265}) was sufficiently stable under these conditions of radiolysis.

The scheme given below shows the basic processes taking place during interaction of high-energy electrons with water, and some important reactions of intermediate radiolysis products [6]:

$$H_2O \rightsquigarrow \bar{e}_{aq},\ OH,\ H,\ H_2,\ H_2O_2,\ H_3O^+;$$
$$\bar{e}_{aq} + O_2 \rightarrow O_2^{\cdot-};$$
$$H + O_2 \rightarrow H^+ + O_2^{\cdot-};$$
$$2\,O_2^{\cdot-} \xrightarrow{pH} O_2 + H_2O_2;$$
$$\bar{e}_{aq} + N_2O + H^+ \rightarrow N_2 + OH;$$
$$HCOO^- + OH \rightarrow CO_2^{\cdot-} + H_2O,\quad CO_2^{\cdot-} + O_2 \rightarrow CO_2 + O_2^{\cdot-}.$$

The addition of high concentrations of HCCONa selectively scavenges OH radicals formed in the course of radiolysis [6]. In the presence of 150 mM sodium formate radiolysis was accompanied by an almost threefold decrease in accumulation of the chromophore with λ_{max} = 265 nm; this indicated that the product is the result of interaction of carnosine with OH-radicals and not with the superoxide anion O_2^-, for during interaction of the hydroxyl group with formate in the presence of oxygen, an additional quantity of O_2^- is formed. The velocity constant of interaction of carnosine with the OH-radical, according to our calculations,

TABLE 1. Velocity Constants of Certain Processes Taking Place during Radiolysis of Carnosine under Aerobic Conditions (M ± m)

Carnosine, mM	pH	K1, $M^{-1} \cdot sec^{-1} \cdot 10^{-5}$	K2, $sec^{-1} \cdot 10^{-2}$
0	7.4	24,3±1*	–
0,02	—	8,0±0,7	—
0,2	—	7,4±0,5	—
2	—	5,3±0,5	—
20	—	4,3±1,5	1,95±0,4
0	5,5	358±40	—
2	—	734±60	—
2	9,7	—	138±45

Legend. Molar extinction coefficient for the superoxide anion-radical at pH 7.4 taken to be 220 $M^{-1} \cdot cm^{-1}$, and at pH 5.5, 2000 $M^{-1} \cdot cm^{-1}$ [8]. Asterisk indicates case of disproportionation of O_2^- in the absence of carnosine.

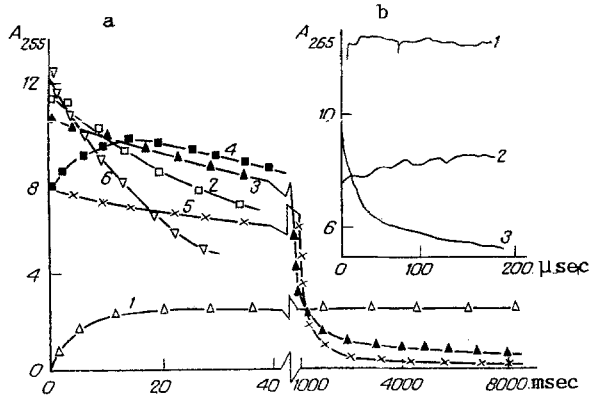

Fig. 2. Kinetics of destruction of superoxide anion and its complex with carnosine. a) concentration of carnosine (in mM): 0 (1), 0.02 (2), 2 (3), or 20 (4), pH 7.4. Theoretical curves describing destruction of complex of carnosine with superoxide anion and P_{265} accumulation on curves 4-5 and 6; b) kinetics of breakdown of Carn... O_2^-, obtained in the presence of 2 mM carnosine at different pH values: 7.4 (1), 9.7 (2), and 5.5 (3). Ordinate, optical density (A) at 255 (a) and 265 (b) nm (in relative units $\times 10^2$).

was $9 \cdot 10^9$ $M^{-1} \cdot sec^{-1}$, in agreement with data obtained for this reaction of carnosine and histidine, published in the literature [14].

Under anaerobic conditions (after 40 min of saturation of the sample with He or N_2O) pulsed radiolysis of aqueous solutions of carnosine led to the formation of a chromophore with λ_{max} = 275 nm (P_{275}) instead of λ_{max} = 265 nm; moreover, optical density at 275 nm increased in medium with N_2O, which increases the concentration of the OH-radical [6], and it was significantly reduced in the presence of formate. Consequently, the formation of the P_{265} product during interaction of carnosine with radiolysis intermediates requires the participation of oxygen in the initial stages of the reaction, whereas the P_{275} product can be formed as a result of direct interaction of carnosine with the OH-radical. Yet another product, whose appearance we recorded in these experiments, had an optical absorption band with λ_{max} = 320 nm.

Radiolysis of aqueous solution of carnosine at pH values less than pK_a for the imidazole ring (pK_a = 6.8) did not lead to the formation of optically active products in the 260-280 nm region, which suggests that it is the nonprotonated imidazole ring that is involved in these reactions. Meanwhile, interaction of carnosine with $O_2^{\cdot-}$ is observed also at acid pH values (pH 5.5).

On chromatographic fractionation of radiolysis products obtained in the presence of carnosine (20 mM, pH 9.7, dose 80 kGy) four compounds were found, whose optical spectra included an absorption band corresponding to the imidazole group. A band with λ_{max} = 265 nm was present only in the first of them (as regards the time of elution from the column), confirming the absence of subsequent conversions of P_{265} during radiolysis. Recording fast spectral changes after a single electron pulse revealed several processes taking place in carnosine solution in the presence of oxygen.

The superoxide anion with spectral characteristics identical with those described in the literature [8] reacts very quickly with carnosine – a bathochromic shift of the $O_2^{\cdot-}$ absorption maximum takes place after less than 5 μsec, even with the minimal concentration of the dipeptide. These results also show that carnosine forms a complex with $O_2^{\cdot-}$ with a low dissociation constant, of similar type to the complex with charge transfer Carn...$O_2^{\cdot-}$ [13]. The molar extinction coefficient calculated for this complex was 1790 $M^{-1}\cdot cm^{-1}$. The kinetics of destruction of this complex can be followed by studying the fall in optical density at 255 nm. This shows that an increase in the carnosine concentration in the solution leads both to lowering of the level of $O_2^{\cdot-}$ formed under these conditions, and measured immediately after the end of the electron pulse, and to a decrease in the rate of its decomposition (Fig. 2a). With a high carnosine concentration in the solution, accumulation of P_{265} also is observed (Curve 4). The continuous curves in Fig. 2a correspond to the calculated kinetics of destruction of the complex of carnosine with the superoxide anion (Curve 5) and accumulation of the P_{265} product (Curve 6) under these conditions.

The rate of destruction of the Carn...$O_2^{\cdot-}$ complex, measured at 265 nm under conditions when P_{265}-formation was very small, changed only a little at neutral and alkaline pH values but rose sharply at pH 5.5 (Fig. 2b), indicating instability of the complex of carnosine with the superoxide anion at acid pH values.

The schemes of interaction of carnosine with radiolysis products are given below:

$$\mathrm{Carn}+O_2^{\cdot-} \rightarrow \mathrm{Carn}\ldots O_2^{\cdot-} \xrightarrow[\mathrm{pH}]{k_1} \text{disproportionation}$$

$$\mathrm{Carn}+\mathrm{OH}\rightarrow \mathrm{Carn}^{\cdot}\cdot\mathrm{OH}\rightarrow^{O_2} O_2\cdot\mathrm{Carn}\cdot\mathrm{OH}\xrightarrow[\mathrm{pH}]{\mathrm{Carn}} P_{265}\rightarrow P_{275}$$

in accordance with this scheme carnosine interacts with $O_2^{\cdot-}$ or with the OH-radical and oxygen independently. The complex of carnosine with $O_2^{\cdot-}$ disproportions without the formation of optically active products. For the formation of the product with λ_{max} = 265 nm, interaction of the adduct of carnosine with the OH-radical and O_2 with the other carnosine molecule is necessary. The constants calculated in accordance with this sequence of reactions are given in Table 1. Assuming that the formation of one P_{265} molecule at alkaline pH values takes place as a result of equimolar interaction of carnosine and the OH-radical, the molar extinction coefficient for this product can be calculated: its value is 1220 $M^{-1}\cdot cm^{-1}$.

The results show that carnosine in aqueous solutions interacts actively with $O_2^{\cdot-}$ and OH-radicals, and as a result stable long-living compounds may be formed, and this mechanism is able to explain the stabilizing properties of carnosine as an effective hydrophilic antioxidant [1, 3, 7, 12]. They also explain differences in the character of the antioxidative effect of carnosine at alkaline and acid pH values [3, 9].

The authors are grateful to A. A. Komissarov for efficient help with the chromatographic fractionation of the radiolysis products.

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EFFECT OF THE LIPOSOMAL FORM OF DIATRIZOATE ON COMPOSITION OF BLOOD AND ORGAN LIPIDS IN EXPERIMENTAL ANIMALS

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UDC 616.153.915-092.9-073.755.4

KEY WORDS: blood lipids; diatrizoate; liposomes.

In recent years there has been a steady increase in the use of liposomes as carriers of drugs for their targeted transport to organs of the reticuloendothelial system, both in the USSR and elsewhere [2, 6]. The use of liposomes in x-ray diagnosis has proved to be particularly promising. It has been shown that with the aid of the liposomal form of diatrizoate (Triombrast) the x-ray contrast of the spleen, liver, blood vessels, kidneys, urine, large intestine, and boundaries of tumors can be increased [1, 7, 8].

However, before liposomes can be used as a transport system for clinical purposes, their safety has to be guaranteed. In this connection it is important to know how the liposomal form of diatrizoate will affect the lipid composition of the organs and tissues, for its introduction into the blood stream in a dose of 2.5 ml of liposomal suspension/kg body weight for man will be accompanied by considerable lipid loading (for example, lecithin 75 mg/kg and cholesterol 35 mg/kg). The aim of the present investigation was accordingly to study the effect of the liposomal form of diatrizoate on the lipid composition of the blood and organs in laboratory animals, as judged by the hydrolysis and utilization of the administered lipids.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats weighing 120-150 g, kept on the standard animal house diet. Intact animals and those receiving the test preparation were deprived of food for 12-14 h before sacrifice.

The liposomal form of diatrizoate was obtained by the method described in [1].

The rats were given an intravenous injection of the liposomal form of diatrizoate (Triombrast, 76% solution, produced by the M. V. Lomonosov Kiev Pharmaceutical Chemical Factory) in the ordinary clinical dose of 1 ml/kg body weight (the doses are comparable for content of diatrizoate).

Central Roentgeno-Radiologic Research Institute, Ministry of Health of the USSR, Leningrad. N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 393-395, October, 1990. Original article submitted July 18, 1989.

 0007-4888/90/0010-1378$12.50