to protect neurons effectively from hypoxic death and widen prospects for the use of these compounds in disorders of cerebral circulation leading to ischemic disease.

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Effect of Carnosine on the Activity of Lipoxygenase Isolated from Rabbit Reticulocytes

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The effects of the histidine-containing dipeptide carnosine and of histidine on the activity of 15-lipoxygenase from rabbit reticulocytes are studied. It is shown that low concentrations of carnosine (0.5-4 mM) activate oxidation of arachidonic acid by lipoxygenase, while high concentrations (>4 mM) inhibit the enzyme activity. Histidine elicits no activatory effect, although its inhibitory activity is comparable to that of carnosine.

Key Words: *15-lipoxygenase; reticulocytes; arachidonic acid; carnosine*

The processing of arachidonic acid (AA) results in the formation of prostaglandins and thromboxanes (the cyclooxygenase pathway) and leukotrienes and lipoxins (the lipoxygenase pathway) [6]. 15-Lipoxygenase of reticulocytes was originally described in 1979 [12]. This enzyme oxidizes AA to leukotrienes and actively produces lipoxin B_4 without the involvement of other lipoxygenases [10]. The lipoxygenase reaction is characterized by activation with lipoperoxides [11]. No other naturally occurring activators of these enzymes have been found. The enzymes are inhibited by various compounds, including antioxidants [1], since oxidation of unsaturated fatty acids is a free-radical process.

We examined the effect of the naturally occurring histidine-containing dipeptide carnosine, which elicits a potent antioxidant effect *in vivo* and *in vitro* [7], on the lipoxygenase pathway of AA oxidation. In addition to antioxidant activity, this dipeptide exhibits antistressor, immunomodulating,

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and radioprotective activities [2,3,5]. *In vivo* its effects are observed at concentrations one or two orders of magnitude lower than those required for an antioxidant effect *in vitro* [8]. Bearing in mind the significance of the AA oxidation cascade as a source of biologically active lipoperoxidase radicals, as well as the possible interaction between lipoxygenase and carnosine in pathological states, we undertook the present study as a tool for better understanding the mechanisms of carnosine action *in vivo*.

MATERIALS AND METHODS

A homogenous preparation of 15-lipoxygenase from rabbit reticulocytes was purified by preparative isoelectrofocusing as described previously [12]. Arachidonic acid in a final concentration of 40 μM was used as a substrate. It was added to the reaction mixture as an ethanol solution (the final concentration of ethanol was less than 5% of the sample volume). The reaction was carried out at 25°C in a thermostatically controlled quartz cuvette (2.5 ml) in 40 mM K/Na-phosphate buffer (pH 7.4 at 25°C), the buffer being partially replaced by the studied compounds, which also have buffering properties. The reaction was started by adding the enzyme, and the rate of diene conjugate production was determined from the increase in light absorbance at 235 nm (ε =25,000 opt.units/M×cm). The measurements were performed in a double-beam Hitachi-557 spectrophotometer in the differential regime, using sample lacking the enzyme as a reference probe. Kinetic characteristics were analyzed using designated software (a generous gift of Dr. L. V. Gurevich). The reaction rate was calculated from the initial linear portion of the curve.

Arachidonic acid and histidine were from Merck. Carnosine (Medical Preparations Plant, St. Petersburg) was 99% pure (repeated recrystallization). Other reagents of chemically pure grade were from domestic sources.

RESULTS

Figure 1 shows typical curves for AA oxidation with 15-lipoxygenase from rabbit reticulocytes and the effects of various carnosine concentrations on this process. The reaction was essentially linear for the first 45-50 sec, and the accumulation of reaction products reached the maximum at 180 sec. The enzyme was activated by low (1-2 mM) and stimulated by high (8 mM) carnosine concentrations.

The reaction rate (V) and accumulation of the reaction products by 120 sec (P) were compared in

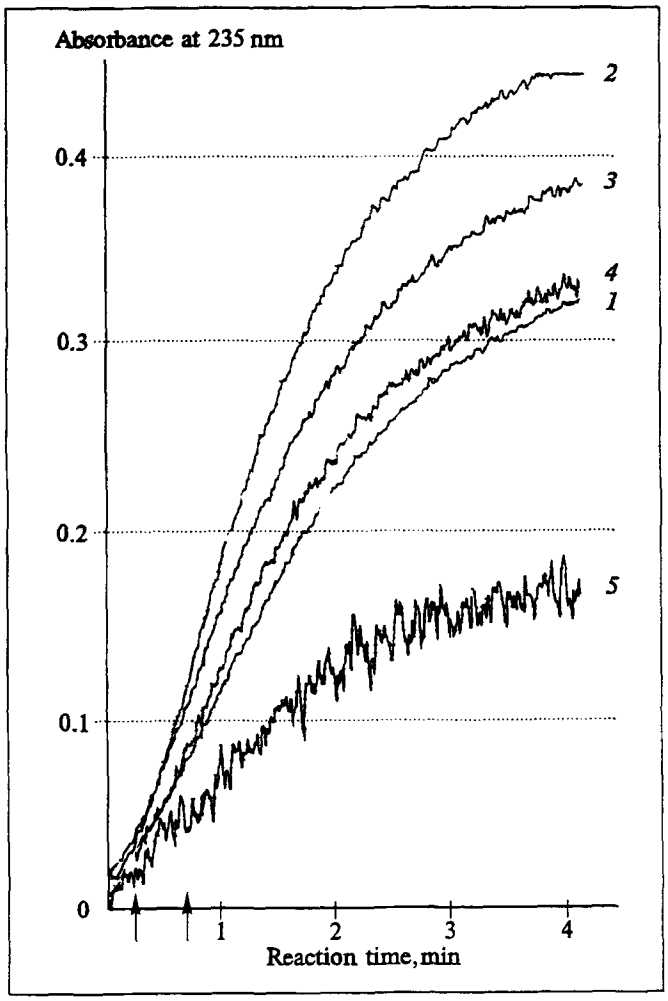

Fig. 1. Kinetics of AA oxidation with 15-lipoxygenase of rabbit reticulocytes in the control and in the presence of different carnosine concentrations. Time intervals used for calculations are indicated with vertical arrows. *1*) control (40 mM phosphate buffer); *2*, *3*, *4*, and *5*) 1, 2, 4, and 8 mM carnosine, respectively (39, 38, 36, and 32 mM phosphate buffer, respectively).

the control and experimental probes (V_c/V_c; P_c/P_c) containing carnosine and histidine in the corresponding concentrations. Histidine was used for comparison with carnosine because it is a natural precursor of carnosine but lacks its specific activity [9]. The results are summarized in Table 1.

At concentrations <4 mM carnosine activated 15-lipoxygenase, while at concentrations >5 mM it inhibited the enzyme. A direct antioxidant effect of carnosine in nonenzyme peroxidation systems *in vitro* has been observed at a concentration of 10 mM and higher [8]. Histidine does not activate lipoxygenase, although the inhibitory effect observed for 8 mM histidine is comparable to that of carnosine. At a concentration of 20 mM (not shown) carnosine almost completely inhibited the lipoxygenase reaction, while histidine suppressed it by 60%.

TABLE 1. Effects of Carnosine and Histidine on the Activity of 15-Lipoxygenase from Rabbit Reticulocytes (Means of 3-5 Measurements Expressed as a Percentage of the Control)

Studied compound, mM	V_e/V_c	P_e/P_c
Carnosine		
0.25	103±8	96
0.50	116±11	119±11
0.75	138±16	132
1.00	122±16	125±25
2.50	114±27	102±42
4.00	102±21	108±26
8.00	74±11	76±15
Histidine		
0.50	106	108
1.00	94±19	98±9
4.00	90±2	91±6
8.00	76±26	68±33

In the *in vivo* experiments all the documented effects of carnosine were observed at concentrations one or two orders of magnitude lower than those providing antioxidant activity *in vitro* [8]. For example, when administered intraperitoneally to stressed animals in a dose of 0.2 mg/kg body weight [2], carnosine inhibited the accumulation of lipid peroxidation products in the blood and brain, i.e., when the tissue content of the preparation did not provide even the minimum antioxidant concentration at which significant inhibition of peroxidation has been observed *in vitro*.

The carnosine concentration inducing activation of lipoxygenase in this study is 10- to 20-fold lower than that required for a direct antioxidant effect and is comparable to serum carnosine levels. This suggests that carnosine is a naturally occurring modulator of lipoxygenase, and its administration *in vivo* generates a biphasic effect: inhibition of blood cell lipoxygenase is followed by its activation as carnosine is hydrolyzed by carnosinase. Thus, the effect of carnosine on the whole organism should not be confined to alterations in the tissue content of the peroxidation products.

Previously we reported that carnosine stimulates the colony-forming activity of hematopoietic stem cells, thus enhancing the organism's resistance to radiation; however, the mechanism of this phenomenon is unclear [3]. At the same time, there is evidence that very low concentrations of lipoxin B_4, a product of the lipoxygenase oxidation of AA, affects the proliferation and differentiation of the granulocyte-monocyte colony-forming units, being a potent biological effector capable of modulating the proliferation and differentiation of the leukocyte precursor stem cells [4]. If this is so, the release of small concentrations of carnosine against the background of the AA oxidation cascade (which occurs in different pathologies, including radiation damage) should create conditions for increasing the efficiency of the hematopoietic system and protect the organism against damaging factors.

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