ANTIRADICAL AND ANTIOXIDATIVE EFFECT OF ARGININE AND ITS INFLUENCE ON LIPID PEROXIDATION ACTIVITY DURING HYPOXIA

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The broad spectrum of regulatory action of arginine is determined by its chemical structure. Several very important metabolites are formed from arginine, and their protective and antioxidant effect is now known [1, 6, 14]. The well-marked cationic properties of the guanidine group of arginine, its ability to form complexes and undergo protonation, or to form cross-linkages with aldehydes, suggest that not only arginine derivatives, but also the amino acid itself, can regulate peroxidation processes in membranes.

Accordingly the antiradical and antioxidative properties of arginine were studied in model experiments, and the effect of exogenous arginine on the intensity of lipid peroxidation (LPO) also was studied in the microsomal membranes of the liver and of the rat testes during hypoxia.

EXPERIMENTAL METHOD

The antiradical activity of L-arginine hydrochloride was estimated from the degree of inhibition of the reaction to form a superoxide anion-radical in two systems. System 1 comprised: 50 mM carbonate buffer, pH 10.2, 0.1 mM EDTA, 1.2 mM nitro-blue tetrazolium (nitro-BT), 1 mM hydroxylamine, and 0.03% Triton X-100 [13]. System 2 comprised: 0.066 M phosphate buffer, pH 7.8; 1 mM EDTA, 0.407 mM nitro-BT, 1.8 μM phenazine metasulfate, 1 mM NADH, and 1 mg gelatin [11]. Incubation was carried out for 10 min under aerobic conditions at 25°C. The optical density of the reduced nitro-BT was measured on a Beckman DU-7 spectrophotometer (USA) in a thermostated cuvette. LPO was induced in blood plasma in a system containing bivalent iron (10^{-8}) and ascorbic acid (10^{-6}); in Tris-HCl buffer, pH 7.4.

Experiments to study the action of altitude hypoxia (9000 m above sea level for 1 h) were carried out on male albino rats. A group of animals was given an intraperitoneal injection of L-arginine hydrochloride in a dose of 120 mg/100 g body weight 30 min before the experiment. Lipids were extracted [10] from the microsomal membranes of the liver and blood plasma, after which concentrations of diene conjugates [2] and Schiff bases [9] were determined. A solution of quinine sulfate in 0.1 N sulfuric acid was used as the standard. Concentrations of total lipids were determined [5]. The numerical results were subjected to statistical analysis [4].

EXPERIMENTAL RESULTS

Addition of 0.5 and 1.0 μmoles of arginine to the system generating the superoxide anion-radical during autooxidation of hydroxylamine inhibited the process of reduction of nitro-BT by 15-17% (Fig. 1). In a system containing NADH and phenazine metasulfate, addition of the same concentrations of arginine inhibited reduction of nitro-BT by 13-15% (Fig. 2). These findings are evidence of the antiradical activity of arginine. Evidently arginine can react directly with the superoxide anion-radical, and it can also serve as an effective trap for singlet oxygen and the hydroxyl radical [8].

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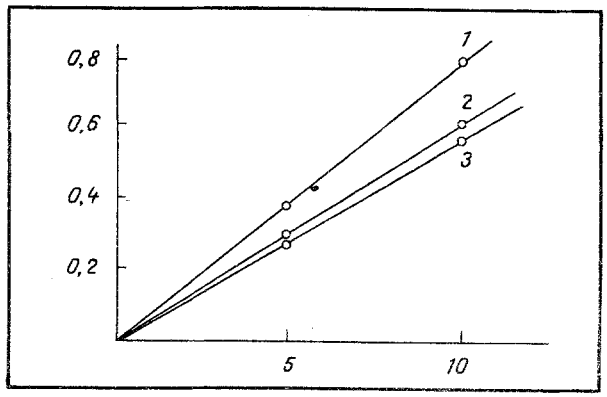

Fig. 1. Kinetics of inhibition of reaction of superoxide anion-radical formation during autooxidation of hydroxylamine. 1) Without arginine; 2) 0.5 μmole arginine; 3) 1.0 μmole arginine. In Figs. 1 and 2: abscissa, time of superoxide anion-radical generation (in min); ordinate, optical density units of reduced nitro-blue tetrazolium.

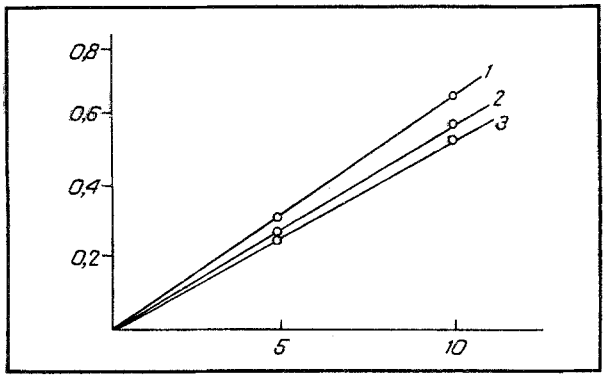

Fig. 2. Kinetics of inhibition of reaction of superoxide anion-radical formation in a system of NADH – phenazine metasulfate. 1) Without arginine; 2) 0.5 μmole arginine; 3) 1.0 μmole arginine.

TABLE 1. Regulation of Ion-Ascorbate-Dependent LPO in Blood Plasma

Parameter	Control	Iron-ascorbate-dependent induction of LPO		
			arginine, μmole	
			0,5	1,0
Diene conjugates, μmoles/mg lipid	5,25±0,32 (11)	7,50±0,45 (9)	3,91±0,39 (8)	4,87±0,61 (10)
p		<0,001	<0,02	
p_m			<0,001	<0,001
Schiff bases, relative units/mg lipid	1,33±0,13 (10)	3,55±0,70 (11)	1,73±0,26 (11)	1,78±0,28 (11)
p	<0,01	<0,01		
p_m			<0,05	<0,05

Legend. p) Significance of differences relative to control, p_1) relative to samples with induced LPO. Number of experiments given in parentheses.

Incubation of the blood plasma for 30 min in the presence of ferrous and ascorbate ions activates LPO (Table 1). Addition of arginine (0.5 and 1.0 μmole) to the incubation system lowered the level of diene conjugates by 48-35% and of Schiff bases by 50-51%. It follows from the data given above that arginine is an inhibitor of both the initial and the final stages of LPO

TABLE 2. Content of Diene Conjugates and Schiff Bases in Plasma and Microsomes of Rat Liver during Hypoxia and Intraperitoneal Injection of Arginine

Number of experiments	Diene conjugates, μmoles/mg lipid		Schiff bases, relative units/mg lipid	
	plasma	liver microsomes	plasma	liver microsomes
Control	4,61±0,52 (14)	9,06±0,93 (8)	1,41±0,12 (11)	2,21±0,22 (8)
Control+arginine	4,23±0,34 (6)	10,57±1,61 (8)	1,83±0,14 (6)	2,00±0,12 (13)
p	>0,1	>0,2	>0,1	>0,5
Plasma	20,21±1,88 (10)	18,20±1,08 (9)	3,40±0,44 (6)	2,83±0,20 (11)
p	<0,001	<0,001	<0,001	<0,05
Hypoxia+arginine	6,74±0,84 (6)	10,53±2,43 (7)	1,47±0,13 (6)	2,42±0,62 (8)
p	<0,05	>0,5	>0,1	>0,5
p_1	<0,001	<0,02	<0,002	>0,1

Legend. p) Significance of differences relative to control, p_1) relative to hypoxic series. Number of experiments given in parentheses.

due to its antiradical activity and to the presence of imino- and amino-groups, reacting with intermediate cross-linking LPO products, i.e., aldehydes [3].

One of the leading mechanisms of the damaging action of hypoxia is intensification of processes of free-radical lipid oxidation [7, 12]. The value of antioxidants in hypoxia of varied etiology has been demonstrated [7]. The discovery of the antiradical and antioxidative properties of arginine enabled us to use it as an antihypoxic agent.

Under altitude hypoxia conditions considerable accumulation of LPO products was observed (Table 2). Preliminary injection of arginine restored the normal intensity of LPO during hypoxia. The concentration of diene conjugates in the plasma and liver microsomes fell by 67-42% and that of Schiff bases by 57-15% compared with animals in a state of hypoxia.

The endogenous metabolite arginine thus possesses antiradical and antioxidative properties, and this may be a basis for its protective effects under extremal conditions.

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