PROTECTION OF MONOAMINE OXIDASE IN BRAIN SYNAPTOSOMES BY LIPID- AND WATER-SOLUBLE ANTIOXIDANTS DURING LIPID PEROXIDATION

A. D. Antipov, V. A. Tyurin,
N. F. Avrova, A. E. Khovanskikh,
and V. E. Kagan

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Monoamine oxidase (MAO) is an enzyme located chiefly in the outer mitochondrial membrane, which catalyzes the deamination of several neurotransmitters and biogenic amines, as well as amine-containing xenobiotics, including drugs [3, 4, 13]. As a result of this reaction active forms of oxygen (O_2^-, H_2O_2) are formed, and in the presence of metals of variable valency, these give rise to hydroxyl radicals and may initiate the process of lipid peroxidation (LPO) [2]. Considering the wide distribution of MAO, this effect may be of great importance despite the very low rate of induction of LPO in the course of MAO-catalyzed reactions. Evidently the nearest target for attack by active forms of oxygen and also by radical and molecular products of LPO may be the enzyme itself, which is a lipid-dependent integral membrane protein [6, 11]. It has been shown that the result of this oxidative modification of MAO is not its inhibition, but its qualitative transformation, manifested as disappearance of activity against some substrates and the appearance of ability to deaminate others, such as diamines [3].

MAO is represented in the membrane in many different forms, differing in their substrate specificity, their sensitivity to inhibitors, and also their lipid dependence [3, 8, 11, 14]. It is considered that MAO, by oxidative deamination, maintains the physiological level of mediators in the presynaptic ending [4]. Considering the central role of MAO in the metabolism of many neurotransmitters and the assumed participation of MAO inactivation in the etiology of many nervous and mental diseases [3, 6], the search for stabilizers protecting MAO against the damaging action of free radicals is an exceedingly important matter.

The aim of this investigation was to compare the effectiveness of the protective action of a lipid-soluble (α-tocopherol) and a water-soluble (the histidine-containing dipeptide, carnosine) antioxidant on the activity of different types of MAO, on the fatty-acid composition of lipids, and hydrophobicity and microviscosity of the membranes during induction of LPO by an Fe^{2+}—ascorbate system in synaptosomal membrane of the rat cerebral cortex.

EXPERIMENTAL METHOD

Synaptosomal membranes were obtained from the rat cerebral cortex by the method of Hajos [9]. Lipid phosphorus was determined by the method in [15] and the protein concentration by a modified method of Lowry et al. [10]. LPO was induced by addition of Fe^{2+} in a concentration of 10 μM and ascorbate in a concentration of 0.5 mM to a medium containing Tris-HCl 40 mM, NaCl 100 mM, pH 7.4. The reaction was stopped by the addition of 4-methyl-2,6-di-tert-butylphenol in a concentration of 0.5 mM. Accumulation of LPO products was recorded by the reaction with 2-thiobarbituric acid (TBA) and by the formation of hydroperoxides of polyene lipids, as in [5]. The fatty-acid composition of the lipids was studied on a Pye-104 gas—liquid chromatograph (England) with flame-ionization detector, identifying fatty acids relative to a standard mixture. To study the molecular organization of the lipids in the synaptosomal membranes, the method of EPR-spin probing was used. The protein concentration in these experiments was 20-30 mg/ml. EPR spectra were recorded on a small EPR spectrometer in capillary tubes 1 mm in diameter. The accuracy of thermostatic control of the specimen was 0.5°C.

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. Institute of Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 169-171, February, 1989. Original article submitted May 30, 1988.

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TABLE 1. Effect of α-Tocopherol and Carnosine on Accumulation of LPO Products and MAO Activity (in nmoles semicarbazone or NH_3/mg protein/min) in Synaptosomal Membranes during Induction of LPO (M ± m)

Experimental conditions	MDA, nmoles/mg protein	Hydroperoxides, nmoles/mg phospholipids	Serotonin	Tyramine	Benzylamine	Tryptamine	Histamine	Cadaverine
Control	1,2±0,1	5,4±0,3	1,4±0,1	2,2±0,3	1,4±0,1	2,7±0,2	—	—
LPO	5,0±0,2	44,8±2,7	0,8±0,1	1,4±0,1	1,2±0,1	2,2±0,1	0,18±0,02	0,11±0,01
LPO + α-tocopherol	4,0±0,2	16,1±0,6	1,3±0,1	1,9±0,1	1,3±0,1	2,6±0,1	0,03±0,01	0,02±0,01
LPO + carnosine	4,4±0,2	25,1±0,9	1,0±0,1	1,3±0,1	1,2±0,1	2,3±0,1	0,14±0,01	0,08±0,01
LPO + α-tocopherol + carnosine	3,5±0,2	12,2±0,7	1,4±0,1	2,1±0,2	1,3±0,1	2,7±0,1	0,02±0,01	0,01±0,01

TABLE 2. Effect of α-Tocopherol and Carnosine on Parameters S and h of Synaptosomal Membranes during LPO Induction (M ± m)

Experimental conditions	S	h
Control	0,73±0,01	0,60±0,02
LPO	0,77±0,01	0,53±0,02
LPO + α-tocopherol	0,73±0,01	0,60±0,02
LPO + carnosine	0,75±0,01	0,53±0,02
LPO + α-tocopherol + carnosine	0,73±0,01	0,60±0,02

The parameters of organization S and hydrophobicity of the medium h were calculated as in [1]. MAO activity was determined as accumulation of ammonia [7] and by a modified semicarbazone method [12]. Semicarbazide was added after incubation of the enzyme. Serotonin creatinine-sulfate ($2.5 \cdot 10^{-4}$-$2.5 \cdot 10^{-5}$ M), benzylamine hydrochloride ($25.0 \cdot 10^{-4}$-$12.5 \cdot 10^{-5}$ M), tryptamine hydrochloride ($2.5 \cdot 10^{-5}$-$10.0 \cdot 10^{-4}$ M), and tyramine hydrochloride ($7.5 \cdot 10^{-4}$-$2.5 \cdot 10^{-5}$ M) were used as MAO substrates.

EXPERIMENTAL RESULTS

Data on the action of LPO and also of antioxidants on MAO activity in synaptosomal membranes are given in Table 1. Clearly as a result of incubation of the membranes in the presence of LPO inducers (Fe^{++}, ascorbate) cumulation took place both of primary LPO products (lipid hydroperoxides) and of secondary products, carbonile compounds interacting with TBA. Activation of LPO was accompanied by lowering of MAO activity for serotonin, tyramine, benzylamine, and tryptamine, but there was a simultaneous increase in diamine oxidase activity, i.e., induction of LPO under the conditions used led to qualitative transformation of MAO, described previously in [3]. In the presence of lipid-soluble (α-tocopherol) and water-soluble (carnosine) LPO inhibitors their protective action was observed against accumulation of LPO products and also against changes in MAO activity. It will be noted that the protective action of carnosine was much weaker, and the degree of prevention of LPO activation corresponded to the protective effect against MAO.

The results confirm data obtained previously on the possibility of preventing qualitative transformation of MAO by ionol [3]. Experiments to study the combined action of water-soluble and fat-soluble antioxidants are the most interesting. In this case the protective effect of LPO inhibition was higher than the action of these compounds separately, although lower than their additive action. It can be postulated that the mechanisms of the inhibitory action of α-tocopherol and carnosine are not identical. In other words, during the combined action of the water-soluble and lipid-soluble antioxidants, scavenging of radicals both in the aqueous phase (oxygen radicals) and in the lipid phase (lipid radicals) leads to more complete inhibition of free-radical oxidation of lipids. It is interesting to note that the combined protective effect of α-tocopherol and carnosine relative to MAO also exceeded the protective action of each of these compounds separately. This probably means that the modifying action of radical and molecular LPO intermediates on MAO can also be realized in both aqueous and hydrophobic phases.

The data in Table 2 confirm that carnosine realizes its effects mainly through interaction with water-soluble radicals. The parameters of the EPR spectra of the hydrophobic

TABLE 3. Effect of α-Tocopherol and Carnosine on Fatty Acid Composition of Synaptosomal Membrane Lipids during Induction of LPO

Fatty acids	Relative content, % of total				
	control	Induction of LPO			
			α-tocopherol	carnosine	α-tocopherol + carnosine
Monoenic	20,9	18,8	20,1	19,7	21,0
Polyenic	34,3	25,7	28,6	27,5	29,6
Saturated	44,7	55,5	51,3	52,8	49,4
Unsaturated	55,2	44,5	48,7	47,2	50,6
Index of unsaturation	188,5	147,0	155,9	151,9	164,3

Legend. When calculating the content of polyunsaturated fatty acids the following acids were taken into consideration: 18:2, 20:3ω6, 20:4ω6, 20:4ω3, 22:4ω6, 22:5ω6, 22:6ω3.

spin 5-doxylstearic acid were evidently strongly modified as a result of LPO: S is increased and h reduced. Data on the increase in polarity of the hydrophobic zone of the membrane and the decrease in molecular mobility of the lipids in it are in agreement with results obtained previously [5]. Carnosine, a hydrophilic LPO inhibitor, exerts only a very weak action on the values of both S and h. Conversely the hydrophobic inhibitor α-tocopherol, when added separately or together with carnosine, prevents the effects of LPO virtually completely. The results of the study of changes in the fatty acid composition of synaptosomal membrane lipids during induction of LPO in the presence and in the absence of antioxidants are in agreement with these views (Table 3). Induction of LPO causes a decrease in the fraction of polyenic fatty acids, and this is reflected in a decrease in the index of unsaturation of the fatty acids. Both antioxidants give a protective effect, but it is more marked in the case of α-tocopherol. During the combined action of α-tocopherol and carnosine the protective effect is virtually identical with that of α-tocopherol alone.

It can be concluded from these results that with the combined use of water-soluble and lipid-soluble antioxidants their protective effects become greater than with the use of either of them separately. This indicates different mechanisms for their interaction with radical intermediates of peroxide oxidation. The added protective effect occurs, apparently, as a result of interception of radicals in the aqueous as well as the lipid phase. Probably the choice of optimal combinations of antioxidants should be based on the establishment of effective combinations of aqueous and lipid soluble compounds.

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