The kinetics of monoamine deamination in the heart and brain of rats thus changes after an exposure of 5 min to hyperbaric oxygen. The kinetics of monoamine deamination in the compensatory and preconvulsive phases of oxygen epilepsy differs considerably; in some cases the disturbances observed in the first phase disappear in the second phase or are reversed. The most marked changes in catalytic properties of MAO due to hyperbaric oxygenation in the preconvulsive period are observed in the rat's heart and not in its brain, evidence of the important role of the cardiovascular system in the genesis of oxygen poisoning, and they lead to the conclusion that, as is reflected also in the electrophysiological data, in the preconvulsive period of oxygen epilepsy definite changes are observed also in neurotransmitter metabolism and, in particular, at the level of their oxidative deamination.

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ACTION OF A HOMOLOGOUS SERIES OF UBIQUINOLS ON LIPID PEROXIDATION IN BRAIN MITOCHONDRIAL AND SYNAPTOSOMAL MEMBRANES

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Radicals of oxygen and lipids and molecular products of lipid peroxidation (LPO), which can damage the membrane apparatus of nerve cells, are nowadays regarded as pathogenetic factors in a whole range of mental and organic diseases of the CNS (schizophrenia, Down's syndrome, senile dementia, Parkinson's disease, etc.), and also in drug-induced diseases caused by the action of antidepressants and neuroleptics [3, 5, 12]. These ideas are based on experimental data which indicate an exceptionally strong damaging action of free-radical reactions on such important neuronal functions as reception and transmembrane transmission of impulses, and also on uptake and release of neurotransmitters [6, 10]. The following factors have been named as possible causes of excessive accumulation of LPO products: hyperproduction of active forms of oxygen, deficiency of enzymic and nonenzymic antioxidative systems and a combination of these two factors. The two most important systems generating active forms of oxygen are the electron-transport chains of the endoplasmic reticulum and mitochondria respectively. Oxygen radicals are generated in the endoplasmic reticulum as a result of NADPH-dependent regeneration of components of the NADPH electron transport chain: cytochrome P-450 reductase and cytochrome P-450 itself. Oxygen reduction products in mitochondria are firmly bound with the active center of cytochrome oxidase, but they can "leak" to other components of the electron-transport chain, from ubiquinone for example.

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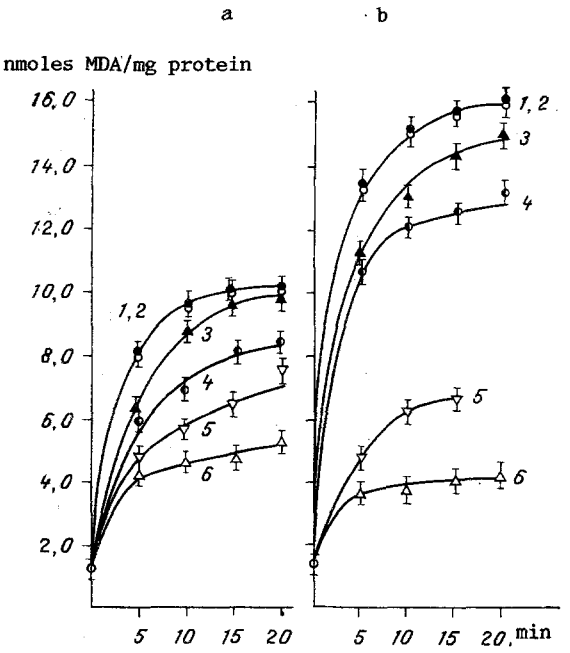

Fig. 1. Accumulation of LPO products in synaptosomal (a) and mitochondrial (b) membranes in presence of oxidized and reuced forms of ubiquinols (10^{-4} M) on induction of LPO in a Fe^{++}–ascorbate system. 1) Control, 2) in presence of oxidized Q_{10}, 3) in presence of oxidized Q_4, 4) in presence of reduced Q_0, 5) in presence of reduced Q_{10}, 6) in presence of reduced Q_4.

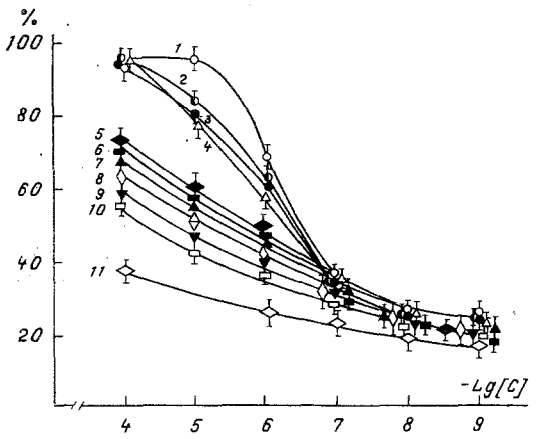

Fig. 2. Dependence of inhibition of LPO in synaptosomal membranes on concentration of ubiquinols with isoprenoid chains of different lengths. Here and in Fig. 3: abscissa, concentration of ubiquinols, M; ordinate, percentage of inhibition of LPO. Compound added: 1) Q_1, 2) Q_2, 3) Q_3, 4) Q_4, 5) Q_5, 6) Q_6, 7) Q_7, 8) Q_8, 9) Q_9, 10) Q_{10}, 11) Q_0.

Activity of NADPH-dependent mono-oxygenases is very low in nerve cells and can hardly be regarded as a realistic mechanism of production of active forms of oxygen. Ubiquinones, electron carriers in the mitochondrial electron-transport chain, are converted during cyclic oxidation and reduction into ubisemiquinone radicals, reducing oxygen to superoxide [15]. The main components of the electron-transport chain are long-chain ubiquinones Q_9 and Q_{10} [9]. Meanwhile short-chain homologs Q_1-Q_3 frequently act as substrates for partial reactions of the respiratory chain [9]. The ubiquinols formed as a result of complete reduction have an antioxidant action like that of tocopherol [8]. There is experimental evidence that both tocopherols and ubiquinones are utilized in the course of LPO as a result of interaction with radical intermediates [8], and they can be regenerated by endogenous reducing agents, such as ascorbate, glutathione, and others, whose concentration in nerve tissue is sufficiently high [11].

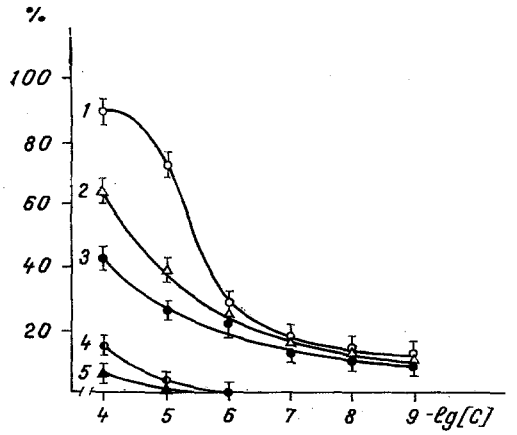

Fig. 3. Dependence of inhibition of LPO in mitochondrial membranes on concentration of oxidized and reduced forms of ubiquinols. In presence of: 1) reduced Q_4, 2) reduced Q_{10}, 3) reduced Q_0, 4) oxidized Q_4, 5) oxidized Q_{10}.

The aim of the present investigation was accordingly to study the action of ubiquinones and ubiquinols with isoprenoid chains of different length (Q_1-Q_{10}) and of benzoquinone (Q_0) on ascorbate-dependent LPO in mitochondrial and synaptosomal membranes of the rat brain.

EXPERIMENTAL METHOD

Mitochondrial and synaptosomal membranes were obtained from the gray matter of the rat brain by the method described in [4] with certain modifications. The protein concentration was determined by the microbiuret method. LPO was induced by an Fe^{++}–ascorbate system in medium of the following composition: 40 mM Tris-HCl, 100 mM NaCl, pH 7.4. The final concentration of Fe^{++} and ascorbate was 20 μM and 0.5 mM respectively. Accumulation of malonic dialdehyde (MDA), a secondary product of LPO, was determined by the reaction with 2-thiobarbituric acid (TBA) [13]. The concentrations of ubiquinones and ubiquinols were determined spectrophotometrically [15]. Homologs of ubiquinones were obtained from Esai Co. (Japan).

EXPERIMENTAL RESULTS

The dynamics of accumulation of LPO products (interacting with TBA) in synaptosomal and mitochondrial membranes of the rat brain on induction of LPO by a Fe^{++}–ascorbate system, and also with the addition of oxidized and reduced forms of ubiquinones (Q_0, Q_4, Q_{10}), is shown in Fig. 1. The velocity of LPO can be seen to be significantly higher in mitochondrial than in synaptosomal membranes. Reduced forms of ubiquinones (ubiquinols) had an inhibitory action on LPO in membranes of both types; oxidized ubiquinones had a very weak action, and the curves of accumulation of LPO products were almost indistinguishable from the controls. Homologs of ubiquinols differed in their inhibitory action, Q_4 being most effective, whereas Q_{10} and Q_0, in the concentration used (10^{-4} M), were less effective. Results relating to effectiveness of action of ubiquinol homologs are shown in more detail in the form of concentrated dependences in Figs. 2 and 3. As these data show, short-chain homologs of ubiquinols (Q_1-Q_4) had a much stronger inhibitory action on LPO in synaptosomal membranes than long-chain compounds (Q_5-Q_{10}).

The following circumstances must be mentioned: a) the effectiveness of action of short-chain ubiquinols decreases in the order: $Q_1 > Q_2 > Q_3 > Q_4$; b) the effectiveness of action of long-chain ubiquinols depends virtually only a little on chain length in the order: $Q_5 \geq Q_6 \geq Q_7 \geq Q_8 \geq Q_9 \geq Q_{10}$, and c) Q_0 is a less effective inhibitor of LPO than the other short-chain homologs, and is actually weaker than the long-chain homologs. Similarly, in the mitochondria, short-chain homologs (Q_4, see Fig. 3) are essentially more effective inhibitors of LPO than long-chain homologs and Q_0.

What are the possible causes of the difference in the action of the ubiquinol homologs? It can be tentatively suggested that the low activity of oxidized forms of ubiquinones is due to the absence of molecules of mobile hydrogen atoms, essential for interaction with the

peroxide radicals of lipids, in the composition of their molecules. Values of the constant K_7 for oxidized forms of ubiquinones are 1000 times less than values of K_7 for reduced forms of these compounds [1]. The very weak activity of these compounds in high concentrations is probably due to possible interaction with active forms of oxygen, mainly with OH^- radicals [2]. Differences in the action of homologs of ubiquinones with different chain length are probably due to three causes. First, a decrease in the transbilayer and lateral mobility of the ubiquinols with an increase in the length of their isoprenoid chain [16], second, the formation of clusters from long-chain ubiquinols Q_5-Q_{10} in the intermonolayer space of the lipid bilayer [7], and third, the inability of long-chain ubiquinols to interact with water-soluble oxygen radicals. The question arises: what are the causes of the low effectivness of Q_0 as an inhibitor of LPO? It can be tentatively suggested that one cause is the low Q_0 concentration in the membranous phase as a result of the fact that most Q_0 added to the membrane suspension remains in the aqueous phase. As a result of this the Q_0 concentration is not high enough either in the aqueous or in the membranous phase. Only at the highest of the concentrations used (10^{-4} M) is the activity of Q_0 closely similar to that of the long-chain homologs. Clearly Q_0 gives a well-resolved spectrum in water and ethanol, whereas Q_{10} gives resolved spectra in ethanol and hexane.

In conclusion we may note that the results relating to the effectiveness of action of ubiquinols agree with earlier results on the action of a homologous series of tocopherols (C_1-C_{16}) with different chain length, and it can be concluded from them that the regarding the mechanisms of action of tocopherols and ubiquinones described on their basis and probably valid for natural membrane-bound LPO inhibitors.

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