12. V. A. Tutel'yan, L. V. Kravchenko, and K. I. Eller, in: Toxigenic Fungi: Their Toxins and Health Hazard, Amsterdam (1984), p. 282.
13. Y. Ueno, in: Trichothecenes: Chemical, Biological and Toxicological Aspects, Amsterdam (1983), p. 135.
14. T. Yoshizawa and T. Sakamoto, Proc. Jpn. Assoc. Mycotoxicol., 14, 26 (1982).
15. T. Yoshizawa, S. Swanson, and C. J. Mirocha, Appl. Environ. Microbiol., 39, 1172 (1980).

RELATIONS BETWEEN NATURAL ANTIOXIDANT CONTENT AND VISCOSITY OF LIPIDS IN NORMAL ORGANELLE MEMBRANES

E. B. Burlakova, A. N. Goloshchapov, and R. F. Kerimov

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KEY WORDS: antiradical activity; natural antioxidants; chemiluminescence; viscosity; structural transition.

Strict correlation between structure and function is a characteristic feature of biological membranes, which are cooperative systems [7]. An important role in the function of membranes is known to be played by lipid–protein interactions, a significant effect of which is exerted by processes of lipid peroxidation (LPO) [12]. Changes in the LPO level in membranes lead to changes or even disturbances of their functional activity [11]. It has been shown that intensification of LPO increases the viscosity of membrane lipids [12]. The "flowability" of the lipid bilayer in turn affects activity of systems generating free radicals in lipids. For instance, it was shown in [8] that LPO is initiated only under conditions permitting high flowability of membrane lipids, whereas an increase in viscosity of the lipids causes slowing of LPO [2].

The system of natural antioxidants (NAO), found in lipids, is one of the systems that controls the LPO level in membranes. NAO inhibit excessive LPO through exchange reactions, and they thereby maintain the structural integrity of the membranes and its functional activity. The view is held that antioxidants, built into the lipid bilayer of the membrane, may also have a direct effect on the structure of the membrane [10], and may thus modify accessibility of the membrane lipids to oxygen.

The aim of this investigation was accordingly to establish relations between the content and "efficiency" of NAO, on the one hand, and the viscosity of membrane lipids of subcellular fractions differing in the velocity of LPO reactions, on the other hand. For this purpose the content and "efficiency" of NAO and the viscosity of the lipid bilayer of the membranes in different organelles of liver cells of intact mice were determined.

EXPERIMENTAL METHOD

Organelles (nuclei, mitochondria, and microsomes) were isolated by differential centrifugation [4, 5, 9] from the liver of noninbred mice weighing 18-22 g. Lipids were extracted from these organelles by Folch's method in Kates' modification [6].

The antiradical activity (ARA) of the lipids and the NAO level were determined on a chemiluminescence model of initiated oxidation of ethylbenzene [1].

The viscosity of the membrane lipids, and also thermoinduced structural transitions taking place in them, were determined by the spin probe method [3]. A stable iminoxyl radical — [2,2,6,6]-tetramethyl-4-caprylolyoxypiperidiene-1-oxyl — was used as the probe. The EPR spectra were recorded on an E-4 EPR spectrometer (Varian, USA).

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EXPERIMENTAL RESULTS

ARA of total NAO was calculated from kinetic curves of changes in the intensity of fluorescence of organelle lipids, which is a qualitative characteristic of the "efficiency" of NAO. ARA for lipids of mitochondria, microsomes, and nuclei was $2.8 \cdot 10^6$, $2.7 \cdot 10^6$, and $2.0 \cdot 10^5$ liters/mole•sec, respectively. Clearly the values of ARA for mitochondrial and microsomal lipids differed significantly from those of nuclear lipids. Meanwhile the equality of the values of ARA for mitochondrial and microsomal lipids suggests that lipids of these organelles contain qualitatively the same set of NAO, which differs from that of the nuclei.

The organelle lipids were found to differ in luminescence quenching efficiency. Curves showing changes in efficiency of quenching of luminescence by lipids depending on their concentration are given in Fig. 1. Mitochondrial lipids were twice as active as microsomal lipids (Fig. 1). The luminescence quenching efficiency of the nuclear lipids was much lower than that of mitochondrial and microsomal lipids: by 24 and 12 times, respectively. Efficiency of quenching is known to be determined both by ARA and by the acting fraction of NAO. Since ARA of the mitochondria and microsomes is equal, the difference in the efficiency of action of mitochondrial and microsomal lipids must be due to differences in their NAO content. In fact, calculations showed that the NAO level in lipids of the organelles differed, and was $6 \cdot 10^{-4}$ and $3 \cdot 10^{-4}$ moles of antioxidant per mole of lipid for mitochondrial and microsomal lipids, respectively. As regards nuclear lipids, the low efficiency of inhibition of chemiluminescence by them was mainly due to their low ARA, for the acting fraction of NAO for nuclear lipids was comparable with that for mitochondrial and microsomal lipids, namely $5 \cdot 10^{-4}$ mole of antioxidant per mole of lipid.

Organelle lipids were thus characterized quantitatively, with respect both to NAO content and to their efficiency, by a chemiluminescence method.

To elucidate the connection between NAO and the structural characteristics of the membranes, the microviscosity of the lipid bilayer and the thermoinduced structural transitions taking place in them were determined.

Curves showing changes in microviscosity τ_c (rotary correlation time) for organelles are given in Fig. 2. It will be clear from Fig. 2 that curves of changes in τ_c on temperature differed from one another. For example, τ_c at 37°C for mitochondria was $0.42 \cdot 10^{-10}$ sec, for microsomes $0.38 \cdot 10^{-10}$ sec, and for nuclei $0.27 \cdot 10^{-10}$ sec. These same relations were preserved over the whole temperature range investigated. In Arrhenius plots of log τ_c as a function of temperature, kinks were observed at 23-24°C, which some workers have explained as the transition from a quasicrystalline to a liquid crystalline state (Table 1). The second kink was observed at temperatures of 41-42°C (Table 1). The lipids of the organelles studied were found virtually not to differ in the temperatures of their structural transitions. The interval 10-25°C characterizes transition of membrane lipids from the quasicrystalline to the liquid crystalline state, the interval 25-40°C characterizes the liquid crystalline state, and the interval 40-60°C characterizes the liquid crystalline state and melting of the lipids. As the calculations showed, E_a for lipids differed in different organelles. The energy required to change the viscosity of the organelle lipids was higher in the first and third intervals than in the second. The "thermostability" of the nuclear lipids was higher than that of the mitochondrial and microsomal lipids.

It was thus demonstrated by the spin probe method that lipids of different organelles differ from one another in viscosity and in E_a.

To sum up results obtained by the two methods, we found that mitochondrial lipids are most efficiently protected against peroxidation: They contain the largest quantity of high efficiency antioxidants, and the value of $K_7 \cdot$NAO is higher for them than for all the other organelles studied. Mitochondrial membranes have lipids of the highest viscosity, and as was shown above, they also prevent LPO. The least protected lipids, according to the results of the study of the two systems of protection, are nuclear lipids, for which both the efficiency of NAO and the viscosity of the lipids are significantly lower. At the same time, it must be recalled that the organelles which we investigated differ in the activity of their enzyme system for activation and inhibition of LPO.

The investigation showed direct correlation between the content of "efficient" NAO and lipid viscosity. This correlation may serve to maintain the steady state of peroxidation. Lipid viscosity is an integral value and it depends on the composition of the phospholipids, the relative content of cholesterol, the content of unsaturated fatty acids, the degree of their

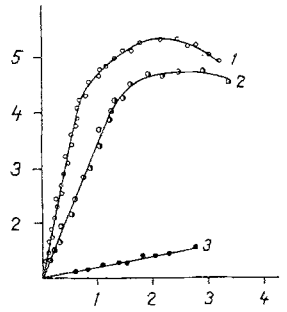

Fig. 1

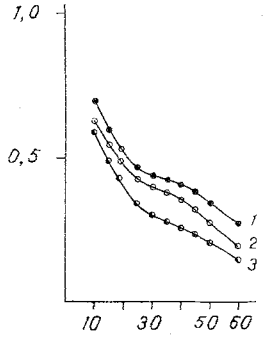

Fig. 2

Fig. 1. Dependence of efficiency of quenching of luminescence on lipid concentration: 1) mitochondria; 2) microsomes; 3) nuclei. Abscissa, lipid concentration (in mg/ml); ordinate, I_0/I, where I_0 is the initial intensity of luminescence, I the intensity of luminescence after addition of the lipids.

Fig. 2. Dependence of change in rotary correlation time of probe in lipids on temperature: 1) mitochondria; 2) microsomes; 3) nuclei. Abscissa, temperature (in °C); ordinate, $\tau_c \cdot 10^{10}$ (in sec).

TABLE 1. Temperature of Structural Transitions (T_s) and Effective Activation Energy (E_a) for Organelle Lipids of Normal Mouse Liver

Organelles	T_s, °C		E_a, kcal/mole		
	first transition	second transition	10—25, °C	25—40, °C	40—60, °C
Mitochondrial	23	42	3,1	0,41	1,93
Microsomal	23	41	2,6	0,8	2,61
Nuclear	24	42	3,2	1,23	2,9

unsaturation, and on lipid—protein interactions [13, 14]. A strict answer to the question of what is the contribution of NAO to the structural characteristics of lipids can therefore be given only in the case of an oriented change in the level of NAO, not affecting the composition of the lipids and proteins of the membranes.

Under normal conditions, the steady state of LPO in organelle membranes is therefore maintained both directly by NAO and indirectly through the flowability of the lipid bilayer.

LITERATURE CITED

1. E. B. Burlakova, S. A. Burobina, N. G. Kharpova, and G. I. Yadykin, Biofizika, 16, 39 (1971).
2. A. N. Goloshchapov, "Physicochemical characteristics of lipids and proteins during growth of experimental tumors," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Moscow (1976).
3. A. N. Goloshchapov and E. B. Burlakova, Biofizika, 20, 816 (1975).
4. I. A. Karuzina and A. I. Archakov, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), p. 43.
5. E. M. Kedrova and L. V. Orlova, in: Modern Methods in Biochemistry [in Russian], Vol. 2, Moscow (1968), p. 59.
6. M. Kates, Techniques of Lipidology, Elsevier (1972).
7. S. V. Konev, in: Regulation of Biochemical Processes in Microorganisms [in Russian], Pushchino (1978), p. 57.
8. D. I. Roshchupkin, Yu. M. Petrenko, G. P. Kuznetsova, and Yu. A. Vladimirov, in: Free Radical Oxidation of Lipids Under Normal and Pathological Conditions [in Russian], Moscow (1976), p. 163.

9. D. Sottocasa, in: Biochemical Investigation of Membranes [Russian translation], Moscow (1979), p. 54.
10. J. A. Lucy, in: Tocopherol, Oxygen and Biomembranes, Amsterdam (1978), p. 109.
11. J. Pasquali-Ronchetti et al., Lab. Invest., 42, 457 (1980).
12. E. Santiago et al., Biochem. Biophys. Res. Commun., 53, 439 (1973).
13. M. Shinitsky, Int. Rev. Cytol., 60, 121 (1978).
14. P. J. Quinn, Prog. Biophys. Mol. Biol., 38, 1 (1981).

DIFFERENT EFFECTS OF LONG-TERM HALOPERIDOL ADMINISTRATION ON $GABA_A$ AND BENZODIAZEPINE RECEPTORS IN VARIOUS PARTS OF THE BRAIN

Ö. Ö. Vasar, A. M. Nurk, M. O. Maimets, A. H. Soosaar, and L. H. Allikmets

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Long-term administration of neuroleptics has been shown to cause substantial structural changes in many neurotransmitter systems of the brain, including the GABA-ergic system and benzodiazepine receptors [1, 4, 6]. On long-term administration of the typical neuroleptic haloperidol, a marked decrease in the density of $GABA_A$- and benzodiazepine receptors has been observed in various structures of the forebrain and cerebellum [1, 2]. Besides these changes, if muscimol, an agonist of $GABA_A$ receptors is used, its sedative action is reversed [2]. Instead of inhibiting orienting-motor activity in mice muscimol had the opposite effect — it stimulated motor activity in animals treated beforehand with haloperidol. However, the paradoxical change in the action of muscimol cannot be attributed entirely to a decrease in the density of $GABA_A$ and benzodiazepine receptors in the forebrain. Direct injection of muscimol into the substantia nigra is known to induce stereotyped behavior analogous to that found after systemic administration of dopaminomimetics [3]. Microinjection of muscimol into the medial nucleus raphe leads to the development of hyperactivity in rats; simultaneous administration of chlordiazepoxide, a benzodiazepine agonist, moreover, potentiates the stimulating effect of muscimol considerably [8]. After long-term administration of neuroleptics neurons of the substantia nigra develop hypersensitivity to GABA agonists [4] and the density of GABA receptors in the substantia nigra is increased [6].

The data described in this paper are evidence that long-term administrationof haloperidol has an opposite effect on the density of $GABA_A$ and benzodiazepine receptors in the fore- and hindbrain. These changes are reflected at the molecular level as reversal of the behavioral effect of the $GABA_A$ agonist muscimol and the benzodiazepine agonist Ro15-1788.

EXPERIMENTAL METHOD

Experiments were carried out on 160 male mice weighing 25-30 g and on 100 male Wistar rats weighing 250-270 g. Haloperidol in a dose of 0.25 mg/kg (from Gedeon Richter, Hungary) or physiological saline was injected intraperitoneally twice a day for 15 days. Behavioral tests and radioligand binding experiments were carried out 12 h after the last injection of the neuroleptic. The sedative action of muscimol (from Serva, West Germany), a $GABA_A$ receptor agonist, was determined in mice by means of a photoelectric actometer. The animals were placed in the actometer 15 min after intraperitoneal injection of muscimol in doses of 0.75 and 1.5 mg/kg, and their orienting-investigative activity was determined for 30 min. The effect of the benzodiazepine antagonist Ro15-1788 (from Hoffmann-La Roche, Switzerland) on the rats' behavior was assessed by the open field method. The animals were placed in the center of an open field (measuring 100 × 100 × 40 cm) 30 min after intraperitoneal injection of 5 mg/kg of Ro15-1788. The animals' behavior was recorded for 5 min. The following parameters were de-

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