manifested as a change in an integral parameter, namely the microviscosity of the bilayer, but with its effect purely on the region of annular lipids. This last hypothesis is perfectly logical if it is recalled that some investigators have demonstrated that free fatty acids and lysophospholipids can exert a direct action on membrane enzymes, i.e., that they participate in the formation of the annular region of membrane proteins [1, 5].

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MECHANISMS OF REGULATION OF MEMBRANE RECEPTOR ACTIVITY BY SYNTHETIC ANTIOXIDANTS OF THE SCREENED PHENOL CLASS

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KEY WORDS: antioxidants; receptors; lipids; synaptosomes; secondary messengers.

The effect of exogenous inhibitors of free-radical processes (synthetic antioxidants) on various aspects of cell metabolism under normal and pathological conditions has recently been extensively studied [1, 2]. A key problem is the mechanism of action of antioxidants on the cell. It is the antiradical properties of these substances that lie at the basis of changes in the physicochemical parameters of the various cell structures [6], but the concrete pathways of realization of these properties in regulation of metabolism of the living cell have so far received little study. In particular, the effect of synthetic antioxidants on biological activity may be due to interaction with the plasma membranes of cells, changes in their physicochemical characteristics and, correspondingly, changes in activity of membrane-bound protein formations, such as membrane receptors. It can be tentatively suggested that the possible receptor-mediated action of inhibitors of radical processes on cell metabolism is realized in two ways: first, by complex formation between synthetic antioxidants with a certain chemical structure and specific binding sites on the cell surface, and second, by nonspecific interaction with the membrane and modification of the lipid component, leading to a change in activity of different receptor complexes integrated into the membrane bilayer.

The aim of this investigation was to study the action of synthetic antioxidants of the screened phenol class (SASP) on conduction of informative signals in several membrane receptor systems of the rat brain.

EXPERIMENTAL METHOD

Rat brain synaptosomes were obtained by the use of the method described previously [9]. The preparation of solubilized opioid receptor complexes was obtained by the method suggested

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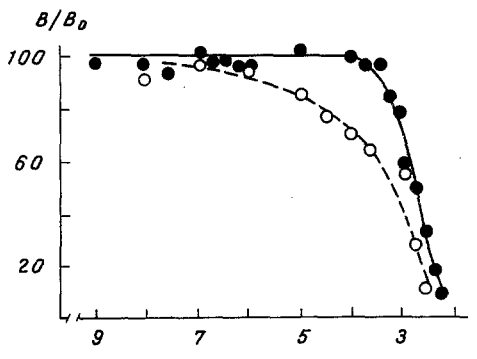

Fig. 1

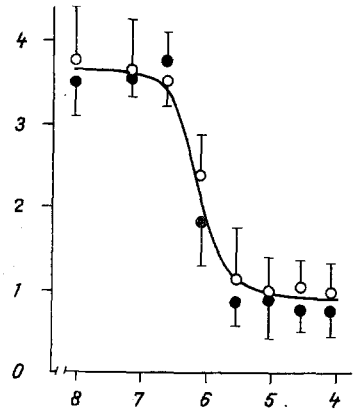

Fig. 2

Fig. 1. Dependence of specific binding of naloxone with rat brain opioid receptors on concentration of phenosan-K. ●) Synaptosomes, preincubation with antioxidant for 3 h at 25°C; ○) opioid receptors, not preincubated with antioxidant. Abscissa, negative logarithm of phenosan-K concentration (in M); ordinate: B and B_0) quantity of specifically bound ligand in presence and absence respectively of antioxidant, B/B_0 (in %).

Fig. 2. Accumulation of conjugated dienes in synaptosomal fraction as a function of concentration of phenosan (●) and phenosan-K (○). Incubation for 15 min at 37°C. Abscissa, negative logarithm of antioxidant concentration (in M); ordinate, ratio of optical density at 233 nm in experiment to that in control.

in [10], by treatment of a suspension of synaptosomes with 1% digitonin solution for 30 min. To determine activity of cAMP synthesis, the method in [5] was used. The cGMP content in the samples was determined by radioimmunoassay using a standard kit from "Amersham International" (England) [14]. Ca^{++}-dependent protein kinase (protein kinase C) was isolated from bovine brain and purified by the method in [15]. The incubation medium to study the action of antioxidants on protein kinase C activity contained: Tris-HCl buffer 20 mM (pH 7.5), $Mg(CH_3COO)_2$ 10 mM, dithiothreitol 1 mM, ATP 0.02 mM, protein kinase C 0.2 μg per sample, and γ-^{32}P-ATP 10^6 cpm ("Amersham"). After incubation, 40 μl of suspension was taken from each 100-μl sample and filtered through "Whatman 3MM" filters (England). The filters were washed in 10% TCA, dehydrated, dried, and placed in toluene scintillator. Azolecithin (0.3 μg per sample) was used as the phospholipid during activation of protein kinase C.

When the effect of antioxidants on complex formation by specific ligands and receptors was analyzed the following preparations were used: ^{3}H-quinuclidinyl benzylate, an antagonist of muscarinic acetylcholine receptors ("Amersham," specific radioactivity 12.3 Ci/mmole); ^{3}H-naloxone, an opioid receptor antagonist ("New England Nuclear," USA; specific radioactivity 50 Ci/mmole); ^{3}H-dihydroalprenolol, a β-adrenoreceptor antagonist ("Amersham," specific radioactivity 78 Ci/mmole). Equilibrium binding of labeled compounds with preparations of synaptosomes and solubilized opioid receptor complexes was studied during incubation for 1 h at 25°C in the corresponding media: HEPES 5 mM (pH 7.4), KCl 125 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, KH_2PO_4 0.5 mM (synaptosomes) and Tris-HCl buffer (pH 7.4) 50 mM, Na_2EDTA 1 mM, NaCl 1 M (solubilized opioid receptors). Specific binding was determined as the fraction of the total which was blocked by a 1000-fold excess of unlabeled ligand, and in the case of ^{3}H-quinuclidinyl benzylate, by an excess of atropine sulfate. Bound and free ligands were separated by vacuum filtration through "Whatman GF/C" paper filters (England).

The content of lipid hydroperoxides in the membranes was determined by measuring absorption of conjugated diene structures at 233 nm after transfer of the extracted lipids into heptane [3]. During investigation of the structural state of the membrane lipids by EPR spectroscopy, two spin probes were used: the doxyl derivative of stearic acid with a nitroxide ring attached to the 5th carbon atom (5-doxyl stearate; from "Sigma," USA) and 2,2,6,6-tetramethyl-4-palmitoylhydroxypiperidine-1-oxyl (15C). Both probes are located mainly in the lipid bilayer of the membranes. The final concentration of the probes in the samples was 100 μM; the protein concentration in the test suspensions of synaptosomes 5 mg/ml. The microviscosity

TABLE 1. Change (experiment/control) in Structural Parameters of Lipid Phase of Rat Brain Synaptosomes under Influence of SASP in Vitro

Concentration of SASP, M	τ_c of probe 15C		S of 5-doxyl stearate	
	phenosan	phenosan-K	phenosan	phenosan-K
10^{-7}	1,00	1,00	1,00	1,00
$5 \cdot 10^{-7}$	0,91	0,93	1,05	1,00
10^{-6}	0,85	0,83	1,12	1,10
$5 \cdot 10^{-6}$	0,80	0,79	1,20	1,18
10^{-5}	0,85	0,84	1,21	1,20

Legend. Incubation for 30 min at 25°C.

TABLE 2. Effect (experiment/control) of SASP on Bovine Brain Protein Kinase C Activity

Concentration of SASP, M	Phenosan		Phenosan-K	
	Ca^{2+}	Ca^{2+}, azolecithin	Ca^{2+}	Ca^{2+}, azolecithin
10^{-6}	1,00	1,00	1,00	1,00
$5 \cdot 10^{-6}$	1,06	1,00	1,08	1,03
10^{-5}	1,20	1,15	1,15	1,10
$5 \cdot 10^{-5}$	1,43	1,22	1,23	1,18
10^{-4}	1,62	1,35	1,40	1,32

Legend. Incubation for 15 min at 30°C.

of the lipid phase was estimated from the rotary correlation time τ_c of the 15C probe from the equation for fast isotropic rotation of spin probes [4]. In the case of 5-doxyl stearate, the parameter of orderliness S was calculated as the measure of anisotropy [4].

The following SASP were used: γ-(4-hydroxy-3,5-di-tert-butylphenol) propionic acid (phenosan) and the potassium salt of phenosan (phenosan-K), generously supplied by V. V. Ershov and L. G. Plekhanova, Institute of Chemical Physics, Academy of Sciences of the USSR.

EXPERIMENTAL RESULTS

The experimental results showed that the action of SASP on transmission of informative signals in a number of receptor systems of rat brain cells is, as a rule, not realized at the level of regulation of complex formation by specific ligands and receptor binding sites. Phenosan and phenosan-K did not affect the process of complex formation by opioid, muscarinic cholinergic, and β-adrenergic receptors and specific ligands in below-millimolar doses. Only on facilitation of access to the hydrophobic part of the receptor, for example, by solubilization of opioid receptor complexes by digitonin, was the action of SASP on the process of complex formation of the specific ligand with the receptor binding sites rendered more effective (Fig. 1). Thus the effect of antioxidants on specific binding of the ligand with the receptor is effective if mediated through the hydrophobic region of the membrane, i.e., through the lipid bilayer. On the basis of these findings we suggested that the modifying action of SASP on transmission of informative signals in receptor systems, in below-millimolar doses, ought most probably to be realized at the level of transmembrane transfer of the signal. Since the molecular processes taking place in the membrane bilayer depend to some degree on its physicochemical state, it was to be expected that a change in the lipid component caused by the action of antioxidants would modify activity of the secondary messenger system of the membrane receptors. Data on the effect of phenosan and phenosan-K on the structural characteristics and oxidative properties of the lipid component of the synaptosomes are given in Table 1 and Fig. 2. Even in micromolar concentrations the SASP modified the physicochemical parameters of the membrane lipid bilayer in vitro.

We know from the literature that the viscosity, composition, and degree of oxidation of lipids determine activity of membrane adenylate cyclase bound in receptor complexes [8, 11, 12];

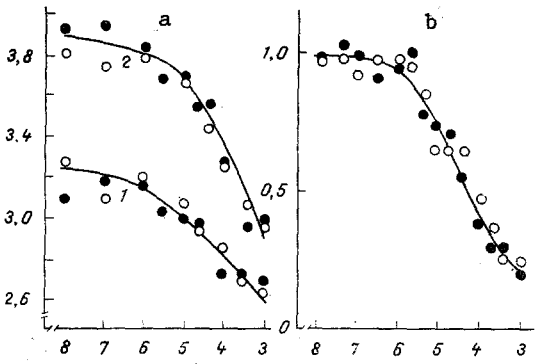

Fig. 3. Effect of phenosan (●) and phenosan-K (○) on activity of synthesis of secondary messengers of several receptor systems in rat brain synaptosomes. a) cAMP. 1) Basal activity; 2) activity stimulated by 10 μM isoproterenol. b) cGMP. Incubation for 60 min at 37°C. Abscissa, negative logarithm of antioxidant concentration (in M); ordinate, level of synthesized cyclic nucleotides (in nmoles/min/mg protein).

activity of protein kinase C depends on the content of phospholipids in the membrane and the ratio between their fractions [7]; activity of quanylate cyclase may be regulated by the concentration of endogenous lipid peroxides [13], and so on. Thus SASP, interacting nonspecifically with the plasma membranes of cells and modifying the physicochemical state of the lipid bilayer, ought to act effectively on the system of secondary messengers in transmembrane transmission of informative signals from receptor to cell. The data in Fig. 3 indicate that phenosan and phenosan-K act effectively on activity of synthesis of secondary messengers of some of the most important receptor systems, namely cAMP and cGMP. In this case, the antioxidants act in vitro in concentrations of up to 10 μM, which agrees approximately with the minimal effective doses in which phenosan and phenosan-K modified the state of membrane lipids. The effect of SASP on signal conduction in receptor systems may evidently be mediated not only by a change in the lipid bilayer of the membranes, but also by interaction with several biological compounds playing a key role in the receptor act. For example, the antioxidants can change activity of lipid-dependent protein kinase C, not only by their action through a change in the membrane lipids, but also by interacting directly with the enzyme (Table 2).

The action of SASP on transmission of informative signals in membrane receptor systems of the brain is thus realized primarily at the level of transmembrane transfer of the informative signal. The effective action of antioxidants on receptor activity is determined by interaction with the hydrophobic part of the receptors, buried in the lipid bilayer, or by their effect on the system of secondary messengers, for example, on activity of specific cyclases. This kind of effect is possible in vitro with SASP in concentrations of over 10 μM, evidently through their nonspecific interaction with the plasma membranes of the cells, and it is mediated through a change in the physicochemical state of the membrane lipids.

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ENZYMATIC UTILIZATION OF LIPID PEROXIDES IN THE ATHEROSCLEROTIC HUMAN AORTA

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The intensity of lipid peroxidation (LPO) in the body changes in certain pathological states associated with disturbance of lipid metabolism [1]. Hyperlipidemias, accompanying the development of atherosclerosis, also are characterized by an increased velocity of LPO. In particular, a marked increase in the concentration of lipid peroxides is found in patients with atherosclerosis in the blood [2] and in zones of atherosclerotic lesions in the aorta [6]. It is suggested that elevation of the lipid peroxide level is one cause of the development of atherosclerosis because of the ability of hydroperoxides to cause damage to the intima of blood vessels [14], to stimulate platelet aggregation and proliferation of smooth-muscle cells [12], and also to inhibit prostacycline synthesis in the vascular wall [7]. An important role in the detoxication of lipid peroxides in the body is played by glutathione peroxidase (GP) and glutathione-S-transferases (GT) [9, 5]. The latter differ from GP in their ability to reduce not only hydroperoxides of free fatty acids, but also the aliphatic acyl groups of membrane phospholipids [5].

Since no systematic investigations of changes in GT activity in atherosclerosis have been undertaken, the aim of the present investigation was to study GT activity in specimens of the intima and media of the human aorta infected with different degrees of atherosclerosis.

EXPERIMENTAL METHOD

The thoracic aortas of persons aged from 27 to 72 years, dying from cardiovascular diseases, were taken for investigation. Autopsies were performed in the A. L. Myasnikov Institute of Clinical Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, 1-6 h after the stated time of death. The intima and media from the intact zone of the vessel, and the regions of lipid stains and lipid-fibrous plaques were separated mechanically, after which the tissue samples obtained were homogenized in 50 mM phosphate buffer, pH 7.4 (1:5), the homogenate was centrifuged for 10 min at 800g, and the supernatant was frozen in liquid nitrogen and kept at −20°C. GT activity was determined with 1-chloro-2,4-dinitrobenzene (CONB) and with p-nitrophenyl acetate (NPA) as described previously [13]. Determination of activity with 1-fluoro-2,4-dinitrobenzene (FDNB) was carried out under standard conditions [13] with addition of the substrates (glutathione and FDNB) in a concentration of 0.1 mM. The unit of GT activity was taken to be the quantity of enzyme catalyzing the formation of 1 μmole of product during 1 min under the conditions of determination.

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