fact, in the region of tissue in which the regulator concentration falls, a reaction arises which attempts to compensate the unfavorable consequences of this action (since the prostaglandin regulators are one of the commonest found in the body).

LITERATURE CITED

1. É. M. Galimov, The Nature of Biological Isotope Fractionation [in Russian], Moscow (1981), p. 246.
2. E. F. Doil'nitsyn, V. I. Zyuzin, and A. P. Pertseva, Physiochemical Methods of Investigation in Geology [in Russian], Novosibirsk (1981), pp. 134-138.
3. A. S. Panosyan, Khim. Prir. Soedin., No. 6, 783 (1983).
4. A. O. Nier and E. A. Gulbransen, J. Am. Chem. Soc., 61, 697 (1939).

ANTIOXIDANT-INDUCED CALCIUM TRANSPORT IN BIOLOGICAL MEMBRANES

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The process of lipid peroxidation plays an important role in the development of several pathological states [1]. Data have recently been published on the side effects of synthetic antioxidants, especially when used in high doses [7, 11]. Since molecules of synthetic antioxidants, which as a rule are arotomatic compounds, do not correspond structurally to the packing of the fatty-acid residues of phospholipids, the formation of defects in the lipid bilayer of the membrane can be expected on their insertion. This, in turn, is bound to affect ionic homeostasis, as has been demonstrated for artificial bilayer membranes [3].

Calcium ions (Ca^{++}) are one of the universal cellular regulators. It can therefore be understood why damage to Ca^{++}-transporting systems may be a cause of various diseases and, in some cases also, of death of the cell [12]. The aim of the present investigation was accordingly to study the effect of antioxidants on the distribution of Ca^{++} and its transport through biological membranes.

EXPERIMENTAL METHOD

Platelets were isolated from the blood of noninbred rats by the method in [9]. Aggregation was induced in medium of the following composition (in mM): NaCl — 134, KCl — 5, $MgSO_4$ — 1, Na_2HPO_4 — 0.5, HEPES — 10, glucose — 5 (pH 7.4, at 37°C). Aggregation was induced with arachidonic acid (50 μM) and recorded as the change in scattering of light (using an aggregometer from "Chronolog Corp.," USA). The rate of aggregation was estimated as the tangent of the angle of slope of the tangent drawn to the curve corresponding to the rapid phase of aggregation.

Crude and purified fractions of synaptosomes from rat cerebral cortex were obtained by the method in [8]. Release of ^{3}H-serotonin (13.2 mCi/mmole, "Amersham International," England) by the crude synaptosomal fraction was stimulated by 50 mM KCl. Incubation and washing were carried out in medium of the following composition (in mM): NaCl — 120, KCl — 5, NaH_2PO_4 — 1.2, $MgCl_2$ — 1.3, $CaCl_2$ — 0.73, Tris-HCl — 20, glucose — 10, pargyline — 10 μM (pH 7.4 at 37°C). The samples were filtered on GF/B filters ("Whatman," England), and washed 3 times with cold Tris buffer. Radioactivity was measured on a "RackBeta" liquid scintillation counter (LKB, Sweden).

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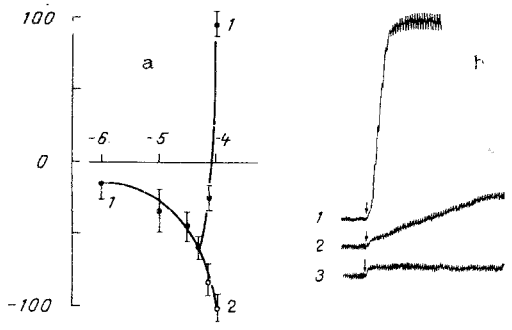

Fig. 1

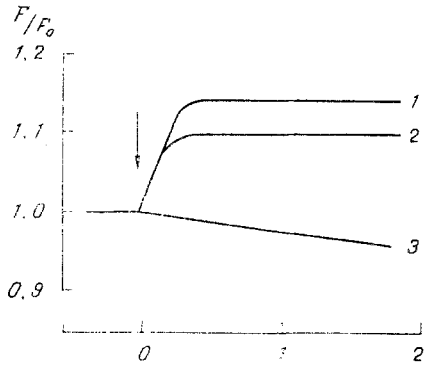

Fig. 2

Fig. 1. Action of antioxidants on platelet aggregation. a) Effect of ionol on platelet aggregation during activation of cells by arachidonic acid (50 μM), in the absence (1) and presence (2) of 0.5 mM EDTA. Abscissa, logarithm of concentration; ordinate: above — activation (in %); below — inhibition (in %); b) activation of platelets by antioxidants. 1) PMC; 2) ionol; 3) TP.

Fig. 2. Effect of antioxidants on transmembrane potential of synaptosomes. 1) PMC, 2) ionol, 3) TP. Abscissa, time (in min); ordinate, relative change in intensity of fluorescence of probe (in relative units).

Changes in the transmembrane potential (TMP) of the purified synaptosomal fraction were recorded with the aid of the fluorescent probe 3,3-dipropyl-2,2-thiodicarbocyanin [diS-C_3-(5)] by the method in [2]. Under these circumstances, an increase in the intensity of potential-dependent fluorescence (F/F_0) of diS-C_3-(5), incorporated into the synaptosomal membranes, corresponded to a decrease in TMP. The fluorometriz measurements were done on a "Hitachi-850" spectrofluorometer (Japan) in a constant-temperature cuvette at 37°C. The concentration of synaptosomes in the cuvette was 300 μg protein/ml and that of the probe 10^{-8} M.

Fragments of membranes of the sarcoplasmic reticulum (SRF) were isolated from rabbit skeletal muscles by the method in [4]. Ca^{++} transport was evaluated as the Ca^{++}/ATP ratio, and activity of Ca^{++}-ATPase was determined in the presence of alamethicin [5].

The protein concentration in the preparations was determined by the method in [6]. Antioxidants were added to the membrane suspension in alcoholic solutions so that the alcohol concentration did not exceed 1% of the original volume and did not affect the parameters recorded.

The following reagents were used: HEPES, sucrose, glucose, D-α-tocopherol, arachidonic acid, and 4-methyl-2,6-di-tert-butylphenol (ionol) were from "Merck," West Germany, pargyline was from "Sigma" (USA), KCl, NaCl, and $MgCl_2$ were from "Koch Light," England. The 6-hydroxy-2,2,5,7,8-pentamethylchromane (PMC) was generously provided by R. P. Evstigneeva (M. V. Lomonosov Moscow Institute of Fine Chemical Technology), and the diS-C_3-(5) by A. Waggoner (USA). The remaining reagents were of Soviet origin and of the chemically pure grade.

EXPERIMENTAL RESULTS

To study changes in Ca^{++} transport in biomembranes two systems were chosen: 1) cellular (blood platelets), and 2) subcellular (brain synaptosomes and SRF). In this way the effect of antioxidants on Ca^{++} transport could be investigated both along the concentration gradient (platelets, SRF, synaptosomes) and against the concentration gradient (Ca^{++}-ATPase) on different types of biological membranes (external and internal). The action of the synthetic antioxidants ionol and PMC was studied and compared with that of one of the principal natural antioxidants, namely α-tocopherol (TP).

Dependence of the rate of platelet aggregation after addition of arachidonic acid on the quantity of ionol in the medium in the presence and absence of Ca^{++} is shown in Fig. 1a. Starting with a concentration of 3×10^{-5} M ionol caused platelet aggregation, and this effect was not observed after the addition of 0.5 mM EDTA. Starting with this concentration and above, ionol in the presence of Ca^{++} induced aggregation even in the absence of arachidonic acid

TABLE 1. Action of Antioxidants on ^{3}H-Serotonin Release by Rat Brain Synaptosomes (M ±m)

Compound, $5 \cdot 10^{-5}$ M	Stimulation of release, %
Control (1% alcohol)	14,8±3,6
Ionol	32,8±3,4
TP	15,8±4,0
Chlorpromazine	34,5±5,5
7-Hydroxychlorpromazine	46,0±5,0

TABLE 2. Action of TP, α-Tocopherol Acetate, and PMC on Ca^{++} Transport in SRF Membranes

Concentration, M	Ca^{++}/ATP, %		
	TP	α-tocopheryl acetate	PMC
—	100	100	100
10^{-6}	100	100	100
$5 \cdot 10^{-6}$	100	100	93
10^{-5}	100	100	86
$5 \cdot 10^{-5}$	100	100	80
10^{-4}	100	100	67
$5 \cdot 10^{-4}$	100	100	45

Legend. Ca^{++}/ATP in control preparations was 1.7.

(Fig. 1b), evidence of disturbance of the barrier properties of the plasma membrane of the platelets, for the aggregation process is determined by the intracellular Ca^{++} concentration [10]. As will be clear from Fig. 1b, PMC also had a powerful aggregating action on platelets, whereas TP, under the same conditions, did not induce aggregation. Thus synthetic antioxidants, unlike TP, caused Ca^{++} ions to flow into platelets along the concentration gradient and, consequently, induce their aggregation.

Data on the effect of antioxidants on serotonin release by the crude synaptosomal fraction are given in Table 1. They show that ionol is an effective activator of release, whereas TP does not possess this property. The effect of ionol which was found is exhibited only in the presence of Ca^{++}, and it evidently is not specific, for other compounds of a different chemical nature have a similar action also (Table 1).

The results show that synthetic antioxidants disturb Ca^{++} homeostasis. It may be expected that differences found in the action of synthetic antioxidants and TP are linked with two processes: with disturbance of the barrier properties of the biomembranes and (or) with a decrease in Ca^{++}-ATPase activity under the influence of synthetic antioxidants. As will be clear in Fig. 2, ionol and PMC, unlike TP, induce depolarization of the synaptosomes, which reflects the disturbance of the barrier properties of their outer membrane. In the same way PMC, unlike TP and α-tocopheryl acetate, causes a disturbance of the barrier functions of the membranes of SRF for Ca^{++}, which is manifested as a concentration-dependent decrease in Ca^{++}/ATP (Table 2). Figure 3, which shows dependence of Ca^{++}-ATPase activity of SRF on the concentration of antioxidants, shows that ionol, unlike TP, has a marked inhibitory action on the enzyme.

Thus both of the above mechanisms of disturbance of Ca^{++}-homeostasis may operate in biomembranes. Ca^{++} escape arises under the influence of synthetic antioxidants of phenolic nature, which have no carbon side chains such as are characteristic of natural antioxidants (tocopherols, ubiquinones, naphthoquinones). This is evidently connected with the appearance of defects in the lipid bilayer due probably to their structural mismatching with the components of the lipid bilayer. Damage to Ca^{++}-transporting systems can be regarded as one mechanism of the appearance of side effects of synthetic antioxidants.

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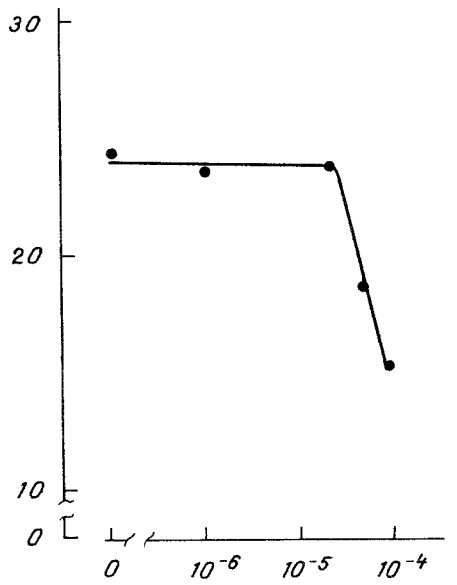

Fig. 3. Effect of ionol on Ca^{++}-ATPase activity of SRF. Abscissa, ionol concentration (in M); ordinate, ATPase activity (in μmoles inorganic phosphorus/min/mg protein).

LITERATURE CITED

1. E. B. Burlakova, A. V. Alesenko, E. M. Molochkina, et al., Bioantioxidants in Radiation Damage and Malignant Growths [in Russian], Moscow (1975).
2. A. N. Erin, V. I. Skrypin, L. L. Prilipko, and V. E. Kagan, Byull. Éksp. Biol. Med., No. 7, 25 (1986).
3. I. I. Ivanov, N. P. Korolev, B. K. Semin, et al., Nauchn. Dokl. Vyssh. Shk., Biol. Nauki, No. 5, 35 (1986).
4. V. B. Ritov, Biokhimiya, 36, 393 (1971).
5. V. B. Ritov and M. K. Murzakhmetova, Biokhimiya, 48, 415 (1983).
6. M. M. Bradford, Anal. Biochem., 72, 248 (1975).
7. K. H. Cheng and J. R. Lepock, Chem. Phys. Lipids, 37, 373 (1985).
8. F. Hajos, Brain Res., 83, 485 (1975).
9. A. Hotteblis, J. Jordan, et al., Biochem. Pharmacol., 30, 2089 (1981).
10. M. B. Feinstein, G. A. Rodan, and L. S. Culter, Platelets in Biology and Pathology, Amsterdam (1981), pp. 437-472.
11. P. S. Kumar, T. George, N. J. Bai, and S. Krishnamurthy, Int. J. Vit. Nutr. Res., 49, 352 (1979).
12. M. Younes and C.-P. Sieger, Biochem. Pharmacol., 33, 2001 (1984).