sol could be changed without disturbing the secretion apparatus. Mg-ATP is essential as the substrate either for contractile ATPases, to enable the secretory granules to move toward the site of exocytosis, or for Ca (CaM)-dependent phosphorylation of the granules on their making contact with the plasmalemma, and to facilitate adhesion and release of the contents of the granules into the extracellular medium.

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STABILIZING EFFECT OF α-TOCOPHEROL ON SYNAPTOSOMES EXPOSED TO PHOSPHOLIPASE A_2

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The stabilizing action of the tocopherols in biological membranes is linked with their ability to inactivate the lipid radicals of lipids [13], to quench singlet molecular oxygen [11], and to organize the lipid bilayer of biomembranes by Van der Waals' interaction with unsaturated acyl groups of phospholipids [8]. It has recently been demonstrated that α-tocopherol (TP) can protect synaptosomal membranes against the harmful action of phospholipase A_2 (PLA_2), as is shown, for example, by restoration of the transmembrane potential (TMP) and the microviscosity of synaptosomal membranes, modified by the action of PLA_2 [2, 5]. However, the concrete molecular mechanisms of the stabilizing effect of TP on synaptosomal membranes have not yet been explained.

It has been shown that the harmful action of PLA_2 on synaptosomal membranes, assessed as the change in TMP, can be quantitatively modeled by the action of free fatty acids (FFA) and is independent of equimolar additions of lysophospholipids [2, 5]. It has also been shown that complexes of TP with FFA are formed in homogeneous systems, and also in lipid bilayers [10]. Investigation of the nature of these complexes in solutions has shown that they own their formation to interaction of a fatty acid with the chromane ring of TP [9]. 4-Methyl-2,6-di-tert-butylphenol (ionol) and 2,2,5,7,8-pentamethyl-6-hydroxychromane (PMC), aromatic

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alcohols which, unlike TP, do not contain a phytol chain, in solution can also form complexes with FFA [3, 9].

To shed light on the mechanisms of stabilization of synaptosomal membranes by TP and the role of individual functional groups of its molecule, in the investigation described below the effects of TP, TP acetate, PMC, and ionol during exposure to PLA_2 were compared.

EXPERIMENTAL METHOD

Synaptosomes were obtained from the gray matter of Wistar rat brain by the method in [12]. The protein concentration was determined by the method in [7]. Changes in TMP were recorded with the aid of the fluorescent probe 3,3-dipropyl-2,2-thiocarbocyanide [diS-C_3-(5)] by the method in [4]; a fall of TMP, moreover, corresponded to an increase in the intensity of potential-dependent fluorescence (PDF) of diS-C_3-(5), incorporated into the synaptosomal membranes. To distinguish PDF from other possible effects (interaction of the probe with additives, quenching by reaction products, and so on) kinetic curves of the change in relative intensity (F/F_0) of fluorescence of diS-C_3-(5), obtained in media I and II, in one of which TMP was established at −70 mV (medium I), and in the other TMP = 0 mV (medium II) [6], were subtracted graphically. Under these circumstances the resultant curves give information only about changes in fluorescence that depend on the value of TMP. The composition of medium I (in mM) was: HEPES 20, NaCl 145, KCl 5, $NaHCO_3$ 5, and $MgCl_2$ 1.3. Medium II differed from medium I in its concentrations of KCl (145 mM) and NaCl (5 mM). The fluorometric measurements were made on a Hitachi-850 spectrofluorometer (Japan) in a constant temperature cuvette at 37°C. The spectral width of the slits of exciting (650 nm) and recorded light (688 nm) was 5 nm. The concentration of synaptosomes in the cuvette was 300 μg protein in 1 ml, and the concentration of the probe 10^{-8} M.

Synaptosomal membranes were treated with PLA_2 in the above-mentioned media and in the presence of exogenous Ca^{++} (1 mM). The diS-C_3-(5), TP, TP acetate, PMC, and ionol were added to the suspension of synaptosomes in alcoholic solutions (the final volume of ethanol did not exceed 2%), so that this did not affect the parameters recorded.

HEPES, Tris, sucrose, glucose, TP, and ionol were obtained from Serva, West Germany, PLA_2 from Boehringer (West Germany), TP acetate from ICN Pharmaceuticals (USA); the diS-C_3-(5) was synthesized by A. Waggoner (USA), and the PMC was generously provided by I. K. Sarycheva (M. V. Lomonosov Moscow Institute of Fine Chemical Technology). The other reagents were of the chemically pure grade.

EXPERIMENTAL RESULTS

In the experiments of series I the action of TP, its derivatives TP acetate and PMC, and also ionol on PDF in synaptosomes treated with PLA_2 was compared. Treatment of synaptosomes with PLA_2 was shown to increase the intensity of PDF of diS-C_3-(5) (i.e., to cause their depolarization), which reached maximal values after incubation for 1.5-2 min (Fig. 1). It was shown previously that under these conditions of incubation of synaptosomes with PLA_2, PDF reaches saturation after hydrolysis of 7-10% of membrane phospholipids [2, 5]. Addition of TP in a concentration of 10^{-4} M (about 20 moles % relative to membrane phospholipids) restored the value of TMP and even caused some degree of hyperpolarization. Addition of the same concentrations of TP acetate, PMC, and ionol did not affect PDF of diS-C_3-(5) in a suspension of PLA_2-treated synaptosomes.

In the experiments of series II preliminary treatment of synaptosomes with TP was found to have the effect that during hydrolysis of phospholipids by PLA_2 no depolarization of the synaptosomal membranes was observed. Preliminary addition of TP acetate to the synaptosomes, unlike TP itself, did not affect the intensity of PDF of diS-C_3-(5), induced by the action of PLA_2 (Fig. 2). PMC and ionol not only did not change the rate of fall of TMP under the influence of PLA_2, but in a concentration of 10^{-4} M it significantly reduced the intensity of PDF of the probe itself, i.e., it lowered TMP (Fig. 2).

The experimental data described above are evidence that TP possesses the ability to protect synaptosomal membranes against the action of PLA_2, but this ability is not shared by its derivatives lacking either the OH-group in the chromane nucleus (TP acetate) or the phytol chain. The screened phenol (ionol) likewise had no protective action on synaptosomes. Moreover, PMC and ionol themselves caused destabilization of the synaptosomal membranes, whereas TP and TP acetate did not possess this destabilizing property (Fig. 2). If it is recalled

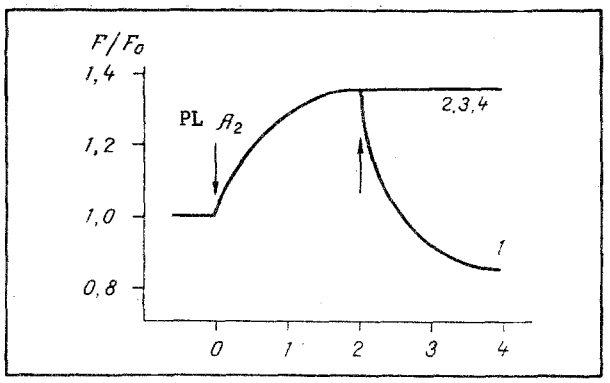

Fig. 1

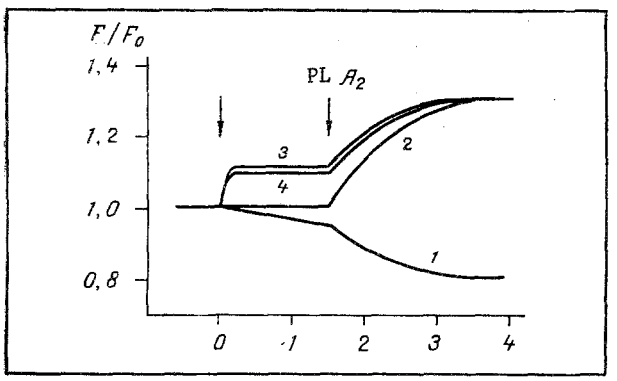

Fig. 2

Fig. 1. Kinetics of change in intensity of PDF of diS-C_3-(5) in suspension of synaptosomes under the influence of PLA_2 and subsequent addition of TP (1), TP acetate (2), PMC (3), and ionol (4). Here and in Figs. 2 and 3: abscissa, duration of incubation (in min). Arrows indicate addition of PLA_2 and TP (TP acetate, PMC, or ionol) in final concentrations of 10 μg/ml for PLA_2 and 10^{-4} M for other compounds.

Fig. 2. Kinetics of changes in PDF of diS-C_3-(5) in suspension of synaptosomes to which 10^{-4} M TP (1), TP acetate (2), PMC (3), or ionol (4) was added previously, under the influence of PLA_2.

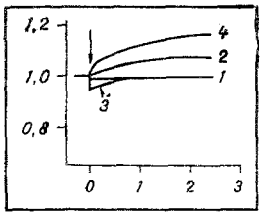

Fig. 3. Kinetics of changes in intensity of fluorescence of diS-C_3-(5) in suspension of synaptosomes in medium II after addition of 10^{-4} M TP (1), TP acetate (2), PMC (3), or ionol (4), and also of 10^{-4} M oleic acid.

that TP, PMC, and ionol in solution can form complexes with FFA, the question arises why PMC and ionol should be without this effect in synaptosomes treated with PLA_2. It can be tentatively suggested that TP and its derivatives, and also ionol, are located differently in the membrane, and consequently, that the effectiveness of their interaction with hydrolysis products of phospholipids (mainly with FFA) also differs. This is confirmed to some degree by the results of measurement of the intensity of fluorescence of the probe in medium II (TMP = 0 mV), where changes in the intensity of fluorescence reflect interaction of the probe with substances introduced into the suspension of synaptosomes (Fig. 3). TP in this case was found not to affect the fluorescence of diS-C_3-(5), just like FFA, whereas TP acetate and PMC induced quenching, and ionol increased the intensity of fluorescence. Without going into a detailed analysis of the mechanisms of these effects, we may note that they are more probably caused by differences in the location of the compounds in the membrane.

Hence, unlike in solutions, in membranes not only the chromane ring of TP with an OH-group, but also the phytol chain, whcih evidently ensures correct orientation and affects the localization of the molecule in the lipid bilayer, are essential for binding hydrolysis products of phospholipids.

It is probably no accident that ubiquinone, which possesses vitamin E activity, also contains a hydrocarbon chain, just as incidentally, do other neutral lipids of biomembranes (cholesterol, vitamin A, etc.). The fact that PMC and ionol not only have no stabilizing action on synaptosomes, but can also disturb their barrier functions, is hardly surprising. Cells are probably not subject to a deficiency of these compounds, which are formed in excess, for example, through the working of the cytochrome P-450-dependent xenobiotic hydroxylation system [1]. If, therefore, aromatic alcohols were able to protect biomembranes against damage caused by PLA_2, there would probably be no need for any similar mechanism for the stabilizing action of vitamin E.

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TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS OF HUMAN ERYTHROCYTE MEMBRANE PROTEINS

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The study of the structure of the human erythrocyte membrane, and in particular, of its constituent proteins, is of some urgency because various hereditary diseases (spherocytic anemias, muscular dystrophies, certain diseases of the CNS) are characterized by disturbances of the protein component of the erythrocyte membrane and, as a result, by disturbance of its structural and functional organization. In connection with the systematic study of products of the expression of human genes using a molecular-anatomical approach, which has now begun, it is extremely important to determine the number and principal properties of the polypeptides forming the erythrocyte membrane and to compile a catalog of proteins. Opportunities for research of this kind, and also the search for the primary biochemical defect in inherited anomalies increased considerably after the widespread introduction of two-dimensional polyacrylamide gel (PAG) electrophoresis, between coordinates of isoelectric point and molecular weight [2, 9]. Nevertheless, so far as we know, there have been very few attempts at two-dimensional electrophoretic study of erythrocyte membrane proteins and their results have proved contradictory [5, 7, 11, 12].

The aim of this investigation was to obtain a reproducible two-dimensional gel by electrophoresis of human erythrocyte membrane proteins and to draw a provisional protein map as the first step toward creation of a catalog of the polypeptides constituting this cell structure.

EXPERIMENTAL METHOD

Packed erythrocytes were obtained by passing heparinized blood (about 20 ml) through a column with HBS-cellulose, after which the erythrocytes were allowed to stand in a 3% solution of 500,000 dextran in 0.15 M NaCl twice, for 45 min each time. Erythrocyte membranes were obtained from erythrocytes by lysis in 5 mM Na-phosphate buffer, pH 8.0, containing 1 μM phenyl-

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