EFFECT OF K^+-DEPOLARIZATION ON THE PHYSICAL STATE OF MEMBRANE LIPIDS IN RAT BRAIN SYNAPTOSOMES

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Depolarization of neuron membranes, including membranes of nerve endings (NE), is accompanied [3] by structural changes and regrouping of protein-lipid complexes. The character of these changes is still largely unexplained, but their understanding is important for elucidation of the functioning of membrane-bound proteins (ionic channels and pumps, ion-exchangers, receptors) during excitation and the mechanism of coupling of depolarization and mediator secretion. In an investigation of the hydrophobic region of the lipid bilayer of synaptic membranes during depolarization, no significant changes in microviscosity of these membranes could be recorded by either fluorescent [9] or spin probes [12]. Depolarization of NE membranes evidently has no action on the hydrocarbon region of the lipid bilayer. Nevertheless, it can be postulated that changes in the physical state of the lipid bilayer are located close to protein subunits of NE membranes on account of increased mobility of synaptic membrane proteins on depolarization [12].

To study the physical state of the lipid bilayer in the region of protein–lipid contact of the synaptosomal membranes during depolarization, we used inductive-resonance energy transfer (IRET) from aromatic groups of proteins to fluorescent probes located in the lipid bilayer of NE membranes.

EXERIMENTAL METHOD

Noninbred albino rats weighing 200 g, kept on a standard animal house diet, were used. Synptosomes were isolated from the cerebral cortex of rats by the method in [11]. After their isolation the synaptosomes were kept in medium: 0.32 M sucrose ("Serva," West Germany), 20 mM Tris-HCl ("Sigma," USA), pH 7.4 at 4°C, and were used within 3-4 h. The protein concentration was determined by Lowry's method with the addition of 1% Na dodecylsulfate ("Serva"). Pyrene ("Fluka," Switzerland) and 4-dimethylaminochalcone – DMCH ("Monokristall Reaktiv" Research-Production Combine Khar'kov) were used as fluorescent probes. The fluorescence spectra of the probes were recorded on an MPF-4 spectrofluorometer ("Hitachi," Japan) in a constant-temperature cuvette 1 mm thick, located along the line bisecting the right angle between the optical axes of the monochromators. The spectral width of the slits did not exceed 5 nm. The incubation medium in which the measurements were made consisted of Krebs–Ringer solution buffered with 20 mM Tris-HCl, pH 7.4, at 37°C [7]. Synaptosomes were depolarized by addition of 30 mM KCl to the medium with replacement of the corresponding concentration of NaCl. Synaptosomes were added to the medium up to a final concentration, as protein, of 0.08 mg/ml, and incubated at 37°C for 10 min. The necessary portion of fluorescent probe was then added to the sample, mixed, and the spectral measurements made. Pyrene and DMCH were dissolved in 96% ethanol, the final ethanol concentration in the sample not exceeding 0.2%. Polarization spectra of 5 μM DMCH were recorded by the system of polarizers supplied by the firm for use with the apparatus. Polarization of fluorescence of DMCH (P) was calculated at the peak of the spectrum, allowing for scattering of light [2].

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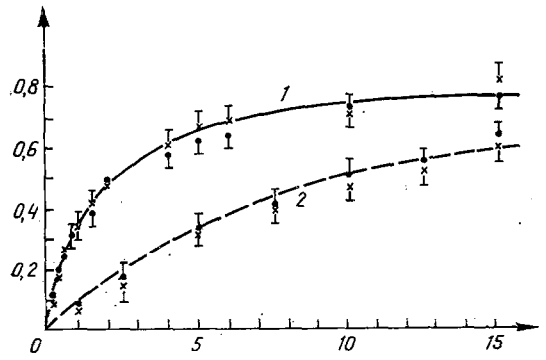

Fig. 1. Dependence of quenching of fluorescence of proteins in synaptosomal membranes by pyrene (1) DMCH (2), during depolarization (crosses) and in control (filled circles). Abscissa, concentration of pyrene or DMCH, in μM; ordinate, natural logarithm of ratio F_0/F, where F_0 and F denote intensities of fluorescence of proteins at 345 nm in the presence and absence of acceptor (probe) respectively.

To study microviscosity of the lipid bilayer of the synaptosomal membranes in the region of protein–lipid contact, IRET from aromatic groups of protein amino acids to the fluorescent probes were used. During excitation of the synaptosomal suspension by light with a wavelength of 285 nm, a fluorescence spectrum with peak intensity at 335 nm was recorded. Under these circumstances the principal fluorescent components are tryptophan residues, located mainly in the hydrophobic environment [1, 8]. Tryptophanyls of proteins were chosen as energy donors, and pyrene and DMCH, which have absorption in the region of fluorescence of tryptophan [4], as energy acceptors. On the addition of pyrene or DMCH to the NE membrane suspension the following phenomena took place: 1) quenching of fluorescence of tryptophanyls of proteins, 2) an increase in the intensity of fluorescence of the probe, 3) the appearance of an excitation band of tryptophan residues in the excitation spectrum of fluorescence of the probe. Thus IRET from tryptophanyls of proteins to the probe takes place in the synaptosomes – pyrene or DMCH system [2, 4, 5]. To calculate the intensity of fluorescence of pyrene monomers (F_m^{285}) and excimers (F_e^{285}) due to IRET, the method described in [6] was used, together with the equations:

$$F_m^{285} = F_{373} - F_{373}^p - F_{373}^n, \quad F_e^{285} = F_{475} - F_{475}^p - F_{475}^n,$$

where

$$F_{373,\,475}$$

denotes the measured intensity of fluorescence with excitation at 285 nm and recording at 373 and 475 nm; $FP_{373,475}$ the intensity of natural fluorescence of proteins after addition of pyrene, with excitation at 285 nm, at points 373 nm and 475 nm of the spectrum; $F^n{}_{373,475}$ the intensity of natural fluorescence of pyrene with direct excitation at 285 nm, calculated like FP, as in [6]. To estimate the microviscosity of the total lipid of the synaptosomal membranes, the ratio of intensities of fluorescence of pyrene monomers F_m^{338} and excimers F_e^{338} with excitation at 338 nm and recording of the fluorescence of the pyrene monomers at 373 nm and of the excimers at 475 nm was used. The degree of polarization of fluorescence of DMCH (P) with excitation at 430 nm and fluorescence at 510 nm was used for the same purpose [2, 4]. The experimental results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Addition of pyrene or DMCH to the synaptosomal suspension led to quenching of natural fluorescence of the proteins on account of IRET. According to [5], on the basis of the character of this quenching it is possible to calculate the efficiency of IRET, the accessibility of tryptophanyl groups of proteins to the probe, and after that, the mean distance from the center of gravity of the energy donors to the center of gravity of its acceptors. The use of DMCH and pyrene as acceptors of the energy of protein fluorescence enabled two different regions of the lipid bilayer of the synaptosomal membranes to be studied, for DMCH

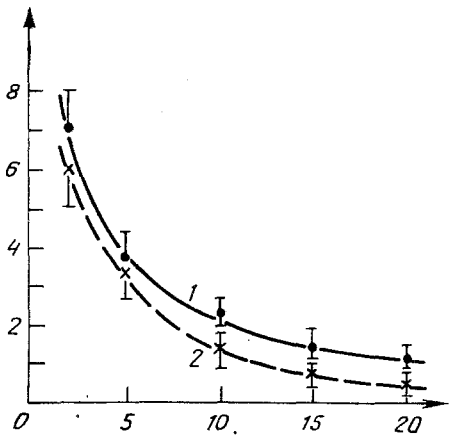

Fig. 2. Change in ratio of IRET-determined intensity of fluorescence of pyrene monomers and excimers in synaptosomal membranes in control (1) and during depolarization (2). Abscissa, concentration of probe, in μM; ordinate, ratio F_m^{285}/F_e^{285} (see: "Experimental Method").

was localized on the solution – membrane phase boundary, whereas pyrene was located in the hydrocarbon region of the membranes [2, 4, 5]. It will be clear from Fig. 1 that K^+-depolarization of the synaptosomal membranes did not lead to any change in the character of dependence of quenching of protein fluorescence by either pyrene or DMCH. The critical radius of energy transfer from tryptophanyl groups of membrane proteins to pyrene does not exceed 3 nm, and for DMCH it is about 2.5 nm [2, 5]. Consequently, for two different regions of the NE membranes in response to polarization there was no change in the efficiency of IRET or the accessibility of the tryptophan of the proteins to the probes. Moreover, the quantum yield of natural fluorescence of the synaptosomal membrane proteins under depolarization conditions did not differ significantly from that in the control, as could be judged by the intensity of fluorescence of proteins at the maximum of the spectrum (335 nm), fluorescence for two functionally different states of the synaptosomes being measured under strictly identical conditions. Accessibility of the tryptophanyl groups of the proteins to DMCH was lower than to pyrene (the angle of inclination of the curve is smaller), probably because DMCH is located in the surface layer of the lipid bilayer of NE membranes. The absence of differences between "resting" and depolarized synaptosomes in Fig. 1 can be explained by the small contribution (not exceeding the error of the method) of structural changes in the parameters studied and (or) the rapidity of the course and reversibility of these changes, so that they cannot be recorded under steady-state conditions.

By using the approach described in [6] it became possible to study lateral mobility of the pyrene molecules in the membrane lipid of NE located on the boundary with protein and to discover the effect of polarization on this region of the membrane. As Fig. 2 shows, depolarization of synaptosomal membranes evokes a decrease in the value of F_m^{285}/F_e^{285}, which signifies an increase in the lateral mobility of the pyrene molecules, evidence of reduction of the microviscosity of the membrane close to the protein subunits. Meanwhile investigation of the fluid properties of the total lipid bilayer by the use of parameters F_m^{338}/F_e^{338} of pyrene and polarization of fluorescence of DMCH showed that K^+-depolarization of NE membranes was not accompanied by any significant decrease in microviscosity for this part of the lipid bilayer (Fig. 3). The value of P in the control, for instance, was 0.25 ± 0.01, whereas during depolarization it was 0.24 ± 0.01 ($p > 0.05$). Consequently, neither in the hydrocarbon (pyrene) nor in the more superficial (DMCH) region of the total lipid bilayer of NE membranes was any change in the microstructure found on depolarization.

Thus in depolarized synaptosomes only that part of the lipid bilayer of the membranes which is in close contact with proteins, forming so-called protein-lipid complexes, undergoes restructuring. It can be postulated that reduction of the microviscosity of lipids in the vicinity of proteins is a result of a change in the character of functioning of the ionic pumps (Na,K- and Ca-ATPases), representing a considerable part of the total membrane protein of the synaptosomes [3]. Another possibility is that regrouping of cytoskeletal proteins anchored in the plasmalemma in response to inflow of Ca^{++} during depolarization of NE can increase the mobility of lipid molecules bordering on these proteins. Addition of colcemid,

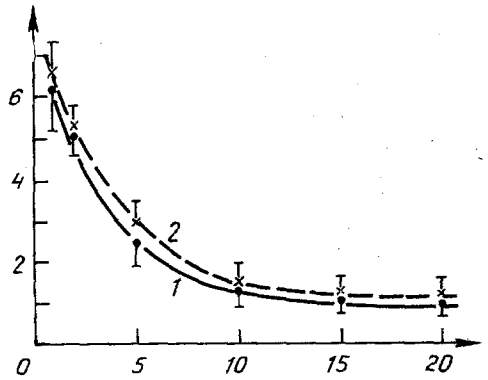

Fig. 3. Changes in ratio of intensity of fluorescence of pyrene monomers to excimers in synaptosomal membranes in control (1) and during depolarization (2) with excitation by light with a wavelength of 338 nm. Abscissa, concentration of probe (in μM); ordinate, ratio F_m^{338}/F_e^{338} (see: "Experimental Method").

which depolymerizes microtubules, to a suspension of Chinese hamster ovarian cells caused an increase in freedom of movement of spin-labeled membrane proteins [10]. As regards the "free" lipid component of the NE membranes, in the present experiments neither colchicine (10-50 μM) nor cytochalasin B (10-20 μM) changed the liquid properties of the lipid bilayer either at rest or during potassium depolarization, as was shown by polarization of fluorescence of DMCH. The latter is in agreement with results obtained on erythrocytes, when colchicine and vinblastin did not affect the microviscosity of erythrocytic membranes [13]. Yet another possible cause of changes in the physical state of the membrane in the region of protein-lipid contact during depolarization of NE may be interaction of membranes of synaptic vesicles with the presynaptic membrane, accompanied by fusion of these membranes [3].

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