12. T. A. Moskvitina, N. S. Kamyshanskaya, T. G. Garishvili, et al., Prep. Biochem., **9**, 171 (1979).
13. A. Szutowicz, P. J. Orsulak, and R. D. Kobes, Biochem. Med., **36**, 1 (1986).
14. M. B. H. Youdim, Experientia (Basel), **44**, 137 (1988).
15. W. F. Young, E. R. Laws, F. E. W. Sharbrough, et al., Arch. Gen. Psychiat., **43**, 604 (1986).

ACTION OF α-TOCOPHEROL AND FREE FATTY ACIDS ON PHYSICAL PROPERTIES OF THE LIPID MATRIX AND ON ADENYLATE CYCLASE ACTIVITY OF RAT BRAIN SYNAPTOSOMES

N. V. Gorbunov, L. A. Kuznetsova, V. I. Brusovanik, and A. N. Erin

UDC 615.356:577.161.3].015.4:612.82.015.152.34

KEY WORDS: synaptosomes; adenylate cyclase; lipids; α-tocopherol; free fatty acids.

Transmembrane transmission of neurotransmitter signals is a complex cascade process, triggered by interaction of ligand with receptor and involving different kinds of response (transmethylation of lipids, activation of phospholipases, prostaglandin synthesis, Ca^{2+} transport, etc.), depending on the type of secondary mediator. In some cases, β-adrenoreception for example, the final step in the mechanism of transmembrane signal transmission is adenylate cyclase (AC) [5, 7]. The β-adrenergic adenylate cyclase system (ACS) in the brain is a protein complex located in the plasma membrane and consisting of β-adrenoreceptor, catalytic subunit (AC itself), and a regulatory guanyl-nucleotide-binding protein (N-protein), of stimulating type [7]. The life span of functional complexes of this kind is limited and the process of their formation requires diffusion of the components of the ACS in the plane of the membrane [5, 6]. It is not surprising, therefore, that it has been suggested that the functional activity of ACS may be regulated by a change in the physical properties (microviscosity) of the membrane matrix, although the experimental data on this question are fairly contradictory and evidently depend largely on the type of membrane [6, 9].

For the reasons given above the aim of the present investigation was to study correlation between the functional state of the ACS of rat brain synaptosomal membranes and the physical state of their lipid matrix.

EXPERIMENTAL METHOD

The investigation was conducted on synaptosomal membranes from the brain of Wistar rats weighing 200-250 g. Synaptosomal membranes were isolated by the method developed previously [1]. The membrane protein content and microviscosity of the lipid matrix were determined and the lipid extract of the synaptosomal membranes obtained as in [1]. AC activity (EC 3.6.1.4) was determined with the aid of a 'Cyclic AMP Kit' (Amersham, England) as in [1].

α-Tocopherol and linolenic acid were added to a suspension of synaptosomes containing about 0.5 mg/ml of membrane protein in a 0.1 M alcoholic solution. The quantity of α-tocopherol incorporated into the membranes was determined by measuring fluorescence of the lipid extracts at λ_{exc} = 295 nm and λ_{fl} = 325 nm (the solvent was hexane). The content of free linolenic acid in the membranes was determined by gas–liquid chromatography, by the change in the characteristic line of the chromatogram of methyl esters of lipids extracted from the synaptosomal membranes.

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 2, pp. 153-155, February, 1990. Original article submitted May 15, 1989.

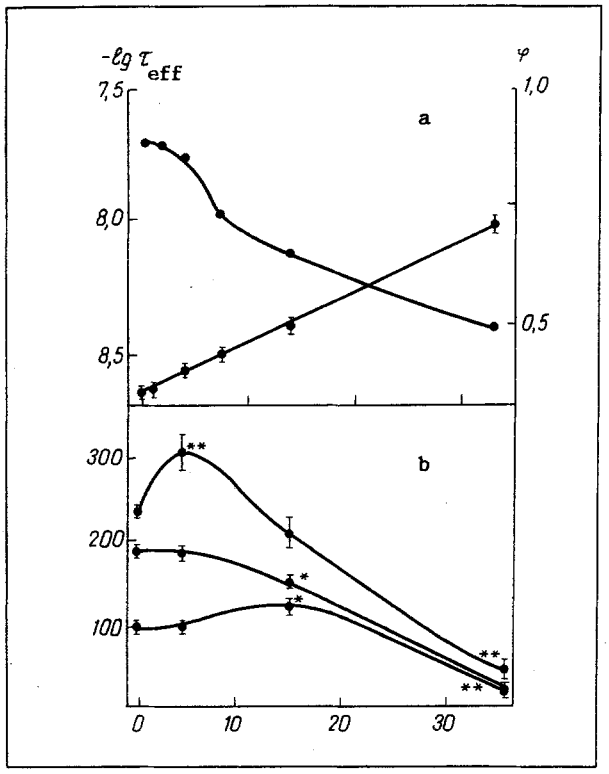

Fig. 1. Dependence of fraction of 'liquid' lipids (A, 1), logarithm of rotary correlation time (A, 2), and also basal AC activity (B, 3) and activity stimulated by isoproterenol (B, 4) or guanylyl-imidotriphosphate (B, 5), depending on α-tocopherol content in synaptosomal membranes. Abscissa, concentration of α-tocopherol (in moles %); ordinate: A) log τ_{eff}, on left [in $-$log (nsec)], and φ, on right (in relative units); B) AC activity (in %). $^{*}p < 0.05$, $^{**}p < 0.01$ Compared with initial values of AC activity.

Changes in lateral mobility of lipids of the membrane matrix as a whole and lipids of the microenvironment of the proteins were estimated by the change in ratio of fluorescence of excimer and monomer forms of pyrene, at λ_{exc} 320 and 280 nm, respectively [2]. Changes in the content of the 'liquid' phase fraction (lipids with a high degree of mobility) in the synaptosomal membranes were recorded as a relative change in amplitude and of the low-field 'fast' component in the EPR spectrum. The possibility of using this method of evaluation was demonstrated by preliminary studies on egg lecithin liposomes; the results are in press.

The α-tocopherol (d, l), linolenic acid, d, l-isoproterenol, and Tris were obtained from 'Sigma,' (USA), and the sucrose, pyrene, and guanylyl-5-imidotriphosphate (GITP) from 'Serva' (West Germany). The remaining reagents were of Soviet origin and of the chemically pure grade.

EXPERIMENTAL RESULTS

In accordance with the aim of the investigation, to change the microviscosity of the synaptosomal membranes we used two known modifiers with opposite actions, namely α-tocopherol and free fatty acids (FFA). The functional state of the ACS was assessed by measuring basal activity of the enzyme (without additional procedures), activity of GITP-stimulated AC, reflecting interaction between N-proteins and the catalytic subunit, and AC activity stimulated by the β-adrenoreceptor agonist, isoproterenol.

In the first series of experiments the effect of α-tocopherol on AC activity and on the physical state of the lipid matrix of the synaptosomal membranes was studied. The data in Fig. 1 show that addition of α-tocopherol to synaptosomal membranes up to 35 moles % caused a proportional increase in the rotary correlation time (τ) and a decrease in the fraction of 'liquid' lipids (φ), i.e., lipids with a high degree of mobility, reflecting an increase in microviscosity of the lipid matrix. AC activity under these

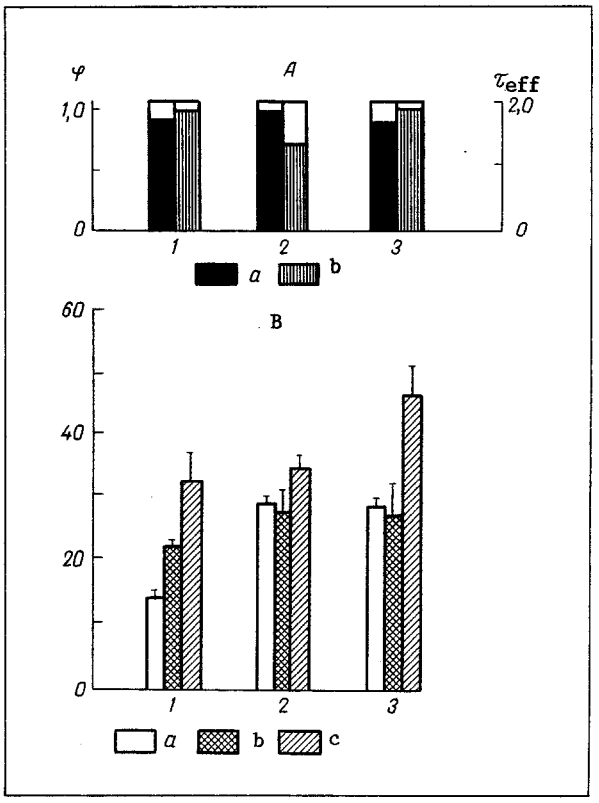

Fig. 2. Changes in effective rotary correlation time of spin probe A, fraction of 'liquid' lipids (A), and AC activity (B) in synaptosomal membranes. 1) Control, 2) linolenic acid (15-18 moles %), 3) the same + 15 moles % of α-tocopherol. A: a) φ (in relative units), b) τ_{eff} (in nsec). B: a) Basal AC activity, b) stimulation by isoproterenol, c) stimulation by GITP (everywhere in pmoles/mg protein/min).

circumstances showed a more complex change. In the presence of relatively low concentrations of α-tocopherol, basal activity and GITP-dependent activity were stimulated whereas AC activity stimulated by the β-agonist was unchanged. This does not accord with the view that an increase in microviscosity must reduce AC activity on account of hindrance of lateral diffusion of receptor, catalytic subunit, and N-protein. With a further increase in the α-tocopherol concentration, stimulation by isoproterenol and GITP coincided with a time of reduced basal activity of the enzyme. As our data show, a change in the functional state in this case may have been due not so much to an increase in microviscosity of the lipid matrix as to phase separation of the lipids and also to a change in the state of the boundary lipids.

In the next series of experiments the action of linolenic acid, which changes the physical state of the synaptosomal membranes in the opposite manner to α-tocopherol, was studied. As the data in Fig. 2 show, addition of FFA led to a decrease in the value of τ and to an increase in the fraction of 'liquid' lipids, reflecting a fall in microviscosity of the synaptosomal membranes. Basal AC activity in this case rose and the effect of stimulation of the enzyme by isoproterenol or GITP was virtually completely abolished. However, the change in activity is also difficult to explain by a change in microviscosity, for normalization of the original parameters characterizing microviscosity as a result of the addition of α-tocopherol, which can protect synaptosomal membranes against the chaotropic action of FFA [4], did not lead to normalization of ACS function (Fig. 2).

The results described above thus indicate that ideas according to which the microviscosity of the lipid matrix of synaptosomes regulates activity of ACS through its effect on the rate of formation of the short-living ACS complex during lateral diffusion of the subunits, are oversimplified. In fact, as the results show, opposite changes in microviscosity can give rise to similar changes in AC activity. If it is recalled that the synaptosomal lipids include highly unsaturated acyl fragments, it seems probable that the process of formation of the ACS complex on account of lateral diffusion of components depending on the microviscosity of the matrix lipids, is not the limiting stage.

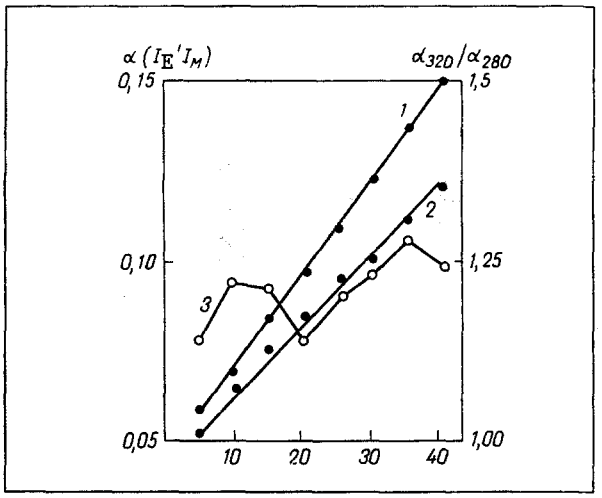

Fig. 3. Temperature dependence of degree of excimerization of pyrene in synaptosomal membranes (α; I_E/I_M) (1, 2), and also relative difference in degree of excimerization of pyrene in liquid microenvironment of proteins and in general lipid matrix of membranes ($\alpha_{320}/\alpha_{280}$; 3). 1 and 2) Excited by light with wavelength of 320 and 280 nm, respectively. Abscissa, temperature (in °C).

The physical properties of lipids on the boundary with proteins, which may differ from the properties of the lipid bilayer, may perhaps be more important for optimal function of the ACS. In fact, as the data in Fig. 3 show, the parameter of excimerization of pyrene inserted into the synaptosomal membranes differs in the case of direct excitation of the probe and of migration of energy to the pyrene molecules from the chromophore protein groups within the temperature range of 5-40°C. These differences are most marked at physiological temperatures, reflecting the difference in the physical state of the general and boundary phases of the lipids. Other workers, using somewhat different experimental approaches, have come to the same conclusion [3]. Taking this nonhomogeneity of distribution of lipids in the synaptosomal membranes into account, it therefore seems most probable that the regulatory effect of lipids on ACS activity is determined by the composition of the 'boundary' lipids. As a result, with the optimal lipid composition, on account of protein–lipid interactions the conditions for steric interaction between receptor, regulatory, and catalytic components are realized.

LITERATURE CITED

1. N. V. Gorbunov, L. A. Kuznetsova, M. L. Borin, and A. N. Erin, Byull. Éksp. Biol. Med., No. 10, 438 (1988).
2. A. D. Dergunov, A. S. Kaprel'yants, and D. N. Ostrovskii, Biokhimiya, **46**, No. 8, 1499 (1981).
3. I. M. Okun', G. V. Kaler, T. M. Volkovets, et al., Biokhimiya, **51**, No. 7, 1132 (1986).
4. A. N. Erin, N. V. Gorbunov, V. I. Brusovanik, et al., Brain Res., **398**, 85 (1986).
5. M. D. Hollenberg, Experientia (Basel), **42**, 718 (1986).
6. M. J. Karnovsky, A. M. Kleinfeld, R. L. Hoover, and R. D. Klausner, J. Cell Biol., **94**, 1 (1982).
7. R. J. Lefkowitz, R. Cerione, J. Codina, et al., J. Membr. Biol., **87**, 1 (1985).
8. P. J. Scarpace, S. W. O'Connor, and I. B. Abrass, Biochim. Biophys. Acta, **845**, 520 (1985).
9. A. K. Sinha, S. J. Shattil, and R. W. Colman, J. Biol. Chem., **252**, 3310 (1977).