EFFECT OF PHOSPHOLIPASE A_2 AND α-TOCOPHEROL ON ADENYLATE CYCLASE ACTIVITY OF RAT BRAIN SYNAPTOSOMES

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In the modern view, transmembrane transmission of certain chemical signals is a complex process, triggered by interaction of ligand with receptor, and including transmethylation of lipids, activation of phospholipases, prostaglandin synthesis, and Ca^{++} transport in the subsequent postreceptor reactions. In some cases, α-adrenoreception for example, an important stage in realization of the action of the mediator on cell metabolism is activation of adenylate cyclase (AC) [9]. Lipids are known to play an essential role in the transmission of chemical signals, in which they are involved in certain stages [2, 8]. To study the character of the effect of lipids on transduction of chemical signals in the cell, the use of agents modifying the properties of the lipid bilayer of the membranes has proved effective. In particular, it has been shown [3] that treatment of synaptosomes with phospholipase A_2 causes changes both in the physicochemical state of the lipid bilayer and in the properties of β-adrenergic receptors — the initial stage of the cascade mechanism. The problem of the effect of phospholipase A_2 on activity of AC — another component of the β-adrenergic system — in the synaptosomes has not been studied.

Accordingly, in the investigation described below on brain synaptosomes, changes in the functional state of the AC system were studied during the action of phospholipase A_2 and α-tocopherol, which can protect the lipid bilayer against injuries caused by this enzyme [6].

EXPERIMENTAL METHOD

Synaptosomes were isolated by the method in [7] from brain tissue of Wistar rats. Determination of the concentration of synaptosomal protein, the preparation of lipid extracts, separation of the phospholipid classes, and determination of the content of lipid phosphorus were carried out as described previously [6]. The microviscosity of the lipid bilayer of the synaptosomal membranes was estimated by measuring the rotary correlation time of a spin probe — the ethyl ester of 7-inoxylstearic acid [10]. EPR spectra were recorded on a small EPR spectrometer in capillary tubes 1 mm in diameter. The spin probe and α-tocopherol were added to the suspension of synaptosomes in alcoholic solution (the quantities of ethanol introduced did not exceed 0.1% of the volume of the suspension).

The synaptosomal membranes were treated with phospholipase A_2 in medium containing 40 mM Tris, 100 mM NaCl, and 1 mM $CaCl_2$ (pH 7.4). Hydrolysis was induced by addition of the enzyme (0.2 μg/mg protein) to the membrane suspension. Hydrolysis was stopped by addition of EDTA (3 mM). The degree of hydrolysis was judged from the lysophosphatidylcholine concentration in the lipid extract.

AC activity was determined by the method in [4]. The reaction was carried out in 200 μl of incubation medium of the following composition: 50 mM Tris, 1 mM EDTA, 5 mM phosphoenolpyruvate, and 3.2 U pyruvate kinase (pH 7.4). The reaction was started by adding the membranes to the incubation medium, and it was stopped after 10 min by boiling (3 min). The samples were then centrifuged to precipitate the denatured protein and the quantity of cAMP formed was determined in the supernatant by means of a "Cyclic AMP Kit" (Amersham International, England).

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TABLE 1. Effect of Phospholipase A_2 and of α-Tocopherol on AC Activity and Rotary Mobility of Spin Probe in Synaptosomal Membranes (M ± m)

Experimental conditions	AC activity				Rotary correlation time (τ), msec
	basal	stimulated by TITP	stimulated by isoproterenol and GITP	stimulated by proterenol	
Control	29±4	74±3 (149)	96±4 (230)	60±3 (98)	2,4±0,1
α-Tocopherol	30±3	101±4 (236)	66±10 (120)	59±5 (94)	2,7±0,1
Phospholipase A_2	30±3	111±16 (273)	67±10 (123)	37±3 (26)	2,0±0,1
α-Tocopherol + phospholipase A_2	47±4	89±5 (88)	139±20 (196)	39±3 (0)	2,6±0,1

Legend. Absolute values of activity in pmoles/mg protein/min and (in parentheses) its excess over the corresponding basal activity (in %) are given. Confidence interval evaluated by Student's test at the $p \leq 0.05$ level of significance (n = 2-3).

Phospholipase A_2 from bee venom, D,L-α-tocopherol, D,L-isoproterenol, and Tris were obtained from "Sigma," (USA) and guanosylyl-5-imidotriphosphate from "Serva" (West Germany). The remaining reagents were of Soviet origin and of the chemically pure grade.

EXPERIMENTAL RESULTS

The AC system of the synaptosomes, sensitive to the action of neurotransmitters, is a protein complex located in the plasma membrane and consisting of a receptor (a β-adrenoreceptor in the case of the β-adrenergic system), an oligomeric guanyl-nucleotide-binding protein (N-protein), and a catalytic subunit [2, 9]. To assess the functional state of the components of this system, basal activity was investigated as a characteristic of the catalytic subunit; AC activity, stimulated by guanylyl-imidotriphosphate (GITP), reflecting interaction of N-proteins with the catalytic subunit, and AC activity stimulated by the β-agonist isoproterenol and by isoproterenol + GITP, characterizing the functional state of the system as a whole, also were studied.

It will be clear from Table 1 that treatment of the synaptosomes with phospholipase A_2, corresponding to 10-12% hydrolysis of phospholipids, led to an increase in the GITP-stimulated AC activity and to a decrease in activity stimulated by isoproterenol alone, or isoproterenol together with GITP. Basal activity was unchanged under these circumstances. Preliminary addition of α-tocopherol (10^{-4} M, protein concentration 1 mg/ml) to the suspension of synaptosomes partially abolished the action of phospholipase on the functional state of AC. This was expressed as normalization of AC activity stimulated by GITP alone, or GITP + isoproterenol, almost to the control level. Meanwhile, α-tocopherol did not affect the reduction of AC activity stimulated by isoproterenol, under the influence of phospholipase A_2. This last fact sheds light on the significance of protein and lipid interactions in β-adrenoreceptor function and confirms conclusions published previously [3].

It can thus be concluded from these experiments that α-tocopherol evidently restores normal interaction of the catalytic and regulatory subunits of AC in the modification induced by phospholipase A_2, both in the presence and in the absence of the β-agonist. This normalizing action of α-tocopherol is evidently unconnected with inhibition of phospholipase A_2, for as the control experiments showed, the quantity of products of phospholipid hydrolysis by this enzyme was unchanged by the presence of α-tocopherol. Treatment of synaptosomal membranes with phospholipase A_2 is known to change the microviscosity of the lipid bilayer, and α-tocopherol can stabilize this parameter during this treatment [5]. It can therefore be expected that normalization of AC activity by α-tocopherol, under the influence of phospholipase A_2, is linked with its effect on the lipid bilayer. To test this hypothesis, effects of the separate action of α-tocopherol and of phospholipase A_2 on GITP-stimulated AC activity and on the microviscosity of the lipid bilayer, and also of their combined action on the same parameters, were compared. It will be clear from Table 1 that the change in microviscosity of the lipid bilayer (as reflected in the rotary mobility of the spin probe) and in GITP-stimulated AC activity takes place independently. For instance, although both α-tocopherol and phospholipase A_2 increase AC activity, they give rise to opposite changes in the microviscosity of the lipid bilayer. Although in the presence of α-tocopherol, phospholipase A_2 has virtually no effect on the microviscosity of the lipid bilayer, the GITP-stimulated AC activity is normalized. In our view this means that normalization of AC function by α-tocopherol during the action of phospholipase A_2 is connected, not with its effect on the lipid bilayer as a whole, which is

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].

LITERATURE CITED

1. S. V. Konev, T. I. Lyskova, I. M. Okun', and S. L. Aksentsev, Abstracts of Poster Communications, 5th All-Union Biochemical Congress [in Russian], Vol. 2, Moscow (1986), pp. 384-385.
2. M. N. Pertseva, Zh. Évol. Biokhim. Fiziol., No. 3, 281 (1981).
3. L. L. Prilipko, V. E. Kagan, V. A. Tyurin, et al., Dokl. Akad. Nauk SSSR, 296, No. 5, 1260 (1983).
4. V. A. Tkachuk and G. N. Baldenkov, Biokhimiya, 43, No. 6, 1097 (1978).
5. V. P. S. Channan, L. S. Ramsammy, and H. Brockerhoff, Biochim. Biophys. Acta, 772, 239 (1984).
6. A. N. Erin, N. V. Gorbunov, V. I. Brusovanik, et al., Brain Res., 398, 85 (1986).
7. J. M. Gurd, L. R. Jones, H. R. Mahler, and W. J. Moore, J. Neurochem., 22, 281 (1974).
8. F. Hirata and J. Axelrod, Science, 209, 1082 (1980).
9. R. J. Lefkowitz, R. A. Cerrione, J. Codina, et al., J. Membr. Biol., 87, 1 (1985).
10. M. Pontus and M. Delmelle, Experientia (Basel), 31, 799 (1975).

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

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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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