ROLE OF GANGLIOSIDES IN PROTECTION OF β-ADRENORECEPTORS AGAINST DAMAGE BY LIPID PEROXIDATION IN SYNAPTOSOMAL MEMBRANES

V. A. Tyurin, L. A. Kuznetsova, Yu. Yu. Tyurina, A. N. Erin, N. F. Avrova, M. N. Pertseva, and V. E. Kagan

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The view most widely held at the present time is that transmembrane signal transmission involves the participation of three main molecular components of signal systems, namely receptor, catalytic, and regulatory [2, 10]. The exceptional importance of the coordinated working of all these components of this system will be evident. Receptor proteins of brain membranes are typical integral proteins, whose properties depend essentially on interaction with the lipid bilayer of the membrane [2, 10, 11]. For example, modification of the lipid bilayer of membranes by products of lipid peroxidation (LPO), formed in many different diseases (Parkinson's disease, senile dementia, schizophrenia, etc.) has a powerful damaging action on neuronal functions such as reception, uptake, and release of neurotransmitters [4, 7, 14, 15]. It was found comparatively recently that besides enzymes of antioxidative protection and antioxidants, signal systems whose effect is realized in the cell through the protein kinase system [8] may also be involved in the regulation of LPO.

The aim of this investigation was to study the action of the monosialoganglioside GM1 (a modulator of protein kinase activity) and of the natural lipid-soluble antioxidant α-tocopherol on the properties of β-adrenoreceptors in brain synaptosomes during induction of LPO.

EXPERIMENTAL METHOD

Synaptosomal membranes were obtained from the cerebral cortex of Wistar rats by the method in [6] with certain modifications. Protein was determined by a modified Lowry's method [12]. Lipids were extracted from the membranes by the method of Folch et al. [5]. Monosialoganglioside GM1 was obtained from hog brain, as described previously [1]. The membranes were incubated with ganglioside GM1 as described in [3], in medium consisting of Tris-HCl 40 mM, NaCl 100 mM, pH 7.4. LPO was induced in a system of Fe^{2+} — ascorbate (10 μM Fe^{2+}, 0.5 mM ascorbic acid). The reaction was stopped by addition of 4-methyl-2,6-di-tert-butylphenol in a concentration of 0.5 mM) Accumulation of LPO products was recorded spectrophotometrically by the reaction with 2-thiobarbituric acid (TBA-active products) [16]. Analysis of ligand receptor interaction was carried out with the aid of [^{3}H]-dihydroalprenolol. Radioactivity was determined by the scintillation method. Specific binding of the ligand was determined as the difference between its binding in the absence and in the presence of an excess of the nonradioactive antagonist propranolol [9]. The fatty acid composition of the lipids were studied on a gas—liquid chromatograph (Pye-104, England) with flame-ionization detector, the fatty acids being identified against a standard mixture. The [^{3}H]-dihydroalprenolol was obtained from Amersham (England) and the propranolol, D,L-α-tocopherol, and TBA from Serva (West Germany). The remaining reagents were of Soviet origin.

*Deceased.

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. Institute of Psychiatry, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. Institute of Physiology, Bulgarian Academy of Sciences, Sofia. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman*.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 6, pp. 597-599, June, 1991. Original article submitted December 7, 1990.

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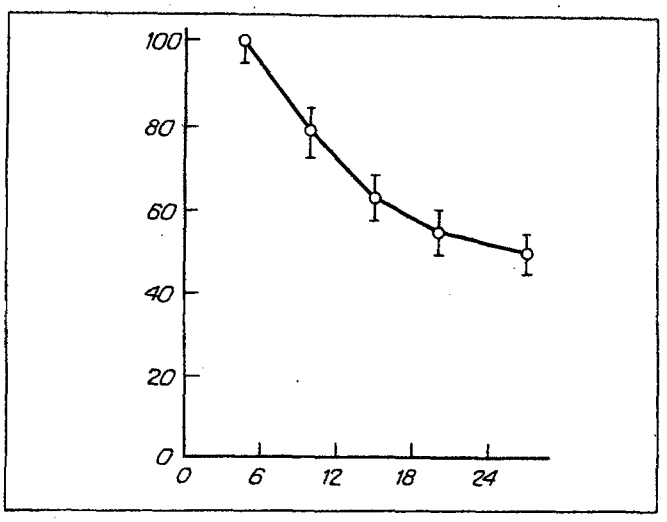

Fig. 1. Effect of induction of LPO on specific binding of [^{3}H]-dihydroalprenolol with β-adrenoreceptors, Abscissa, concentration of LPO products (in nmoles MDA/mg protein); ordinate, specific binding of [^{3}H]-dihydroalprenolol (in %)

TABLE 1. Action of Induction of LPO on Specific Binding of [^{3}H]-Dihydroalprenolol with β-Adrenoreceptors of Synaptosomal Membranes (M ± m)

Parameter	Control	Induction of LPO, 30 min		
			In presence of α-tocopherol (10^{-6} M)	In presence of monosialoganglioside GM1 (10^{-8} M)
B_{max}, nmoles/mg protein	1,28±0,01	0,51±0,02	1,19±0,02	1,00±0,02
K_d, nM	0,20±0,05	1,80±0,15	0,10±0,19	0,23±0,05

EXPERIMENTAL RESULTS

The results in Fig. 1 and Table 1 indicate that as a result of chemical modification of the lipids, due to induction of LPO, changes in ligand-receptor interaction take place in the synaptosomal membranes. This is manifested by the fact that there was a significant decrease in the level of specific binding of [^{3}H]-dihydroalprenolol, in agreement with results obtained by other workers [9] who studied the effect of induction of LPO on β-adrenoreceptor activity in erythrocytes. Under these circumstances not only lowering of the level of maximal binding B_{max} was recorded, but also an increase in the values of the dissociation constant K_d. These changes in the properties of the β-adrenoreceptors depend on the quantity of phospholipids undergoing modification, and they are intensified with an increase in the concentration of LPO products.

Previously we demonstrated the dose-dependent regulatory effect of various gangliosides relative to free-radical processes in nerve tissue [3, 15]. In the next series of experiments we therefore chose that concentration of monosialoganglioside GM1 (10^{-8} M) at which maximal inhibition of LPO is observed. Curves of accumulation of LPO products (TBA-positive products) in synaptosomal membranes in the course of their incubation in a system of Fe^{2+}-ascorbate, in the absence and in the presence of ganglioside GM1 and α-tocopherol, are shown in Fig. 2. It will be clear from these data that preincubation of synaptosomal membranes with ganglioside GM1 and α-tocopherol leads to marked inhibition of LPO, which is reflected in a decrease in malonic dialdehyde (MDA) accumulation by 46 ± 7 and 34 ± 5% respectively.

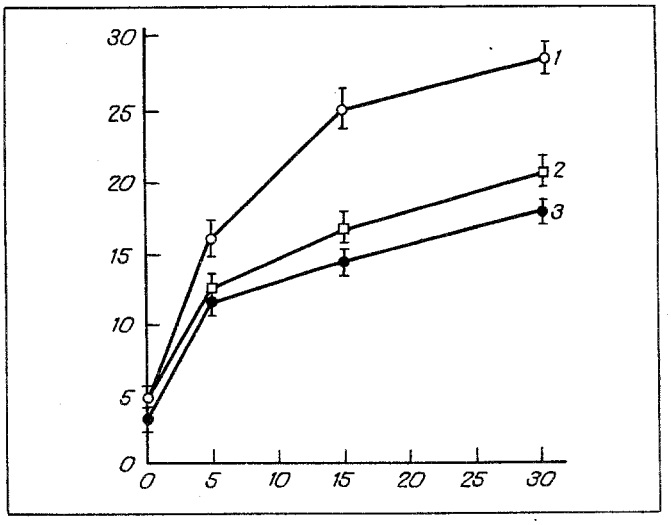

Fig. 2. Accumulation of LPO products in synaptosomal membranes during induction of LPO in a system of Fe^{2+} — ascorbate. 1) Control; 2) in presence of α-tocopherol (10^{-6} M); 3) in presence of ganglioside GM1 (10^{-8} M). Abscissa, time (in min); ordinate, concentration of LPO products (in nmoles MDA/mg protein).

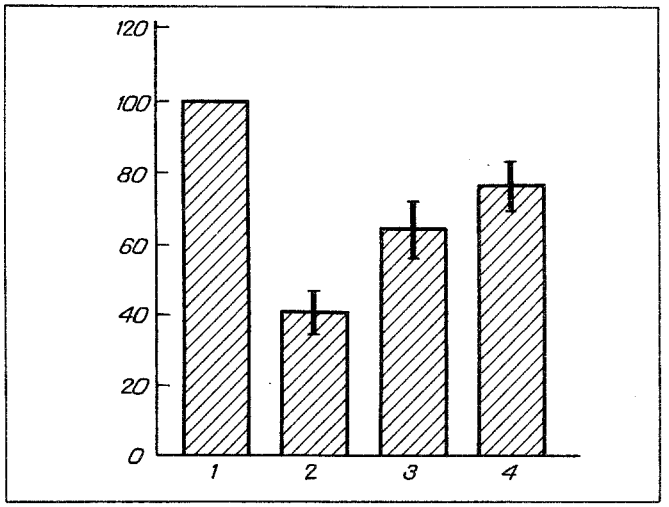

Fig. 3. Effect of ganglioside GM1 and α-tocopherol on specific binding of [^{3}H]-dihydroalprenolol with β-adrenoreceptors in synaptosomal membranes during induction of LPO. 1) Control; 2) induction of LPO; 3) induction of LPO + α-tocopherol (10^{-6} M); 4) induction of LPO + GM1 (10^{-8} M). Ordinate, specific binding of [^{3}H]-dihydroalprenolol (in %).

In these same experiments protection of the β-adrenoreceptors against the damaging action of LPO in synaptosomal membranes was observed. For instance, during induction of LPO in the absence of GM1 and of α-tocopherol the level of specific binding of [^{3}H]-dihydroalprenolol fell by 60% whereas in the case of preincubation with 10^{-8} M GM1 or 10^{-6} M α-tocopherol this decrease amounted to only 23 and 35% respectively (Fig. 3). Protection of β-adrenoreceptors against LPO products was manifested by a decrease in the value of B_{max} on induction of LPO to 60% whereas under the influence of ganglioside GM1 and α-tocopherol it reverted to normal values, on average 1.00 and 1.19 pmole/mg protein respectively. It will be clear from Table 1 that these compounds also restored normal affinity of the receptors or the

TABLE 2. Action of Ganglioside GM1 and α-Tocopherol on Fatty Acid Composition of Lipids of Synaptosomal Membranes during Induction of LPO

Parameter	Control	Relative content, % of total		
		induction of LPO, 30		
			In presence of α-tocopherol (10^{-6} M)	In presence of ganglioside GM1 (10^{-8} M)
Fatty acids				
monoene	22,6	26,5	27,0	26,8
polyene	34,0	19,4	28,1	29,6
saturated	43,4	54,1	44,9	43,6
$\frac{C_{16:0}+C_{18:0}}{C_{20:4}+C_{22:0}}$	1,52	3,87	2,13	1,99
Index of unsaturation	187,0	113,1	158,2	164,3

Legend. During calculation of index of unsaturation the following fatty acids were taken into account: 18:1, 18:2, 20:1, 20:3ω6, 20:4ω6, 20:4ω3, 22:4ω6, 22:5ω6, 22:6ω3.

ganglioside. We know that the properties of β-adrenoreceptors depend essentially on the physicochemical state of the biological membranes [2, 9, 10, 11]. Modification of the lipid bilayer may alter ligand—receptor interactions and lead to both an increase and a decrease in the efficacy of transmembrane signal transmission [2, 10, 11].

Considering that the hydrophobic bilayer of the membranes is formed by hydrocarbon chains of fatty acids of lipids, in the next series of experiments we studied the fatty acid composition of lipids of synaptosomal membranes. The data given in Table 2 show that induction of LPO causes a decrease in the fraction of polyene fatty acids, as reflected in reduction of the index of unsaturation of the fatty acids, In the presence of exogenous ganglioside GM1 (10^{-8} M) or of α-tocopherol (10^{-6} M) protection of the polyunsaturated fatty acids from oxidative destruction takes place.

Comparison of the inhibitory action of ganglioside GM1 and of α-tocopherol on LPO shows that the inhibitory effect of GM1, manifested as a decrease in the rate of accumulation of LPO products, is not characterized by the presence of a lag-period, which is observed in the case of high concentrations of α-tocopherol and other antioxidants, interacting with lipid radicals [3, 13]. In addition, the inhibitory effect of α-tocopherol rises in a straight line with an increase in its concentration, whereas the dependence of the inhibitory action of ganglioside GM1 is biphasic in character. In the presence of polymixin B (an inhibitor of protein kinase C) GM1 has virtually no effect on induced LPO [13]. These facts suggest that although ganglioside GM1 and α-tocopherol have a similar protective action on ligand-receptor interactions during induction of LPO, this action is effected through different mechanisms. Indirect evidence in support of this conclusion is given by our data showing that the penetrating analog dibutyryl-cAMP (10^{-6} M) partially abolishes inhibition of LPO by ganglioside GM1 and does not affect the inhibitory action of α-tocopherol in synaptosomal membranes. These data suggest that inhibition of LPO in the synaptosomal membranes is not realized through direct interaction of ganglioside GM1 with lipid radicals or with active forms of oxygen, but is connected with its indirect action, probably through activation of protein kinases involved in the regulation of LPO.

The results are thus evidence that preincubation of synaptosomal membranes with ganglioside GM1 or with α-tocopherol protects the membranes against oxidative destruction, and thereby exerts a protective effect in relation to β-adrenoreceptors.

It can be tentatively suggested that this effect of protection of β-adrenoreceptors by ganglioside GM1 and α-tocopherol which we found during induction of LPO is of great importance for our understanding of the mechanisms of maintenance of adrenoreceptor reactivity of the cell and its correction during the development of pathological states accompanied by activation of LPO in brain membranes.

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ROLE OF ESTROGENS IN REGULATION OF THE BILE ACID COMPOSITION OF THE ENTEROHEPATIC SYSTEM OF RABBITS AND MONKEYS WITH EXTRAHEPATIC CHOLESTASIS

Yu. A. Bogdarin and N. P. Goncharov

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The study of the action of estrogens on lipid metabolism in cholestasis is extremely important because this disease is sex-dependent [8, 10]. The link between induction of cholecystasis by retention of bile and its formation after the use of contraceptives is known [7]. During the formation of cholesterol biliary calculi (CBC) a leading role is ascribed to changes in the metabolism of bile acids on whose biosynthesis and secretion into the bile complex formation between biliary lipids in a solution of bile depends, in the hepatocytes [5]. Since the liver is an extragenital organ for endogenous and exogenous sex steroids, and is influenced by these substances [1], analysis of the bile acid composition of the enterohepatic system (EHS), which depends to a certain degree on the estrogen level [9], is important in the study of the mechanism of CBC formation. This paper gives the results of a comparative study of bile acid levels in EHS of rabbits and monkeys with extrahepatic cholestasis associated with estrogen deficiency and excess.

Laboratory of Biochemistry, Research Institute of Pediatrics, Ministry of Health of the RSFSR, Nizhnii Novgorod Laboratory of Biochemical Endocrinology, All-Union Endocrinologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. A. Pankov.)

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