ROLE OF GANGLIOSIDES IN REGULATION OF FREE-RADICAL REACTIONS IN BRAIN MEMBRANES

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In the modern view initiation of lipid peroxidation (LPO) processes in membrane structures is determined by the efficiency of generation of active forms of oxygen by the various electron-transport systems [3, 11]. Products of single- and double-electron reduction of oxygen ($O_2^{\cdot-}$, H_2O_2) are themselves ineffective lipid oxidizing agents [1, 3, 11]. However, as a result of the breakdown of these molecules or their interaction, especially in the presence of variable valency metals, highly toxic hydroxyl radicals ($OH^{\cdot}$) are formed [1, 3, 11]; these, interacting with polyunsaturated lipids at high velocity, generate radical lipid intermediates ($R^{\cdot}$, $RO^{\cdot}$, $ROO^{\cdot}$) and lead to the formation of a variety of molecular LPO products [1, 3, 11, 14]. Excessive accumulation of LPO products is caused, on the one hand, by hyperproduction of active forms of oxygen, and on the other hand, by a deficiency of enzymic and nonenzymic antioxidant systems. It has been shown that besides antioxidant systems, systems of signal transduction, realizing their effect at the protein kinase level, may also play a role in the regulation of LPO [12]. Gangliosides are modulators of protein kinase activity (calmodulin-dependent, ganglioside-dependent, etc.) [7, 8]. We showed recently that gangliosides, after preincubation with brain synaptosomal membranes, can protect β-adrenoreceptors and polyunsaturated fatty acids against oxidative destruction and reduce accumulation of LPO products [4, 5].

To further elucidate the mechanism of inhibition of LPO by gangliosides we have studied the action of the monosialoganglioside GM1 on free radical formation, recorded as luminol-dependent chemiluminescence induced by $Fe^{2+}-H_2O_2$ in synaptosomal membranes and brain myelin.

EXPERIMENTAL METHOD

Synaptosomal membranes and brain myelin were obtained from Wistar rats by the method in [10] with certain modifications. The protein concentration was measured by a modified Lowry's method [13]. Lipids were extracted from the membranes by Folch's method [9]. The fatty-acid composition of the lipids was studied on a gas–liquid chromatograph ("Pye-104," U.K.) with flame-ionization detector, capable of identifying fatty acids in a standard mixture. Gangliosides were extracted from bovine brain by Folch's method, with additional treatment by Suzuki's method [16]. Gangliosides were fractionated by preparative column chromatography on silica-gel KSK in a system of chloroform:methanol:water (65:25:4 and 60:35:7). The purity of the isolated ganglioside fractions was not less than 98-99%, as shown by thin-layer high-resolution chromatography. The concentration of gangliosides was determined as N-acetylneuraminic acid by the resorcin method [6]. Preincubation of the synaptosomal membranes and myelin with the monosialoganglioside GM1 and α-tocopherol was carried out at 37°C in medium of the following composition: 40 mM Tris-HCl, 100 mM NaCl, pH 7.4. The formation of active forms of oxygen was induced in

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TABLE 1. Content of Principal Polyunsaturated and Saturated Fatty Acids in Synaptosomal and Myelin Membranes of the Rat Brain

Fatty acids	Synaptosomal membranes	Myelin membranes
C20:4ω6, % of total sum of fatty acids	15.8	8.7
C22:6ω3, % of total sum of fatty acids	14.1	4.3
C20:4ω6+C22:6ω3 / C14:0+C16:0+C18:0	0.68	0.35

Legend. C14:0 myristic, C16:0 palmitic, C18:0 stearic, C20:4ω6 arachidonic, C22:6ω3 docosahexaenic fatty acid.

the Fe^{2+}-H_2O_2 system and determined by the luminol-dependent chemiluminescence method, with recording on an LKB-1251 chemiluminometer (Sweden). The final protein concentration in the cuvette was 1 mg/ml, of Fe^{2+} 20 μM, of H_2O_2 400 μM, and luminol 50 μM. The following reagents were used: luminol and 12-myristate-13-acetate phorbol ester (PMA, from "Sigma," USA), and D,L-α-tocopherol (from "Serva," Germany). The remaining reagents were of Russian origin and of the chemically pure and highly pure grades.

EXPERIMENTAL RESULTS

Typical curves of luminol-dependent chemiluminescence, induced by the Fe^{2+}-H_2O_2 system in Tris-HCl buffer (a) and in synaptosomal membranes and brain myelin (b) are shown in Fig. 1. Clearly during generation of active forms of oxygen in the buffer system which we used, the "fast" flash of chemiluminescence, due to interaction of luminol with oxygen radicals is recorded: the superoxide anion-radical $O_2^{\cdot-}$ and the hydroxyl radical ($OH^{\cdot}$) [2, 15]. The formation of a fast flash of chemiluminescence also is observed in synaptosomal membranes and myelin, but at the same time a "slow" component of the response (slow flash) is recorded, due to interaction of luminol with lipid radicals, and reflecting the reaction of disproportioning of peroxide radicals ($RO_2^{\cdot}-RO_2^{\cdot}$) [1, 2]. Under these circumstances the character of the chemiluminescence kinetics in myelin membranes, and also in synaptosomal membranes before and after their preincubation with ganglioside GM1 and α-tocopherol does not differ significantly (Fig. 1). Comparison of the level of chemiluminescence (the slow component of the response) in the preparations studied, under identical conditions of generation of active forms of oxygen, shows that the intensity of chemiluminescence in synaptosomal membranes is significantly higher than in myelin membranes. This is probably due to the higher content of polyunsaturated fatty acids: arachidonic and docosahexaenic (the main sources of lipid radicals) in synaptosomal membranes compared with myelin membranes (Table 1). Preincubation of synaptosomal and myelin membranes with ganglioside GM1 leads to a fall in the level of chemiluminescence (Figs. 1 and 2). Reduction of the intensity of chemiluminescence also takes place in the presence of exogenous lipid-soluble antioxidant α-tocopherol (Figs. 1 and 2). The observed inhibition of luminol-dependent chemiluminescence may be the result of capture of directly free radicals or activation of enzymes of the antioxidant defense system. The data given in this paper may be evidence of differences in the mechanisms of action of the monosialoganglioside GM1 and α-tocopherol. We know that the mechanism of action of the natural inhibitor of free-radical reactions α-tocopherol is realized through its interaction with the superoxide and hydroxyl radical, and also with lipid radicals [1, 3, 11, 14]; moreover, the higher the concentrations in which it is used and the greater its inhibitory effect. The study of dependence of the chemiluminescence response of synaptosomal membranes on the GM1 concentration shows that the curve characterizing the inhibitory effect is biphasic in character and reaches peak values at a concentration of 10^{-8} M (Fig. 3). It must be pointed out that the inhibitory effect of α-tocopherol is exhibited immediately after its addition to the membranes, whereas for GM1, preincubation with the membranes for at least 30-60 min is necessary (Fig. 2). Some differences in the mechanism of the inhibitory action of gangliosides on LPO (absence of a lag-period, absence of effect after

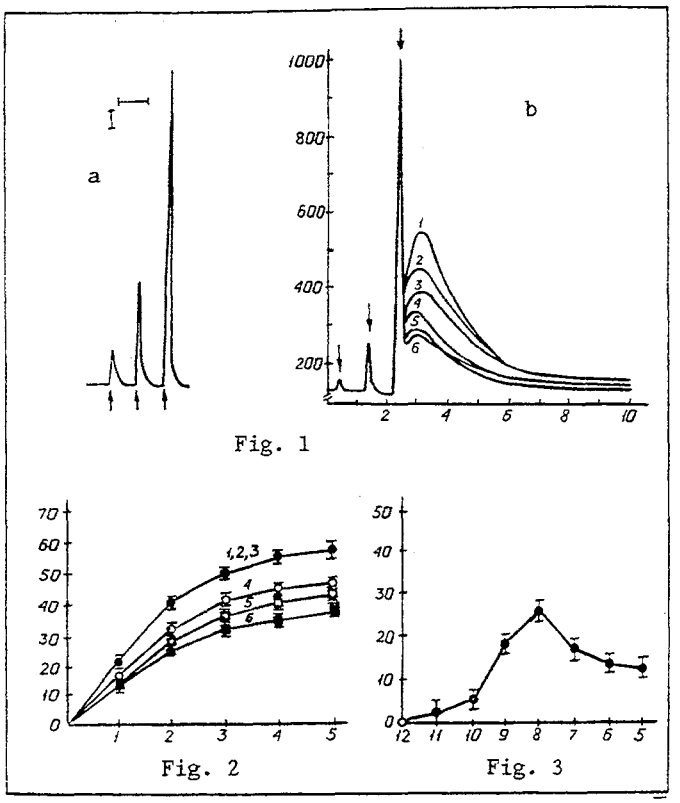

Fig. 1. Effect of GM1 and α-tocopherol on luminol-dependent chemiluminescence induced by $Fe^{2+}-H_2O_2$ system in Tris-HCl buffer (a) and in synaptosomal and brain myelin membranes (b). Abscissa, time (in min); ordinate, intensity of chemiluminescence (a – calibration, 10 mV, b – 1000 mV). 1) Synaptosomal membranes, control; 2) synaptosomal membranes, preincubated with GM1 (10^{-8} M) for 60 min at 37°C; 3) synaptosomal membranes + α-tocopherol (10^{-6} M); 4) myelin membranes, control; 5) myelin membranes, preincubated with GM1 (10^{-8} M) for 60 min at 37°C; 6) myelin membranes + α-tocopherol (10^{-6} M). Arrows indicate 50 μM luminol, 400 μM H_2O_2, 20 μM Fe^{2+} respectively.

Fig. 2. Action of GM1, α-tocopherol, and phorbol ester on light-sum of luminol-dependent chemiluminescence induced by the $Fe^{2+}-H_2O_2$ system in brain synaptosomes. Abscissa, time (in min); ordinate, light-sum (in relative units). 1) Synaptosomal membranes, control; 2) synaptosomal membranes without preincubation with GM1 (10^{-8} M); 3) synaptosomal membranes without preincubation with phorbol ester (10^{-6} M); 4) synaptosomal membranes preincubated with phorbol ester (10^{-6} M) for 60 min at 37°C; 5) synaptosomal membranes preincubated with GM1 (10^{-8} M) for 60 min at 37°C; 6) synaptosomal membranes + α-tocopherol (10^{-6} M).

Fig. 3. Inhibitory action of GM1 on luminol-dependent chemiluminescence induced by the $Fe^{2+}-H_2O_2$ system in brain synaptosomes. Abscissa, log of concentration of GM1 (in M); ordinate, per cent inhibition.

heat-inactivation of synaptosomes, weak effect in liposomes, etc.), compared with α-tocopherol, and also the need for preincubation with GM1 and the biphasic character of the curve depending on concentration, have been demonstrated also in a study of the action of GM1 on induced formation of secondary LPO products (carbonyl compounds) [4].

We also demonstrated previously strengthening of the inhibitory action of GM1 in synaptosomal membranes during induction of LPO in an Fe^{2+}–ascorbate system in the presence of Ca^{2+} ions, necessary for activating lipid-dependent protein kinases [8], and also the negligible effect of GM1 in the presence of polymixin B (an inhibitor of protein kinase C) [4]. These results may be evidence that the inhibitory effect of nanomolar concentrations of gangliosides on induced LPO in synaptosomal membranes may be the result of activation of enzymes of the antioxidant defense system, which is evidently realized by signal transduction systems. The protein kinase C activator phorbol ester, in phagocytic cells, causes generation of superoxide anion-radicals $O_2^{\cdot-}$ [2]. On the other hand, in membrane structures of the liver, and of skeletal and heart muscles, and in brain synaptosomes phorbol esters have an inhibitory effect on LPO processes [12], which may reflect different mechanisms of involvement of the signal systems in the regulation of LPO. In a special series of experiments we found that treatment of human neutrophils with 12-myristate-13-acetate phorbol ester (10^{-7}-10^{-6} M) evokes a powerful flash of luminol-dependent chemiluminescence, which is intensified many times over if the membranes are preincubated with nanomolar concentrations of GM1 (data not given). Meanwhile, in membranes of brain synaptosomes phorbol ester not only does not stimulate chemiluminescence but, on the contrary, inhibits it (Fig. 2).

The results suggest that inhibition of free-radical reactions by ganglioside GM1 (in nanomolar concentrations) in brain membranes is not directly connected with its interaction with lipid radicals, but is due mainly to involvement of the signal transduction systems.

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