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GANGLIOSIDE-DEPENDENT FACTOR INHIBITING LIPID PEROXIDATION IN SYNAPTOSOMAL MEMBRANES

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Experiments in vitro have shown that if standard systems are used to generate active forms of oxygen, significantly less accumulation of lipid peroxidation (LPO) products takes place in the plasma membranes than in the intracellular membranes (fraction of microsomes and mitochondria) [9]. Among the possible causes of resistance of the plasma membranes to LPO inducers at least three may be mentioned: the higher level of saturation of the phospholipids of these membranes and the fact that they contain predominantly glycolipids with a higher degree of saturation [2], the higher cholesterol concentration [3], and effective inhibition of LPO by an enzymic mechanism, coupled with activation of protein kinase C [10].

Gangliosides not only largely determine the structural and functional organization of the plasma membranes of cells, and play an essential role in processes of intercellular interaction and reception of biologically active substances, but they also take part in the regulation of differentiation, plasticity, and regeneration of nerve cells. However, the biochemical mechanisms of the neuronotrophic and neuroregenerative effect of gangliosides have not been elucidated [1].

It has recently been shown that gangliosides are modulators of the activity of various protein kinases, including protein kinase C [12]. With these considerations in mind, and also the fact that gangliosides are localized mainly in the outer monolayer of the plasma membranes of synaptosomes, where they may account for up to 10-15 moles % of the total lipids [4], the investigation described below was carried out to study the action of exogenous monosialoganglioside GM1 (accounting for about 30% of the total mammalian brain gangliosides) on synaptosomes of the rat brain.

EXPERIMENTAL METHOD

Synaptosomal membranes were obtained from the cerebral cortex of Wistar rats by the method described in [8], with certain modifications. The protein concentration was determined by a modified Lowry's method [11]. Gangliosides were extracted from hog brain by Folch's method, with additional treatment as described in [15]. Separation of the gangliosides into fractions

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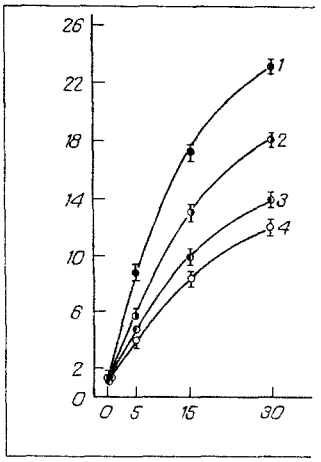

Fig. 1

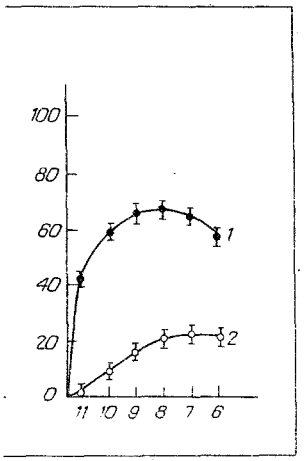

Fig. 2

Fig. 1. Dependence of accumulation of LPO products in synaptosomal membranes at 37°C. 1) Control, 2) control + GM1 (10 pM), 3) control + GM1 (100 pM), 4) control + GM1 (1 nM). Abscissa, time (in min); ordinate, LPO level (in nmoles MDA/mg protein). Protein concentration 1 mg/ml.

Fig. 2. Dependence of inhibition of LPO in membranes on GM1 concentration at 37°C (induction time of LPO 5 min). Abscissa, log [C] GM1; ordinate, degree of inhibition of LPO (in percent). 1) Synaptosomal membranes (protein concentration 1 mg/ml), 2) liposomes prepared from lipids of synaptosomal membranes (concentration of lipids 1 mg/ml).

was carried out by preparative column chromatography on silica-gel KSK in a system of chloroform:methanol:water (65:25:4 and 60:35:7). Concentrations of gangliosides were determined as N-acetylneuraminic acid by the resorcin method [4]. Liposomes obtained from membrane lipids of rat brain synaptosomes were used. Control preparations of liposomes did not contain endogenous gangliosides. The liposomes were obtained by evaporating the lipids under argon in the presence of different concentrations of ganglioside GM1, followed by shaking in 0.1 M Na-phosphate buffer, 0.1 M NaCl, pH 7.4, and treatment on a UZDN-2 ultrasonic disintegrator (22 kHz) at 0-4°C, To reduce destruction of the lipids, the membranes were disintegrated in a cylindrical resonator, ruling out any direct contact between resonator and solution of liposomes. LPO was induced by the addition of Fe^{2+} (10 and 40 μM) and ascorbate (0.5 mM), in a medium of 0.1 M Na-phosphate buffer, 0.1 M NaCl, pH 7.4. Accumulation of LPO products was recorded by the reaction with 2-thiobarbituric acid [13].

EXPERIMENTAL RESULTS

Curves showing accumulation of LPO products (TBA-reactive compounds) in synaptosomal membranes in the course of their incubation in a system of Fe^{2+} ascorbate are illustrated in Fig. 1. In the course of 30 min the malonic dialdehyde (MDA) concentration increased, to reach 23 nmoles MDA/mg protein in the control. On the addition of ganglioside GM1, inhibition of LPO was observed, and depended both on the concentration of added GM1 (Fig. 2) and on the preincubation time before addition of the LPO inducers (Table 1). It will be clear from the data in Fig. 1 that the degree of inhibition of LPO by GM1 decreases with an increase in the LPO induction time. Since the maximal effect was achieved after 90-120 min of preincubation, in the subsequent experiments these times of preincubation were used. It must be pointed out that the inhibitory action of GM1 was manifested as a decrease in the rate of accumulation of LPO products, but it was not accompanied by the appearance of a lag period, characteristic of the action of antioxidants, interacting with lipid radicals [14]. The action of GM1 shows that an inhibitory effect appeared when the concentration was as low as 10^{-11}-10^{-10} M and it reached saturation in the region of 10^{-9}-10^{-7} M, falling in the presence of higher concentrations (in individual experiments the maximal effect reached 80-85%). The important point is that dependence of the action of GM1 on concentration in monolamellar liposomes prepared from membrane lipids of rat brain synaptosomes was different in character: the inhibitory effect was much weaker (at its peak it did not achieve

TABLE 1. Dependence of Inhibitory Action of GM1 (10 nM) on LPO on Duration of Preincubation of Rat Brain Synaptosomes at 37°C (LPO induction time 5 min, $M \pm m$)

Preincubation time, min	Inhibition, % of maximal effect
0	0,0
30	0,0
60	0,0
90	83,5±3,7
120	100,0±4,2

TABLE 2. Dependence of Inhibitory Action of GM1 (1 nM) on LPO on Keeping Time of Rat Brain Synaptosomes at 0°C (LPO induction time 5 min, $M \pm m$)

Keeping time, h	Inhibition, %
4	65,4±2,9
20	60,8±2,7
60	29,2±1,3

TABLE 3. Action of PMA, Polymyxin B, Ca^{2+} and GM1 on (Fe^{2+} + Ascorbate)-Induced LPO in Rat Brain Synaptosomes ($M \pm m$)

LPO induction time, min	Additives	Inhibition, %
15	—	0,0
	PMA (1 μM)	52,6±2,3
	PXB (100 μM)	9,1±0,4
	GM1 (10 nM)	48,7±2,0
	PMA + GM1	58,3±2,6
	PXB + GM1	13,3±0,5
5	—	0,0
	$CaCl_2$ (2 mM)	61,5±2,7
	GM1 (1 nM)	65,4±2,9
	$CaCl_2$ + GM1	70,9±3,2

25%) and the character of the concentration curve was different: in the region of concentrations up to 10^{-9} M it virtually did not appear, and it did not fall at concentrations exceeding 10^{-8} M. It must also be pointed out that no inhibitory action of GM1 on LPO was observed in synaptosomal membranes subjected to thermal denaturation (90°C, 2 min). Keeping the synaptosomes at 0°C led to weakening or loss of the inhibitory action of GM1 (Table 2). Taken as a whole, these results indicate that inhibition of LPO in synaptosomal membranes is realized, not through the direct interaction of GM1 with lipid radicals or with active forms of oxygen, but to its indirect action, possibly through activation of a particular enzyme system involved in LPO regulation. The possibility that LPO is inhibited through the formation of complexes of the ganglioside with Fe^{++} ions also seems unlikely, because the inhibitory effect was manifested at incomparably low concentrations.

It was shown previously that the active enzyme system capable of inhibiting LPO in membrane structures of the liver and skeletal and heart muscle may be protein kinase C [10]. Considering that GM1 is an effective modulator of protein kinase C activity [12], in the subsequent experiments we studied the action of phorbol-12-myristate-13-acetate (PMA), a protein kinase C activator, and also of polymyxin B (PXB), an inhibitor of protein kinase C, on LPO in synaptosomes in the presence and absence of GM1.

The results in Table 3 show that PMA, when used in a concentration of 1 μM, caused 50% inhibition of LPO, and that GM1 had about the same action in the preparations used in a concentration of 10 nM. A combination of GM1 and PMA led to inhibition of LPO, but the effect was only a little stronger than that of these compounds given separately. This suggests that the action of PMA and of GM1 is realized through a common mechanism of protein kinase C activation. This hypothesis is supported also by the fact that the inhibitory action of GM1 in the presence of polymixin B was much weaker than the action of GM1 alone (under these circumstances polymyxin B itself had only weak inhibitory activity).

Potentiation of the inhibitory action of GM1 on LPO in the presence of Ca^{2+} ions (Table 3), which are essential for protein kinase C [7], also will be noted. Finally, yet another argument in support of activation of protein kinase C is the very slight weakening of the effect as a result of the action of micromolar concentrations of GM1, which, according to data in the literature, induce inhibition of protein kinase C [7].

To sum up the results of these experiments it can be postulated that the observed effect of GM1 on LPO in brain synaptosomal membranes is linked with activation of protein kinases, of which the most likely candidate can be considered to be protein kinase C, although the possibility of activation of specific ganglioside-dependent protein kinases stimulated by different gangliosides, including by GM1, cannot be ruled out [12].

Protein kinase C is located on the cytoplasmic surface of the outer membrane of the synaptosomes [7], and in this respect the actual mechanism of the activating action of GM1 on protein kinase C is not fully clear (this difficulty does not apply to ganglioside-dependent protein kinases, which may be ectoprotein kinases). However, it can be tentatively suggested that the effects of GM1 on protein kinase C are realized with the participation of ganglioside receptors, whose existence has recently been postulated [12]. The participation of receptors of this kind in the regulatory action of GM1 would explain the "sensitivity" in synaptosomal membranes to extremely low concentrations of GM1, against the background of higher concentrations of gangliosides present in the membrane.

LPO products are known to lower the breakdown voltage of membranes [3]. Strict regulation of the LPO process is therefore exceptionally important for the maintenance of cell function. In synaptosomes this effect may be connected with the mechanism of mediator release [5]. The results are important for an understanding of the mechanisms of the neuronotrophic and neuroregenerative effects of gangliosides, especially in conjunction with existing evidence of the inhibitory action of LPO on regeneration of organs and tissues [16]. In this way we can understand the possible physiological role of gangliosides as specific LPO inhibitors in nerve tissue.

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