BIOPHYSICS AND BIOCHEMISTRY

Effect of Chronic γ-Irradiation and β-Carotene on Lipid Metabolism in Presynaptic Membranes in Rat Brain

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Lysophosphatidylcholine is involved in radiation-induced modulation of presynaptic membranes in rat brain. High sensitivity of the cortical integrative functions to chronic low-dose γ-irradiation is demonstrated. β-Carotene produced a protective effect during chronic irradiation.

Key Words: *presynaptic membrane; lipids; behavior; β-carotene*

Various adverse factors considerably modulate lipid composition in CNS [9]. Disturbances in lipid composition in the cerebral cortex caused by chronic low-dose irradiation are of particular interest because these lipids play a role in the modulation of nerve impulses [2]. However, little is known on the involvement of lipids in CNS reactions to acute and chronic effect of ionizing radiation. It is important to study the sensitivity of lipid metabolism in the presynaptic membranes in the rat cerebral cortex to long-term low-dose γ-irradiation (a model of environmental contamination with γ-emitting isotopes) and to assess behavioral reactions, characterizing integrative cerebral functions. Among substances capable to prevent the metabolic and functional effects of low-dose radiation, of particular interest are regulators of lipid metabolism affecting membrane structure and cell growth and differentiation, for instance carotenoids [14]. Protective and therapeutic effects of β-carotene are demonstrated during acute irradiation, as well as its capacity to modify the lipid composition of cell nuclei in the thymus and liver when added to the diet for a long time [1,4,5]. Taking into consideration the possible role of carotenoids in adaption to adverse environmental factors [14], it is of interest to use β-carotene for modification of metabolic and functional reactions of CNS to chronic γ-irradiation.

Our aim was to determine the sensitivity of lipid composition in the presynaptic membranes in rat cerebral cortex and behavioral reactions to chronic effects of γ-irradiation (3 sGy/day), as well as the potency of β-carotene to prevent the radiation-induced effects. γ-Irradiation doses of 1-3 sGy/day during life does not affect the mean lifespan [6], although it may be expected to induce structural and functional changes in CNS due to its high functional sensitivity to radiation.

MATERIALS AND METHODS

Male Wistar rats weighing 90±10 g were placed in special γ-chamber with ^{137}Cs at a distance from the radiation source providing dose intensity of 3 sGy/day. Dosimetry was carried out with a VA-J-18 counter. The source was turned off once a day for 15 min to care for the rats. Control and experimental animals were kept under the same conditions and maintained

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on a standard diet. One group was fed synthetic β-carotene (26% emulsion in olive oil with 5% palmitic acid) with curds in a daily dose of 3 mg/kg. When the dose of radiation attained 50 and 200 sGy, the lipid content in the presynaptic membranes of the cerebral cortex was assayed. Synaptosomes were isolated as described elsewhere [13] and shocked in hypotonic solution. Synaptosomal membranes were precipitated by centrifugation. Measuring of protein, lipid isolation and purification, separation of neutral lipids and phospholipids, and measuring of individual lipids were performed as described elsewhere [8,10]. The purity of membrane fraction was controlled under an electron microscope. To this end, the membrane precipitate was fixed in 2.5% glutaraldehyde and postfixed in 0.48% osmium tetroxide (2 h for each reagent). Both fixatives were prepared on 0.1 M cacodylate buffer (pH 7.2-7.4). The specimens were dehydrated in increasing ethanol concentration and in 100% acetone, and then embedded in Epon-Araldite. The specimens were examined under a JEM 100B electron microscope (Jeol). Ultrathin sections (70-90 nm) were prepared on an LKB ultramicrotome with glass knifes. The sections were transferred onto copper grids and contrasted with uranyl acetate and lead citrate. Magnification was controlled with a special grid (2160 lines/mm, Polyscience). Behavioral reactions were studied after the total dose of 40, 75, 100, and 150 sGy (irradiation was interrupted for 4 h). Sensory attention to stimuli of various modality was assessed as described elsewhere [8]. The results were analyzed statistically using Student's *t* test.

RESULTS

The content of lipid phosphorus, cholesterol, phosphatidylcholine (PC), phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and lysophosphatidylcholine (LPC) in the control coincided with our previous [8] and published [7,12,15] data (Table 1). The content of sphingomyelin (SM) considerably surpassed that observed in previous studies [7,8,12,15]. However, the increased level of SM by myelin contamination (Fig. 1). It can be hypothesized that the content of SM cannot be explained in the presynaptic membranes of rat cerebral cortex varies with age. For example, in the cited papers lipid content in the presynaptic membranes of the cerebral cortex was determined in 6-7-month-old rats, while we used 2-month-old rats. Probably, the high level of SM is due to peculiarities of cerebral metabolism at this age. γ-Irradiation for 17 days decreased lipid phosphorus, the content of LPC also tended to decrease (Table 1). Lysophospholipids play an important role in the mod-

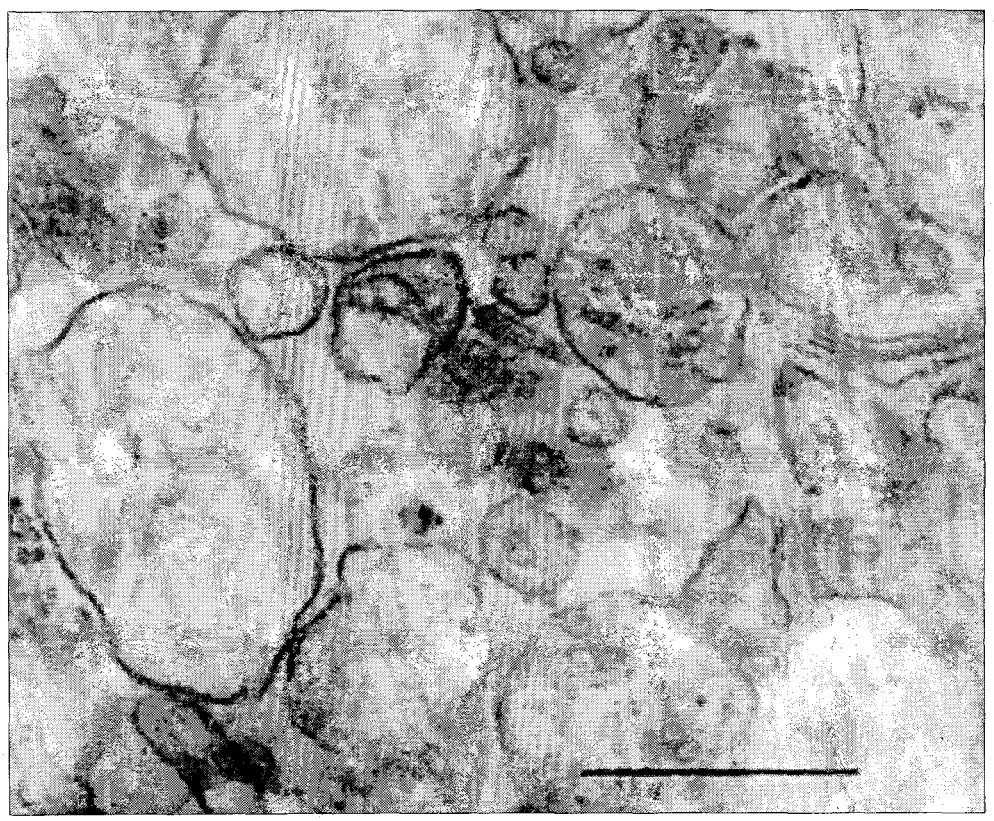

Fig. 1. Presynaptic membranes from rat cerebral cortex, ×80,000. Membrane suspension is practically free of myelin.

TABLE 1. Effect of γ-Irradiation and β-Carotene on Content of Phospholipids and Cholesterol (mg lipid/mg protein) in Presynaptic Membranes of Rat Cerebral Cortex (*M*±*m*, *n*=3-6)

Lipid fraction	Control	50 sGy	50 sGy+ β-carotene	200 sGy	200 sGy+ β-carotene
Lipid phosphorus	707±6	655±13*	664±67	734±49	832±38
Cholesterol	94.7±2.2	94.8±2.5	93.7±6.6	110±6	121±5
SM	151±7	148±13	125±20	170±23	189±26
PC	52.5±7.2	47.5±9.7	56.0±12.7	21.0±12.8	22.7±6.8
Phosphatidylethanolamine	165±3	159±4	166±14	191±15	205±23
Phosphatidylinositol	18.5±0.9	21.8±2.3	17.1±1.3	19.2±1.0	18.9±1.1
Phosphatidylserine	66.0±2.1	64.8±1.4	64.5±4.4	72.0±4.6	77.6±6.3
LPC	20.7±3.8	13.3±2.9	27.6±3.1+	27.2±1.0*	22.2±1.6+
Cardiolipin	14.1±4.1	14.3±0.5	13.8±2.5	16.9±1.2	19.2±1.3

Note. $p<0.05$: *vs.* the control, +*vs.* the corresponding irradiation dose without β-carotene. The brain from one rat was used in each experiment.

ulation of presynaptic membrane permeability and choline metabolism [10,11], therefore these changes in LPC content in the presynaptic membrane are very important. β-Carotene prevented the radiation-induced effect: in irradiated rats maintained on β-carotene-supplemented diet, the content of lipid phosphorus did not significantly differ from the control, while LPC content in this group was significantly higher than in irradiated rat maintained on a standard diet (Table 1). In the rats irradiated for 67 days, the content of PC in the presynaptic membrane was significantly lower than that in 2-month-old rats, while the level of other lipids remained unchanged. Significant differences in PC content in the presynaptic membranes in different age groups can result from age-related variation in the lipid content of presynaptic membranes or seasonal changes (measurements in group 1 and 2 were performed in autumn and winter, respectively). Seasonal variations in the lipid content in the presynaptic membranes in gopher cerebral cortex were demonstrated previously [3]. Irradiation in a dose of 200 sGy increased LPC content (Table 1). Changes in LPC level in presynaptic membranes were also observed after attaining a dose of 20 Gy in rats irradiated with a dose intensity of 12.9 sGy/day [8]. Under these conditions, there was a decrease in the content of phospholipids metabolically related to choline synthesis (PC, phosphatidylcholine, and LPC) [11]. The level of SM, phosphatidylinositol, and phosphatidylserine did not change. When comparing the tendency to decrease in LPC level at a dose of 50 sGy, its growth at 2 Gy, and decrease at 20 Gy [8], it can be concluded that the initial stage of lipid metabolic response to irradiation in the presynaptic membranes includes changes of LPC

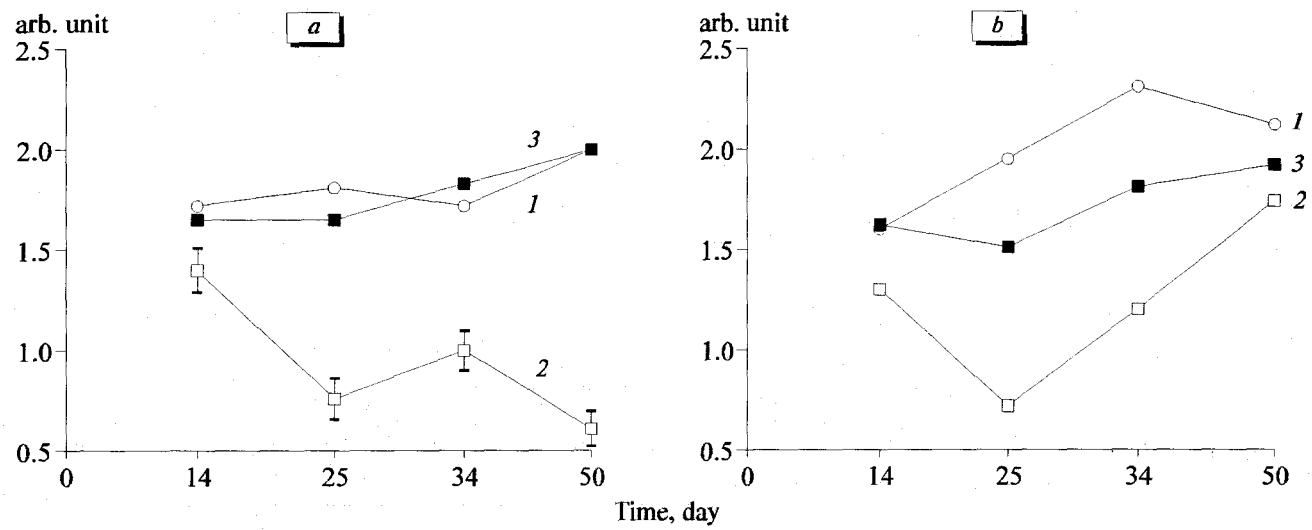

Fig. 2. Effect of chronic γ-irradiation and β-carotene on sensory attention to visual (*a*) and somatosensory (*b*) stimulation. *1*) control; *2*) γ-irradiation; *3*) γ-irradiation+β-carotene. *$p<0.05$ *vs.* the control.

metabolism, whose concentration varies nonmonotonously. LPC is a key element in choline neuronal synthesis [11]. It can be assumed that these changes in LPC content in the presynaptic membranes of irradiated rats are not probably directly related to changes in LPC pool, but are caused by fine membrane rearrangements and involvement of LPC in radiation-induced modulation of the nerve conduction. Phospholipid deacylation-acylation is the key stage in the effect of some hormones and neurotransmitters on synaptic transmission [10].

Comparison of the data on lipid content in presynaptic membranes with behavioral reactions of rats exposed to γ-irradiation in a dose of 3 sGy/day shows that the tendency to decrease in LPC level in the presynaptic membranes (at 50 sGy) appears simultaneously with the disturbances in sensory attention (40 sGy). Irradiation in a dose of 40 sGy impaired attention to somatosensory and visual stimuli by 15 and 20%, respectively. Further accumulation of the radiation dose impairs sensory attention in all behavioral tests (Fig. 2). Treatment with β-carotene preserved behavioral reactions of irradiated animals at the control level (Fig. 2).

Thus, behavioral reactions and metabolism of LPC and lipid phosphorus in presynaptic membranes of rat cerebral cortex are sensitive to low doses of chronic ionizing radiation, while β-carotene corrects the radiation-induced disturbances. Further studies of the mechanisms of high radiation sensitivity of cerebral metabolism to low doses of ionizing radiation and biological effects of chronic low-dose irradiation are of vital importance.

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