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ACTIVATION OF ENDOGENOUS LIPID PEROXIDATION IN THE BRAIN DURING OXIDATIVE STRESS INDUCED BY IRON AND ITS PREVENTION BY VITAMIN E

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The key functions of nerve cells, namely creation and maintenance of the transmembrane potential, reception and subsequent transmission of the signal, production and regulatory action of secondary messengers, and uptake and release of neurotransmitters, are highly sensitive to excessive accumulation of endogenous products of lipid peroxidation (LPO) in membrane structures [1-4]. Accordingly LPO is regarded nowadays as an etiologic or pathogenetic factor in several diseases of the CN (Parkinson's disease, Down's syndrome, schizophrenia, epilepsy, etc.), and also in complications caused by administration of neuroleptics and antidepressants [5, 6]. The role of activation of free-radical reactions and of LPO in particular, in the process of brain aging, accompanied by depression of activity of enzymic and nonenzymic antioxidative systems, also has been discussed [7-9]. The most important of the latter in the membrane structures of the brain is vitamin E, a lipid-soluble interceptor of free radicals, the amount of which falls much more slowly in animals kept on a vitamin E-deficient diet than in peripheral tissues [7-11].

Administration of LPO inducers to animals causes the development of oxidative stress and rapid exhaustion of vitamin E reserves in peripheral tissues, although membrane structures of the brain are much more resistant and under the same conditions activation of LPO and depression of the vitamin E level cannot be recorded in them [7, 12]. The obtaining of an effect of vitamin E-dependent activation of endogenous LPO in the membrane structures of the brain in vivo is interesting in connection with the formation of a model of premature brain aging.

This paper describes an attempt to induce oxidative stress in the rat brain by repeated injections of iron into the animals, the standard substance used as a catalyst of breakdown of lipid hydroperoxides with the formation of radicals in vitro, and to estimate the sensitivity of the developing endogenous LPO to exogenous vitamin E. In view of data showing the

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TABLE 1. Substances Used, with Times and Doses

Group of animals	Substance	Dose, mg/kg	Mode of administration	Duration of administration, days
1-	0,9 % NaCl	—	Perorally	6
2-	Fe	50	Intramuscularly	4
3-	Vitamin E	50	Perorally	6
4-	Nifedipine	20	»	6
5-	Vitamin E + iron	50 50	»	6
6-	Nifedipine + iron	20 50	Intramuscularly Intramuscularly	4* 4*
7-	Vitamin E + nifedipine	50 20	Perorally »	6**
8-	Vitamin E + nifedipine + iron	50 20 50	Perorally » Intramuscularly	6** 4*

Legend. *) Iron given after 6-day course of corresponding substances. **) Substanes given together at the same time.

TABLE 2. LPO Products in Rat Brain Homogenate (relative extinction of samples compared with control sample, receiving parallel treatment, ± standard deviation)

Group of animals	Diene conjugates	Fluorescent products
1-	0,44±0,12	1,80±0,43
2-	0,97±0,09*	2,90±0,30*
3-	0,38±0,19	0,94±0,16*
4-	0,63±0,27	1,40±0,31
5-	0,50±0,14	1,30±0,26**
6-	0,73±0,33	2,05±0,33**
7-	0,59±0,23	0,98±0,23
8-	0,52±0,29	1,06±0,13

Legend. *$p < 0.01$ compared with control group (1); **$p < 0.01$ compared with corresponding control group.

participation of secondary messengers in the cellular pathologies of oxidative stress [3], and also on calcium accumulation in the cells as a result of exposure to stress and, in particular, oxidative stress, we also studied the action of the calcium antagonist nifedipine on LPO formation in this same model.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-180 g and aged 4-5 months were used. In the experiments of series I the effects of a single intramuscular injection of the iron preparation in a dose of 50 mg/kg body weight were evaluated. In the experiments of series II the iron preparation was injected repeatedly: all the animals were divided into 8 groups with 7 animals in each group. The times and doses of the substances injected are given in Table 1. An ampul preparaiton of iron, namely Ferrum-Haussman (Farmakhin, Bulgaria) was used. Vitamin E (D, L-α-tocopherol, from "Serve") was dissolved in purified sunflower oil (free from vitamin E). The nifedipine preparation (from "Farmakhin," Bulgaria, 5 mg of active substance), was emulsified in 0.05 ml Tween-80 and mixed with physiological saline to the required concentration. A brain homogenate was prepared in K-Na-phosphate buffer (pH 7.4, at 4°C, 1:4 by volume). Protein was determined by the biuret method. Lipids were extracted from the homogenate (10 mg protein to 1 ml) with chloroform-methanol mixture (2:1) with a ratio of homogenate to mixture by volume of 1:7, using Wortex (2 min). After separation of the phases (for 2 h at −2°C) 0.5-ml samples of the chloroform phase were taken and the intensity of fluoroscence of the end product of LPO measured (λ_{exc} = 360 nm, λ_{em} = 420 nm) [13]. The content of primary LPO products, namely hydroperoxides with conjugated double bonds, was determined after dissolving the brain lipids in cyclohexane and recording the UV spectra, and also their second derivative, in the region of wavelengths of 190-300 nm [14]. The results were subjected to statistical analysis by the t test of significance.

EXPERIMENTAL RESULTS

A single intramuscular injection of iron into the animals did not cause accumulation of LPO products in brain lipids, although significant activation of free-radical oxidation was observed in the skeletal muscles themselves and in the liver [12]. The results of measurements of the concentration of endogenous LPO products in rat brain homogenate after 4 injections of iron are given in Table 2. Clearly during this prolonged (for 4 days) administration of iron the level of primary molecular products of LPO and of lipid hydroperoxides with conjugated double bonds was doubled, and the level of end products of LPO, of fluorescent "intermolecular cross-linkages," was 1.6 times higher than in the control. Preliminary "saturation" of the animals with vitamin E (a 6-day course, 44 mg/kg daily) not only completely prevented iron-induced activation of LPO, but also significantly lowered the level of endogenous LPO products, including in rats not treated with iron, compared with the control group of animals.

Previous experiments in vitro showed that activation of protein kinase C by phorbol esters causes inhibition of LPO, whereas the adenylate cyclase activator forskolin abolishes this effect [3]. Because of these data on the possible role of the secondary messenger

system in the regulation of LPO, there are good grounds for assessing the possibility of modulating the endogenous LPO level by the action of compounds which change the intracellular Ca^{++} concentration. The results in Table 2 show that nifedipine itself does not lower the concentration of LPO products in the rat brain and does not prevent the activation of LPO following administration of iron to animals. Nifedipine in combination with vitamin E has the same action as vitamin E alone.

It can be concluded from the results as a whole that repeated intramuscular injection of iron causes the development of oxidative stress in the membrane structure of the brain, which is sensitive to the action of the natural antioxidant, vitamin E. These effects can be used as a model of premature aging of the brain and also for screening substances for antioxidant therapy for pathological states of the brain induced by free radical oxidation.

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