ROLE OF LIPID PEROXIDATION IN SEROTONIN RECEPTOR INJURY AND ONSET OF EPILEPTIFORM SEIZURES DURING HYPEROXIA

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Epileptiform seizures are a characteristic manifestation of the toxic action of oxygen (hyperoxia) on the brain [9, 13]. An essential role in seizure formation can be played by damage to the serotoninergic system of the brain [7]. However, the concrete mechanisms through which injury to this system in hyperoxia is realized have not yet been discovered [2]. Two groups of facts obtained recently accordingly appear to be important: 1) intensification of generation of active forms of oxygen during hyperbaric oxygenation [12, 13], which can activate lipid peroxidation (LPO) in membrane structures of the brain [3], 2) the ability of LPO products to lower the level of binding of [^{3}H] serotonin with receptors in brain microsomal fraction *in vitro* [15]. The following hypothetical chain of events, essential for the pathogenesis of hyperoxygenation brain damage and the appearance of epileptiform seizures can be submitted on the basis of these findings: increased pO_2 → increased concentration of active forms of oxygen ($O_2^{\bullet}$, $^{\bullet}OH$) → activation of LPO → inhibition of serotonin binding with receptors → disturbances of function.

The object of this investigation was to test this hypothesis experimentally. Under hyperoxic conditions accumulation of LPO products in brain tissues and the possibility of preventing it by antioxidants, changes in the antioxidant system of the brain, changes in binding of [^{3}H] serotonin with brain membrane receptors, and the possibility of its correction by antioxidants, and the action of inhibitors and stimulators of LPO on the frequency of appearance of epileptiform seizures induced by hyperbaric oxygenation in animals were studied.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 200-220 g, subjected to hyperbaric oxygenation in a pressurized chamber under an oxygen pressure of 5 atm. The animals were divided into four groups (14-16 rats in each group). Rats of group 1 received physiological saline, those of group 2 received α-tocopheryl acetate (perorally, 40 mg/kg daily for 1 week), group 3 received 4-methyl-2,6-di-*tert*-butylphenol intraperitoneally for 3 days in a dose of 120 mg/kg daily, and group 4 received a mixture of unsaturated fatty acids (oleic, linoleic, linolenic, and arachidonic, in the ratio of 1:2:2:1) in a dose of 1 ml per rat daily for 1 week. In each experiment animals of different groups were placed in the pressure chamber, and the times of onset of seizures were observed for 60 min. The concentrations of α-tocopherol and also of primary (lipid hydroperoxides) and end (fluorescent Schiff's bases) products of LPO in the brain tissues was determined by methods described in [4, 17]. Superoxide dismutase (SOD) activity in the samples was determined by the method in [16], using an MPS-50L spectrophotometer (Shimadzu, Japan). Specific binding of [^{3}H] serotonin was determined in microsomal fractions of rat cerebral cortical membranes obtained by the method in [10]. Incubation with [^{3}H] serotonin continued for 20 min at 37°C, the concentration of the labeled ligand in the samples was 25 nM, and the volume of the incubation medium 0.5 ml. After incubation the samples were filtered through gF/B filters (Whatman,

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England) and their radioactivity measured. Specific binding was determined as the difference between binding of the ligand in the absence and presence of an excess (1 μM) of nonradioactive serotonin.

EXPERIMENTAL RESULTS

During hyperoxia considerable accumulation of primary and end products of LPO takes place in the brain, i.e., LPO is activated (Table 1). Hyperoxia caused no changes in SOD activity but it led to a decrease in the brain tissue concentration of α-tocopherol, i.e, it was accompanied by the development of antioxidant deficiency. Accordingly, LPO activation induced by hyperoxia was practically completely prevented by preliminary administration of the synthetic anitoxidant 4-methyl-2,6-di-tert-butylphenol (MDTB).

Preliminary administration of LPO inhibitors (MDTB and α-tocopherol) reduced the frequency of the seizures in the animals (Table 2). When α-tocopherol was used, for instance, the frequency of seizures decreased significantly, and injection of MDTB prevented their appearance almost completely during 40 min of action of oxygen. Differences in the effectiveness of the antioxidants are in good agreement with the greater effectiveness of MDTB than of α-tocopherol, established previously, during inhibition of LPO *in vivo* [1]. Conversely, injection of LPO stimulators (polyene fatty acids) caused a marked increase in the frequency of epileptiform seizures (Table 2). The results are evidence that LPO activation is an important pathophysiological link in the mechanism of the toxic action of hyperoxia on the brain, expressed as the appearance of epileptiform seizures. This conclusion is confirmed by the results of other investigations [5, 6], which demonstrated accumulation of LPO products in penicillin-induced foci of epileptic activity, and in which MDTB was observed to have a protective action.

There is no doubt now about the important role of the brain serotoninergic system in the formation of seizures. A fall in the brain serotonin level has been shown to be accompanied by the appearance of seizures or by a marked lowering of the threshold of their appearance; the severity of the seizures correlates in this case with the fall in the serotonin level [8]. However, a similar effect may also arise as a result of a change in sensitivity of the effector cells to serotonin, which can take place as a result of a change in affinity of the receptors for serotonin or as the result of a decrease in their concentration in the effector sites of nerve cell membranes. Evidently if either of these possibilities is realized, nerve cells forming the brain serotoninergic system will respond just as to a fall in the serotonin concentration. With these considerations in mind, and also the fact that LPO products can cause significant changes in the properties of integral bio-membrane proteins, including the receptor proteins of nerve cells [3], it was decided to study the effect of LPO activation, induced by hyperoxia, on the ability of nerve cell membranes to bind serotonin. Hyperoxia was shown to lower the level of specific binding of [^{3}H]serotonin with membranes of brain microsomal fraction significantly, whereas injection of MDTB into the animals largely prevented this effect (Fig. 1). Consequently, during hyperoxia, as a result of LPO activation the properties of the serotonin receptors are modified, and this may be an important cause of the onset of epileptiform seizures.

TABLE 1. Effect of Hyperoxia on Concentration of LPO Products and α-Tocopherol and on SOD Activity in Rat Brain (M ± m)

Parameter studied	Control	Hyperoxia	Hyperoxia + MDTB
Diene conjugates, nmoles/mg lipids	6,7±0,5	24,5±2,4	8,6±1,0
Schiff's bases, relative units	7,8±0,9	22,8±2,6	7,0±3,7
α-Tocopherol, μg/mg lipids	0,10±0,02	0,04±0,01	—
SOD, activity units/mg protein	0,21±0,02	0,25±0,03	—

TABLE 2. Effect of Antioxidants and LPO Promotors on Frequency of Seizures (in %) in Rats during Hyperoxia

Experimental conditions	Time of observation, min					
	10	20	30	40	50	60
Physiological saline	0	40	80	80	100	100
MDTB	0	0	0	0	10	30
α-Tocopheryl acetate	0	20	30	40	50	70
Mixture of unsaturated fatty acids	0	60	90	100	100	100

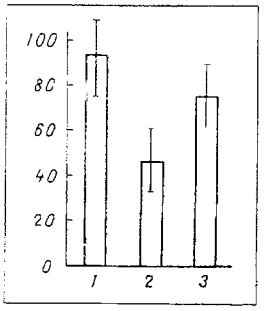

Fig. 1. Effect of hyperoxia and protective action of MDTB on specific binding of [^{3}H]serotonin with rat cerebral cortical cell membranes. Abscissa: 1) MDTB; 2) hyperoxia; 3) hyperoxia + MDTB; ordinate, specific binding of [^{3}H]serotonin (in % of binding of ligand with brain membranes from intact animals).

The appearance of seizures, it will be noted, was accompanied by an increase in the concentration of unsaturated fatty acids in the brain and their prostanoid peroxidation products (prostaglandins, thromboxanes) [11]. It is unlikely, however, that this effect is related to the mechanism of appearance of convulsions, for the appearance of epileptiform seizures is not prevented by administration of indomethacin, which blocks prostaglandin synthesis [14].

The results thus indicate that LPO activation and the resulting modification of the properties of serotonin receptors in the brain are important pathogenetic components of the mechanism of the toxic action of hyperoxia on the CNS and they open up prospects for prevention of hyperoxic brain damage with the aid of antioxidants.

LITERATURE CITED

1. S. A. Aristarkhova, E. B. Burlakova, N. G. Khrapova, E. N. Kukhnina, E. K. Sarycheva, and R. P. Evstigneeva, Vopr. Pitan., No. 5, 34 (1974).
2. A. G. Zhironkin, Oxygen. Physiological and Toxic Action [in Russian], Nauka, Leningrad (1972), p. 272.
3. V. E. Kagan, R. A. Kopaladze, L. L. Prilipko, M. L. Libe, N. F. Turova, and Yu. P. Kozlov, Biol. Nauki, No. 5, 26 (1982).
4. V. E. Kagan, L. L. Prilipko, V. M. Savov, V. A. Pisarev, I. A. Eluashvili, and Yu. P. Kozlov, Biokhimiya, 44, No. 3, 482 (1979).
5. G. N. Kryzhanovskii, E. V. Nikushkin, V. E. Braslovskii, and R. N. Glebov, Byull. Éksp. Biol. Med., 89, 14 (1980).
6. E. V. Nikushkin, V. E. Braslovskii, and G. N. Kryzhanovskii, Byull. Éksp. Biol Med., 90, 696 (1980).

7. N. K. Popova, E. V. Naumenko, and V. G. Kalpakov, in: Serotonin and Behavior [in Russian], Nauka, Novosibirsk (1978), p. 252.
8. I. B. Prakh'e, "Audiogenic convulsions in rats and effects of neurotropic drugs on them," Author's Abstract of Candidate's Dissertation, Leningrad (1971), p. 21.
9. S. I. Prikladnovitskii, Byull. Éksp. Biol. Med., 14, No. 8, 46 (1942).
10. J. P. Bennet and S. H. Snyder, Mol. Pharmacol., 12, 373 (1976).
11. A. Chapman, M. Ingvar, and B. Siesjo, Acta Physiol. Scand., 110, No. 3, 335 (1980).
12. P. H. Deracke, S. Mityavila, et al., Ann. Nutr. Alim., 34, 599 (1980).
13. L. Frank and D. Massargo, Am. J. Med., 69, No. 1, 117 (1980).
14. C. Galli, C. Spagunolo, and A. Petroni, Adv. Prostagl. Thrombox. Res., 8, 1235 (1980).
15. C. Kellog, J. Neurobiol., 2, 209 (1971).
16. B. Lippitt and I. Fridovich, Arch. Biochem. Biophys., 159, 738 (1973).
17. S. L. Taylor, M. P. Lamden, and A. L. Tappel, Lipids, 11, No. 7, 530 (1976).

EFFECT OF CHRONIC PRESYNAPTIC NEUROMUSCULAR TRANSMISSION BLOCK ON PROPERTIES OF FROG MUSCLE FIBER MEMBRANES

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Denervation of a muscle, disturbing neurotrophic control, changes the properties of the frog muscle fiber membrane [4]. Extrasynaptic sensitivity to acetylcholine (ACh) appears, the resting membrane potential (RMP) falls, and the cable properties of the membrane are modified [2, 4, 18].

Chronic postsynaptic blocking of neuromuscular transmission by curare or α-bungarotoxin has been shown not to change the properties of frog muscle fiber membranes [6]. This suggests that nervous impulses and synaptic ACh do not play a decisive role in maintenance of the functionally necessary state of muscle fibers determined by the nervous system. Meanwhile it has been shown that presynaptic blocking of ACh secretion by botulinum toxin causes a series of denervation-like changes in the muscle membrane, although to a less marked degree than denervation of the mucle [8], and this is regarded as proof of the participation of ACh in trophic influences on muscle. Meanwhile in experiments with botulinum toxin not only does ACh secretion cease, but the outflow of substances brought into nerve terminals by axonal transport, and exerting a trophic influence on muscle, from the terminals also is disturbed [10]. At the same time the muscle is excluded from motor activity. All these considerations make it difficult to evaluate the role of both synaptic ACh and of nervous impulses and the associated motor activity of the muscle in neurotrophic control of muscle fibers.

It was accordingly decided to make a further study of the contribution of ACh and nervous impulses in neurotrophic control of the muscle fiber membrane in frogs, using different experimental approaches for the purpose, and details of this study are given below.

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