LIPID PEROXIDATION IN EXPERIMENTAL DEGENERATION OF THE RETINA

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Pigmented degeneration of the retina or, as this severe eye disease is nowadays known, pigmented tapeto-retinal abiotrophy, is one of the most serious diseases of the organ of vision, for it may cause blindness and disablement. Although this problem has been a subject for continuous study by ophthalmologists, much regarding the etiology and pathogenesis of this eye disease remains unexplained.

Experimental investigation of physicochemical processes lying at the basis of the development of various forms of retinopathy in animals, induced by iodine compounds, phenothiazide, azide, bromoacetate, etc., is a highly promising method of elucidating the mechanism of degenerative diseases in man [4].

One such physicochemical process is lipid peroxidation (LPO). The importance of the study of changes in the rate of LPO during degeneration of the retina is accounted for by several arguments presented in the literature. In particular, one such argument is the relationship between the rate of LPO of biological membranes and metabolism of the minor thiols, which undergo significant changes during degeneration of the retina [10, 11]. Moreover, intensification of LPO during degeneration of the retina can considerably aggravate the damage to the retina, for highly toxic LPO products can induce after-effects in the cells such as inactivation of many enzymes, increased permeability, oxidation of thiols, etc. [2, 5].

It has been suggested [7, 8] that factors inducing degeneration may act in photoreceptor membranes as "chaotropic agents," weakening hydrophobic and electrostatic bonds in the membranes and breaking down lipoprotein complexes, thus leading to an increase in the velocity of LPO.

The aim of this investigation was to study the rate of LPO during degeneration of the retina caused by injection of monoiodoacetic acid (MIA) and by oxygen poisoning.

EXPERIMENTAL METHOD

Degeneration of the retina was induced by injection of MIA in a dose of 18-24 mg/kg into the auricular vein of 95 rabbits weighing 3-4 kg. To create experimental degeneration of the retina of different degrees of severity MIA was injected between 1 and 4 times at intervals of 6-7 days. Altogether 180 guinea pigs weighing 250-300 g were subjected to oxygen poisoning in a special pressure chamber for 24 h.

The electroretinogram (ERG) of isolated guinea pig retinas was recorded as in [3]. Vitamin E (tocopherol acetate) in a dose of 120-160 mg/kg, which was injected intramuscularly, was used as antioxidant. The velocity of LPO was judged by changes in the malonic dialdehyde (MDA) concentration [1].

EXPERIMENTAL RESULTS

The results show that 20-30 min after a single injection of MIA the velocity of LPO in the retina was substantially increased: the MDA concentration was raised (Fig. 1a). During

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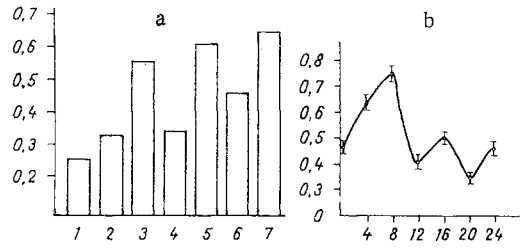

Fig. 1

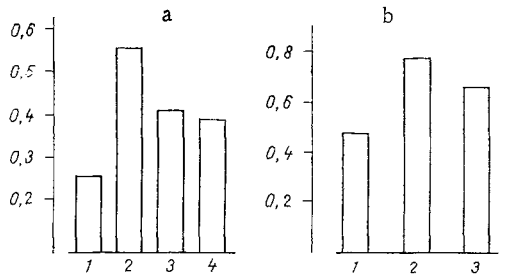

Fig. 2

Fig. 1. Changes in MDA concentration during experimental degeneration of the retina: a) after injection of MIA: 1) control, 2-4) 30 min, 24 h, and 7 days, respectively, after a single injection, 5) after a second injection, 6) after a third injection, 7) after a fourth injection. Ordinate, MDA level (in nmoles/mg protein); b) during oxygen poisoning. Abscissa, length of exposure (in h); ordinate, MDA level (in nmoles/mg protein.

Fig. 2. Effect of vitamin E on changes in MDA concentration in experimental degeneration of the retina: a) during injection of MIA: 1) control, 2) 24 h after injection of MIA, 3) injection of MIA into animals 24 h after injection of vitamin E, 4) injection of vitamin E into animals 2 h after injection of MIA; b) during oxygen poisoning: 1) control, 2) after exposure to oxygen for 8 h, 3) after exposure to oxygen for 8 h preceded by vitamin E administration. Ordinate, MDA level (in nmoles/mg protein).

the 24 h after injection the MDA concentration continued to rise and reached more than twice the control level. However, 1 week after a single injection the MDA concentration fell almost to the control level, evidently because of the protective ability of the body.

After two injections of MIA the MDA level was definitely high. After three injections of MIA, however, the MDA concentration fell a little. In our opinion the fall in the MDA concentration in this case was due to complete destruction of the rods and cones, whose lipids undergo LPO more intensively. After four injections of MIA the MDA level again rose considerably, evidence of involvement of more deeply lying layers of the retina in LPO.

The mechanism of the increase in the velocity of LPO under the influence of different poisons has not been completely explained. Intensification of LPO during administration of MIA may be connected with its chaotropic action, and also with the possibility of formation of free radicals, during lipoprotein breakdown, which actively intervene in cell metabolism [6], on the one hand, and in disturbance of antioxidative protective mechanisms, on the other hand.

The results showed that during oxygen poisoning the MDA concentration in the guinea pig retina increased during exposure for 8 h, after which it fell close to the control level (Fig. 1b).

Evidently induction of chain oxidation of structural lipids of the photoreceptor membranes by such oxygen intermediates as the superoxide radical, hydrogen peroxide, and the hydroxyl radical, lies at the basis of the mechanism of action of intensification of LPO during oxygen poisoning.

Analysis of the kinetics of accumulation of LPO products in the retina depending on the duration of exposure to oxygen indicates that the rate of LPO is reduced after exposure for 8 h (Fig. 1b). The concrete mechanism of this process is far from clear. Nevertheless, it can be definitely stated that the slowing of LPO during the late period of exposure (12-24 h) was not due to substrate exhaustion, for after addition of the $FeSO_4$—ascorbate complex the MDA level rose again appreciably. The fall in the MDA level was evidently due to a decrease in the concentration of polyunsaturated lipids, intensification of MDA breakdown, their interaction with amino groups of amino acids, proteins, and aminophospholipids and, finally, a condensation reaction [5, 9].

The experimental results show that administration of vitamin E, whether before or after injection of MIA, significantly reduced the MDA concentration (Fig. 2a). Similar results were obtained during oxygen poisoning. As Fig. 3 shows, preliminary injection of vitamin E lowered the MDA concentration in oxygen poisoning.

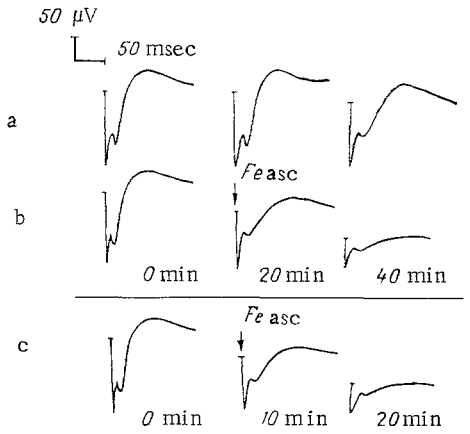

Fig. 3. Action of $FeSO_4$—ascorbate complex on parameters of ERG of guinea pigs during oxygen poisoning. a) Control, b) $FeSO_4$—ascorbate, c) $FeSO_4$—ascorbate after exposure to oxygen for 8 h.

To study the connection between changes in the velocity of LPO and the functional state of the retina, changes in ERG of the isolated guinea pig retina were investigated during oxygen poisoning. The results show that isolated guinea pig retinas during perfusion with oxygen-saturated Ringer's solution maintain the amplitude of all ERG waves almost unchanged for 1 h or more (Fig. 3).

Almost the same rule was observed when the ERG of the isolated retina was recorded before exposure to oxygen for 8 h. However, the action of the $FeSO_4$—ascorbate complex on the ERG of the control and experimental retinas differed significantly (Fig. 3). Whereas addition of $FeSO_4$—ascorbate complex to Ringer's solution with normal retinas lowered the amplitude of the waves of the ERG after 20-25 min, a fall in amplitude in the experimental retinas began after only 5-10 min.

If the animals were kept for a long time (16-24 h) in an atmosphere of oxygen, leading to more severe disturbances of their photoreceptor membranes, the components of the ERG were strongly inhibited. Addition of sodium selenite to the perfusion fluid in a concentration of 0.01% under these conditions did not restore electrical activity of the retina.

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