ANTIRADICAL ACTIVITY OF RETINAL LIPIDS DURING ILLUMINATION

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Free-radical oxidative processes taking place in lipids of photoreceptor membranes are controlled by various regulatory systems [4]. One such system is that of natural antioxidants, inhibiting excessive oxidation on account of metabolic reactions with peroxide radicals, and thereby maintaining the functional activity of the membranes.

However, the role of this system both in the regulation of lipid peroxidation (LPO) and in structural and functional characteristics of photoreceptor cells has not yet been adequately studied. The aim of this investigation was to study the antiradical activity (ARA) of retinal lipids under normal conditions and during intensive illumination.

EXPERIMENTAL METHOD

Lipids were isolated from the bovine retina as in [5]. ARA of the lipids and the quantity of endogenous antioxidants contained in them were determined on a chemiluminescence model of induced oxidation of ethylbenzene [1].

To study the action of intensive illumination on ARA of the retinal lipids, they were kept for 15 min under direct sunlight (0.8 J/sec, area of object $8.0 \cdot 10^{-3}$ m^2).

The effect of exogenous antioxidants on characteristics of the system of natural antioxidants of retinal lipids was studied by incubating them for 1 h at 4°C in incubation medium, with periodic shaking. The incubated retinas were then removed from the medium and washed twice with physiological saline. The retinas, after washing to remove the antioxidants, were illuminated in the same way. Preparation of the incubation medium: solutions of antioxidants in ethanol were diluted with physiological saline to 0.1 mM, then exposed to ultrasound in the cold for 3-5 min, using a UZDN-2T ultrasonic disintegrator (44 kHz, 0.5 A).

α-tocopherol (TP), potassium phenosan (P), and ionol (I) were used as antioxidants.

To calculate values of ARA of the lipids and of the endogenous antioxidants contained in them, equations deduced in [6] to calculate velocity constants of synthetic antioxidants with an inhibitory action, and used in [1], were adopted, allowing for the particular features of the lipids as complex multicomponent systems.

EXPERIMENTAL RESULTS

It was found that an increase in the concentration of retinal lipids reduces the intensity of luminescence. The S-shaped kinetic curves of changes in the intensity of luminescence had periods of induction and a high rate of change of the intensity of luminescence.

Analysis of the kinetic curves showed that the effective constant K_7, characterizing ARA of the sum total of substances composing lipids, was $3.0 \cdot 10^6$ liters/mole · sec. Comparison of our data with values of the constant K_7 or TP ($4.0 \cdot 10^6$ liters/mole · sec) [3] leads to the conclusion that K_7 for retinal lipids and for TP are comparable and are values of the same order of magnitude. This suggests that the main contribution to ARA of the retinal lipids is brought by TP, whose presence in photoreceptor membranes was demonstrated in [4, 7]. However, the effective constant K_7 for retinal lipids was nevertheless lower than that for the individual antioxidant TP, possibly due to the presence of other antioxidants with a lower constant K_7 retinal lipids.

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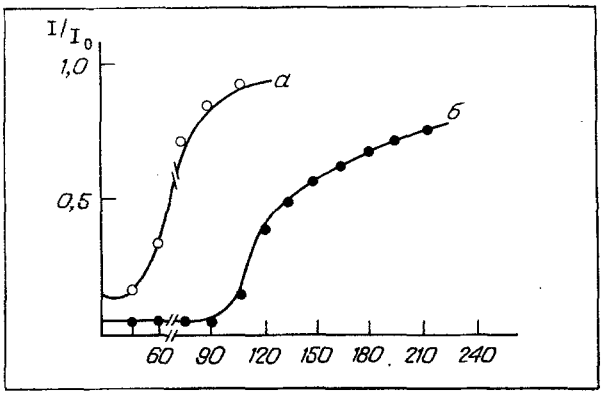

Fig. 1. Kinetic curves of change in intensity of luminescence of lipids of intact retinas depending on their concentration. a) 0.44 mg/ml, b) 1.87 mg/ml. Abscissa, time (in sec); ordinate, reduced to initial intensity of chemiluminescence (in relative units).

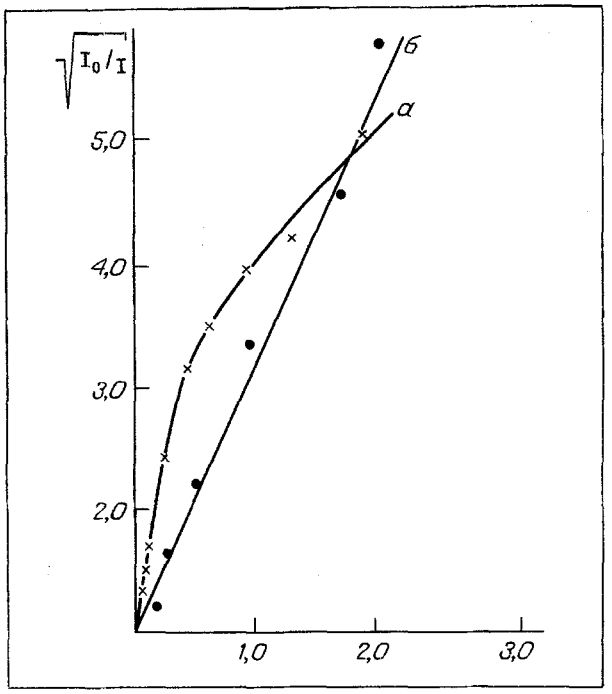

Fig. 2. Dependence of effectiveness of quenching of luminescence on lipid concentration: a) intact retina, b) illuminated retinas. Abscissa, lipid concentration (in mg/ml).

Determination of the content of antioxidants (the actual fraction, α) found in the retinal lipids showed that it was $2.5 \cdot 10^{-4}$ mole of antioxidant/mole of lipid. Incidentally, this is the amount of these antioxidants which possess high efficiency. Since the inhibitory action of antioxidants is determined by both their amount and their efficiency, the product of these characteristics will reflect the total inhibitory activity of natural antioxidants (TIA-$K_7 \cdot \alpha$). TIA for retinal lipids was $7.5 \cdot 10^2$ liters/mole · sec · mole of antioxidant/mole of lipid.

The study of the effect of intensive illumination on the retina showed that values of the parameters of the system changed after illumination, i.e., values of the effective constant K_7 and the content of antioxidants were depressed. Whereas normally the efficiency of quenching of luminescence of the lipids was 5.2, after illumination it fell to 2.5 (Fig. 2). ARA of the retinal lipids became $2.4 \cdot 10^6$ liters/mole · sec. The active fraction of antioxidants of the experimental retinas fell by 40% compar-

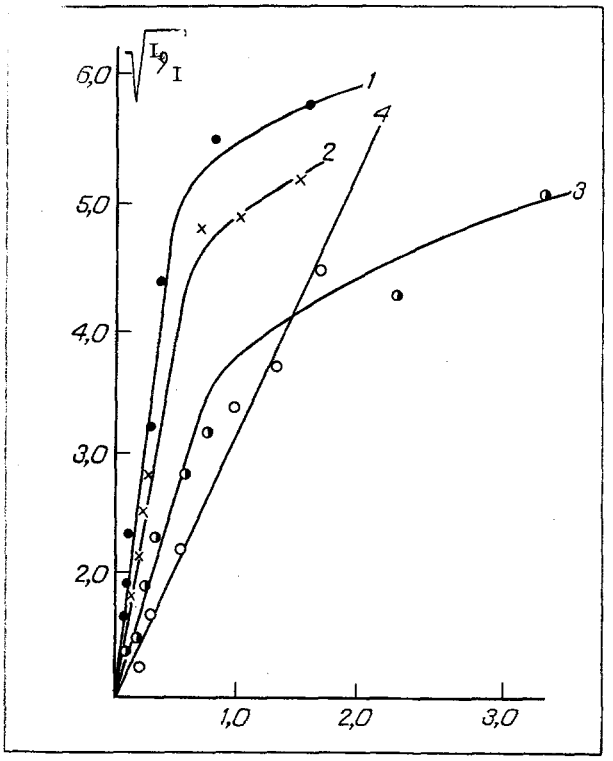

Fig. 3. Dependence of efficiency of quenching of luminescence of retinal lipids incubated with antioxidants (0.1 mM, 1 h) and exposed to the action of strong illumination (15 min, 8.8 J/sec) on concentration. 1) Incubation with TP, 2) with P, 3) with I; 4) incubation without antioxidant (control). Abscissa, concentration of lipids (in mg/ml).

ed with normal, i.e., their content became $1.8 \cdot 10^{-4}$ mole antioxidant/mole of lipid. TIA of natural antioxidants present in the lipids of illuminated retinas was $4.3 \cdot 10^2$ liters/mole · sec · mole antioxidant/mole of lipid.

Reduction of the values of the characteristics of the system of natural antioxidants of retinal lipids after exposure to strong illumination is connected with the fact that illumination in the retina induces intensification of LPO, as a result of which antioxidants are utilized in metabolic reactions with peroxide radicals.

The possibility of correcting the characteristics of the system of natural antioxidants of the retinal lipids by antioxidants after the modifying action of strong illumination was studied by incubating retinas with antioxidants (TP, P, I) taken in equimolar amounts (0.1 mM) and their subsequent illumination for 15 min at 0.8 J/sec.

It was shown that despite the effect of light, which lowers both the efficiency of antioxidants and their content, antioxidants contained in the incubation medium and incorporated into the retinal membranes increased values of characteristics of the system of natural antioxidants. In Fig. 3, for instance, which shows curves of the change of efficiency of quenching of luminescence as a function of lipid concentration, it will be clear that lipids isolated from retinas incubated with TP had the strongest quenching action on luminescence.

Table 1 gives the results of investigations into the effect of strong illumination and antioxidants on characteristics of the system of natural antioxidants of retinal lipids. It was shown that the antioxidants studied affected equally both ARA of the lipids and the content of antioxidants. TP, which increased the content of antioxidants in retinal lipids and, correspondingly, their antiradical activity, had the greatest penetrating power into the retinal membrane of all the antioxidants studied. The least efficient was I, whose constant K_7 was three orders of magnitude lower than that of TP [2].

Comparison of the value of TIA of the antioxidants present in retinal lipids with the content of LPO products — malonic dialdehyde (MDA, Table 1) shows that the higher the values of TIA of the antioxidants, the lower the level of MDA. In other words, negative correlation exists between these parameters, and this serves to stabilize the structural-functional state of the retinal membranes when exposed to strong illumination.

TABLE 1. Characteristics of System of Natural Antioxidants of Retinal Lipids under Normal Conditions and under the Influence of Strong Illumination and Antioxidants

Experimenal conditions	Antiradical activity, K_7 ($\times 10^6$)	Active fraction of antioxidants, α($\times 10^{-4}$)	Total inhibitory activity of antioxidants ($\times 10^2$)	MDA, nmoles/mg protein
Normal	3,0	2,5	7,5	0,6
Illumination	2,4	1,8	4,3	1,2
Incubation + TP + illumination	3,3	5,2	17,2	0,12
Incubation with P + illumination	2,9	3,3	9,6	0,22
Incubation with I + illumination	2,6	1,8	4,7	0,35

The investigation thus showed that strong illumination potentiates retinal LPO and the utilization of antioxidants connected with it, leading to reduction of the antiradical activity of the lipids. It is shown that the damaging action of strong illumination on the retinal lipids can be corrected by the use of antioxidants.

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