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EFFECT OF INDUCED LIPID PEROXIDATION ON DARK ADAPTATION OF PHOTORECEPTORS OF THE ISOLATED FROG RETINA

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Very little is yet known about the mechanisms of visual adaptation of vertebrate photoreceptors. An important role in this process is ascribed to intracellular mediators [6, 8]. Three principal parameters characterizing adaptation in photoreceptors are distinguished: initial concentration of intracellular mediator, release of mediator into the intracellular space and the length of its life, and changes in conductance of Na leakage channels in the plasma membrane of the rods taking place during adaptation to light and darkness (membrane, or M, adaptation [8]). Consequently, besides mechanisms somehow changing the concentration of intracellular mediator, a definite role also is played by the plasma membrane and, in particular, by changes in conductance of its leakage channels. Meanwhile an unusually high concentration of polyunsaturated fatty acids, capable of being oxidized under the influence of light [3], has been observed in the vertebrate photoreceptor membrane, and lipid peroxidation (LPO) products can increase the permeability of both model [5] and biological membranes [4].

The aim of this investigation was to study the effect of LPO on dark adaptation of photoreceptors of the isolated frog retina.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana ridibunda) adapted to darkness for 16-18 h. The retina was isolated from the pigmented epithelium in weak red light in a solution of the following composition (in mM):

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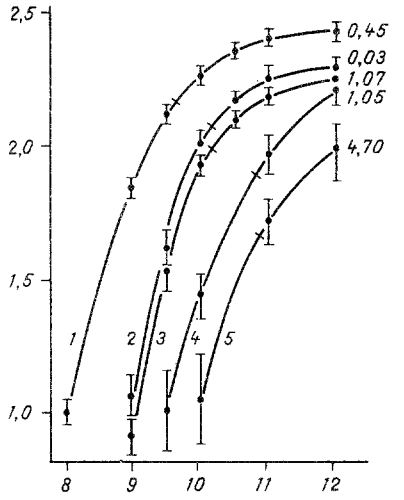

Fig. 1. Dependence of amplitude of LRP on stimulus intensity. Abscissa, log of stimulus intensity (in quanta/cm^2/sec); ordinate, log of amplitude of LRP (in μV). 1) Dark-adapted decolorized retinas; 2-5) partial decolorization at 35th-40th minute of dark adaptation; 2) incubation of retinas in presence of ionol; 3) control; 4) incubation of retinas in presence of ionol + Fe^{++} + ascorbate; 5) the same, in presence of Fe^{++} + ascorbate only. Vertical lines along curves denote values of semisaturation constants. Numbers on right show concentration of MDA (in nanomoles/mg protein).

NaCl – 100, KCl – 3.0, $MgSO_4$ – 1.2, $NaHCO_3$ – 25.0, $CaCl_2$ – 2, glucose – 5.6, sodium aspartate – 5.0, Tris-HCl buffer – 10; pH 7.8, placed on filter paper with the receptor side uppermost, and introduced into a chamber between two Ag-AgCl electrodes. The late receptor potential (LRP) was recorded on an S1-69 oscilloscope by means of a K544UD1A amplifier with integrated microcircuit. Flashes of light passed through an absorption filter with maximum of transmission at 490 nm, with a duration of 50 msec, were used for stimulation. The stimulus intensity was controlled by neutral filters.

Dark adaptation was judged by the shift of the stimulus-response curve, showing the relationship between log of LRP amplitude and log of stimulus intensity, the position of which was recorded before and after decolorization, when dark adaptation reached the steady state [8]. The conditions of decolorization were: yellow light, 50 lx, 6 min.

Permeability of the plasma membrane of the rods was estimated from the change in amplitude of LRP after treatment of the retina with 0.1 M strophanthin K [2].

The intensity of LPO in the retina was estimated from accumulation of malonic dialdehyde (MDA), measured by the reaction with 2-thiobarbituric acid [9].

An Fe^{++}–ascorbate system in concentrations of 10^{-5} and $8 \cdot 10^{-4}$ M respectively was used as LPO initiator, and an alcoholic solution of ionol in a concentration of $8 \cdot 10^{-5}$ M as the inhibitor. The final concentration of alcohol in the incubation solution was 0.5%.

EXPERIMENTAL RESULTS

The stimulus–response curve plotted between logarithmic coordinates can be described by a Michaelis–Menten equation

$$V(I) = V_{max} \frac{I}{I + \sigma},$$

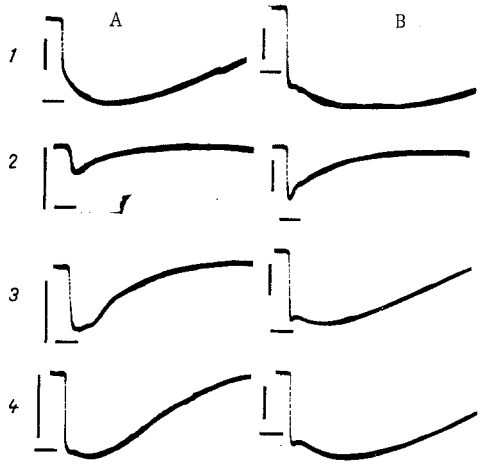

Fig. 2. LRP of isolated frog retina obtained in response to stimuli with intensity of 11 log units (A) and 12 log units (B). 1) Initial responses in state of dark adaptation; 2) immediately after illumination; 3) after 15 min of dark adaptation; 4) after 35 min of dark adaptation. Calibration: 100 μV, 0.5 sec.

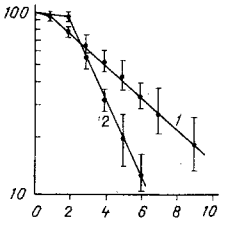

Fig. 3. Effect of ascorbate-induced LPO on permeability of rod plasma membrane. Abscissa, time (in min), ordinate, relative amplitude of LRP after addition of 0.1 M strophanthin K to solution (in percent). 1) Control; 2) Fe^{++} ascorbate.

where V denotes the amplitude of LRP; V_{max} its maximal amplitude; σ is a semisaturation constant, numerically equal to stimulus intensity for which $V = \frac{1}{2}V_{max}$. Thus, having determined the basic constants, such as the saturation amplitude and semisaturation constant, and by recording their changes, it is possible to judge the state of the photoreceptors when exposed to various influences.

The stimulus–response curve for adapted decolorized retinas in the present experiments had a definite shape and position (Fig. 1). Decolorization changed the shape of the responses (Fig. 2) considerably and shifted the curve toward high intensities and low amplitudes of LRP. Later in the course of dark adaptation the stimulus–response curve gradually returned to its initial position. Since this process is exponential in character, and since intervals between stimuli could not be shorter than 1-1.5 min [8], it was difficult to determine the shape and position of the curve during dark adaptation, especially in the initial period. However, after the end of 35-40 min, when the dark adaptation process reached the steady state, no further shift of the curve was observed. Incidentally, when the control curve reached the steady state it always returned to its initial shape, but never regained its initial position (Figs. 1 and 2).

Addition of ionol to the incubation medium of the retina caused no appreciable change in the shape or position of the stimulus–response curve for dark-adapted, nondecolorized retinas. However, after decolorization and subsequent dark adaptation the curve was shifted to the left and upward relative to the control ($P < 0.02$; Fig. 1). The semisaturation constant under these circumstances remained unchanged.

During dark adaptation and with optimal preservation of retinal function, ascorbate-induced LPO led to a very small decrease in amplitude of the LRP: usually by 15-20% after an exposure of 30 min. However, after decolorization of the retina in the presence of induced LPO, restoration of the amplitude of LRP during dark adaptation was abruptly slowed. The position of the stimulus–response curve obtained in the steady state of dark adaptation, for retinas treated with the Fe^{++}–ascorbate system, differed appreciably from the position of the control curve ($P < 0.001$; Fig. 1).

Preincubation of the isolated retina in medium containing the antioxidant, followed by addition of the LPO initiator, led to more rapid restoration of LRP after decolorization in the course of dark adaptation. The stimulus–response curve in this case was shifted relative to that for retinas treated with the LPO initiator only, mainly upward (Fig. 1), as shown by an increase in the maximal amplitude of LRP and by no change in the semisaturation constant.

According to the model mentioned above, such a situation could arise if the LPO initiators (the Fe^{++}–ascorbate system) affected either the initial concentration of intracellular mediator (c_i-adaptation) or the permeability of the photoreceptor plasma membrane after decolorization (M-adaptation) [8]. Since the LPO initiator, like the antioxidant, was added to the incubation medium, their action, in the writers' opinion, must have been limited to the plasma membrane of the photoreceptors.

Proof of a change in permeability of the rod plasma membrane under conditions of induced LPO is given by experiments to measure the rate of decrease of amplitude of LRP after treatment of the retina with strophanthin. LPO products probably increase the permeability of the photoreceptor plasma membrane, causing more rapid disappearance of the responses (Fig. 3).

Accumulation of LPO products in the isolated retina may therefore affect dark adaptation processes in the rods. Judging from the change in position of the stimulus–response curves obtained in the steady state after completion of dark adaptation, and also on the basis of results of experiments to measure the rate of decline of the amplitude of LRP after treatment of the retina with strophanthin, it can be concluded that the most likely mechanism of this effect is a change in permeability of the rod plasma membrane.

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