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EFFECT OF EMOXYPIN ON BASAL CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITY AND LATE RECEPTOR POTENTIAL OF THE ISOLATED RETINA

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Key Words: emoxypin; cyclic nucleotide phosphodiesterase; frog retina.

In pathology of the organs of vision and, in particular, in hereditary degenerations and dystrophies [7, 9], disturbances in the cyclic nucleotide system are observed. It has been shown [4, 5] that emoxypin (a preparation of the 3-hydroxypyridine class) causes an increase in visual acuity in patients with hereditary degeneration of the retina (Stargardt's disease) and with central chorioretinal dystrophies, in whom total electrical activity is depressed. However, the mechanisms of action of emoxypin in the treatment of degenerations and dystrophies of the retina remain unexplained.

The writers showed previously [3, 8], that 3-hydroxypyridine (3-HP) derivatives, including emoxypin (2-ethyl-6-methyl-3-HP), inhibit cyclic nucleotide phosphodiesterase (PDE) in the rabbit heart and platelets. PDE inhibitors are known to cause an increase in amplitude and to delay the appearance of the receptor response of the rods in the isolated vertebrate retina, to raise their threshold sensitivity, and to shift the curve of response amplitude as a function of photic stimulus intensity toward lower levels of illumination, simulating the action of dark adaptation [2].

To determine the possible mechanism of the therapeutic action of emoxypin we studied its effect on the late receptor potential (LRP) of the isolated retina and on basal PDE activity of the outer segments of the retinal rods.

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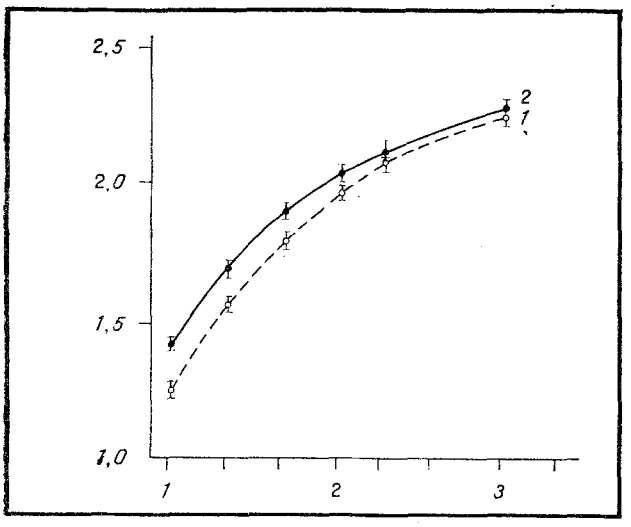

Fig. 1. Amplitude of LRP as a function of stimulus intensity. Abscissa, logarithm of stimulus intensity (in quanta per rod per flash); ordinate, logarithm of amplitude (in μV). 1) Control, 2) emoxypin (10^{-3} M).

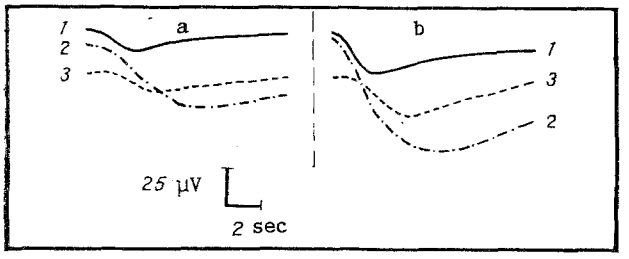

Fig. 2. Effect of PDE inhibitors on LRP of isolated frog retina with stimulus intensity of 45 (a) and 85 quanta (b) per rod per flash. 1) Control; 2) isobutylmethylxanthine (10^{-4} M); 3) emoxypin (10^{-3} M).

EXPERIMENTAL METHOD

Experiments were carried out on isolated retinas of dark-adapted frogs (*Rana ridibunda*).

The retina was separated from the pigmented epithelium in weak red light, laid out in Ringer's solution on a strip of filter paper with the receptor side uppermost, placed in a chamber and covered with black plastic with a hole 4 mm in diameter to admit light [1]. The chamber was filled with the following solution: NaCl 85 mM, aspartate 20 mM, $NaHCO_3$ 10 mM, KCl 2.5 mM, $MgSO_4$ 1 mM, $CaCl_2$ 1 mM, Tris-HCl 10 mM (pH 7.8, volume 2 ml). A gas mixture (95% O_2 and 5% CO_2) was passed through the solution. Emoxypin was added in the form of low concentrations in alcoholic solution. The final concentration of ethanol was 0.5%, and in this concentration ethanol had no action on LRP [1].

LRP was recorded transretinally by two Ag—AgCl electrodes and led to the input of a dc amplifier on KR 544 UD 1A integral microcircuit and photographed from the screen of an S1-69 oscilloscope. The oscilloscope beam was triggered and the screen of the electromechanical galvanometer deflected, producing a flash of light, by means of a two-channel DK/ÉS-4M electrostimulator, working on single operating mode. The maximal wavelength was 490 nm and the duration 100 msec. Stimulus intensity was controlled by neutral filters. The outer segments of the rods of the frog retina were isolated by the method in [10], PDE activity was determined by the method in [6], and protein as in [9].

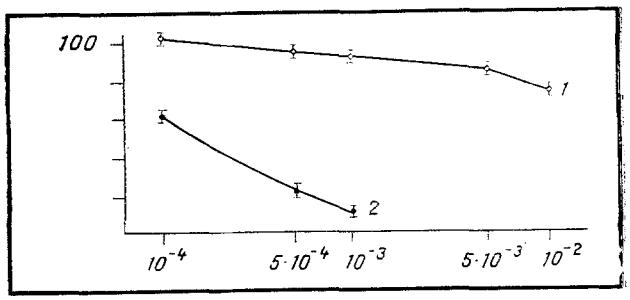

Fig. 3. Effect of isobutylmethylxanthine and emoxypin on basal cyclic nucleotide PDE activity from outer segments of retinal rods. Abscissa, logarithm of concentration of inhibitor (in M); ordinate, activity (in percent of control, taken as 100%). Substrate cyclic GMP, 10^{-6} M. 1) Emoxypin, 2) isobutylmethylxanthine.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that emoxypin caused a significant increase in amplitude of the LRP of the isolated retina and shifted the curve of response amplitude toward a lower intensity of illumination. For instance, with a stimulus intensity of 10 quanta per rod per flash, the amplitude of the response of LRP increased from 17.5 to 25.5; with an intensity of 45 quanta per rod it increased from 35.6 to 49.5; with 85 quanta per rod from 66.8 to 77.8, and with 100 quanta per rod from 95.2 to 113.5. With a further increase in stimulus intensity the difference between the experiment and control decreased and was no longer significant.

More marked changes were found in LRP in the course of the responses. An increase in amplitude of LRP and in the time taken to reach maximal amplitude under the influence of emoxypin and of isobutylmethylxanthine is shown in Fig. 2. Maximal amplitude of LRP was recorded in the control 2.6 sec after the stimulus (45 and 85 quanta per rod per flash), whereas in the presence of emoxypin and isobutylmethylxanthine it was recorded after 4 and 6 sec (with the same stimulus intensities).

It will be clear from Fig. 3 that emoxypin and isobutylmethylxanthine inhibited basal PDE activity of the outer segments of the frog retinal rods (isobutylmethylxanthine was used for comparison with emoxypin as a classical PDE inhibitor).

The results are evidence that emoxypin, in its action on perfused isolated retinas, causes an increase in amplitude and slowing of the receptor response of the rods; it increased their threshold sensitivity (shifted the curve of dependence of responses on stimulus intensity toward lower intensities of illumination), simulating the action of dark adaptation. Emoxypin also behaves like the classical PDE inhibitor, isobutylmethylxanthine; emoxypin, like isobutylxanthine, increases the sensitivity of the retina to light in vitro.

We also showed that emoxypin inhibits the transducing-stimulated PDE activity of the outer segments of the retinal rods by 52% (results not given).

The increase in retinal functional activity in vivo under the influence of emoxypin, expressed as an increase in amplitude of the *a*- and *b*-waves of the electroretinogram [5], can evidently be explained by its ability to inhibit cyclic nucleotide PDE.

The results indicate that one way in which the therapeutic action of emoxypin may be mediated and, in particular, an increase in functional activity of the retina, is by its influence on the cyclic nucleotide system, which plays an important role in the transformation of the photic stimulus into an electrical stimulus through cyclic nucleotide PDE. It can be tentatively suggested that, together with its antioxidant properties, an essential role tn the complex mechanism of action of emoxypin on retinal metabolism may be played by the ability of emoxypin to modify the cyclic nucleotide system.

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