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BIOGERONTOLOGY

Effect of Epithalon on Age-Specific Changes in the Retina in Rats with Hereditary Pigmentary Dystrophy

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The effect of peptide bioregulator Epithalon on the course of hereditary pigmentary retinal degeneration was studied in Campbell rats. Administration of epithalon starting from birth protected morphological structure, increased its bioelectrical activity, and improved its function.

Key Words: *Epithalon; pigmentary retinal degeneration; Campbell rats*

Degenerative changes in the retina are the main causes of vision impairment and blindness. At present, there are no effective methods for treating degenerative diseases of the retina. Gene engineering research in this sphere is in progress, but the results are not yet introduced into medical practice [7,13], and therapy is aimed at improvement of retinal trophics, without consideration for the pathogenetic mechanisms of the disease development [14]. It is noteworthy that degenerative changes in the retina are caused by specific disturbances in protein metabolism in the retinal pigment epithelium and other layers of the retina [10,11].

Therefore, mechanisms of tissue-specific peptide regulation of expression of genes determining structural and functional cell specialization are very important in this respect. Among physiologically active substances, peptides with tissue-specific effects attracted much recent attention [1]. Tetrapeptide Epithalon (Ala-Glu-Asp-Gly) [3] was synthesized on the basis of amino acid analysis of complex preparation isolated from animal retina (Retinalamine, State Pharmacopoeia of the Russian Federation) [2,5]. Four amino acids present in Retinalamine in the highest concentrations were used for the synthesis of new peptide. Interestingly, the composition of peptides constructed on the basis of amino acid analyses of preparations isolated from the epiphysis (Epithalamine) and retina (Retinalamine) was completely identical. This fact attests to existence of common mechanisms of metabolism regulation in the epiphyseal and retinal cells, which is due to their development from the same embryonal leaflet (neuroectoderm). Moreover, it was proven that epiphyseal secretory cells (pinealocytes) are homologous to the retinal photoreceptor cells. In higher vertebrates and in humans pinealocytes lost their capacity to directly react to light stimuli; their reaction consists in secretion of two humoral regulators (melatonin and serotonin), depending on the time of the day [6]. In turn, daily rhythm of renewal of external segments of photoreceptors, rods and cones (rods at dawn and cones at sunset) also indicates the relationship between these cells [12].

Stimulatory effect of Epithalon on the retina due to its capacity to regulate cell metabolism at the level of impaired vascular-tissue membranes was discovered previously [3]. In addition, Epithalon possesses pronounced antioxidant and geroprotective effects [4,8].

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We studied the effect of epithalon on the course of genetically determined [9] pigmentary retinal degeneration in Campbell rats.

MATERIALS AND METHODS

Campbell rats were obtained from London University College of Surgery.

The study was carried out on 124 rats of different age (from birth to 72 days). Experimental animals (n=62) were daily injected with 0.1 ml epithalon (1 μg, 0.2 ml per animal) into both eyes (in parabulbar tissue) throughout the entire experiment. Controls (n=62) were injected with 0.2 ml sterile 0.9% NaCl according to the same protocol.

The effect of epithalon on the course of hereditary retinal degeneration in rats was evaluated electrophysiologically and histologically. All experiments were performed under urethane analgesia (1500 mg/kg). Electroretinogram (ERG) was recorded with a 3-channel electrophysiological device in a dark shielded cage. After 10-min adaptation to darkness, light flashes (500 cd/m^2, 50 msec, at least 1 flash per 5 sec repetition frequency) were presented. ERG were recorded in the control and experimental groups starting from day 17 of life (eye opening) until the time when no response could be recorded.

For morphological examination the control and experimental animals were sacrificed on days 17, 29, 35, 41, 56, 59, 63, and 72 of the experiment (4-5 animals per term). The eyeball was fixed in 10% formalin in 96% ethanol. Deparaffinated sagittal sections of the eyeball were stained with hematoxylin and eosin. The preparations were examined at ×700 using a computer image analysis system. The thickness of the inner plexiform layer, inner and outer nuclear layers, and receptor layer was evaluated.

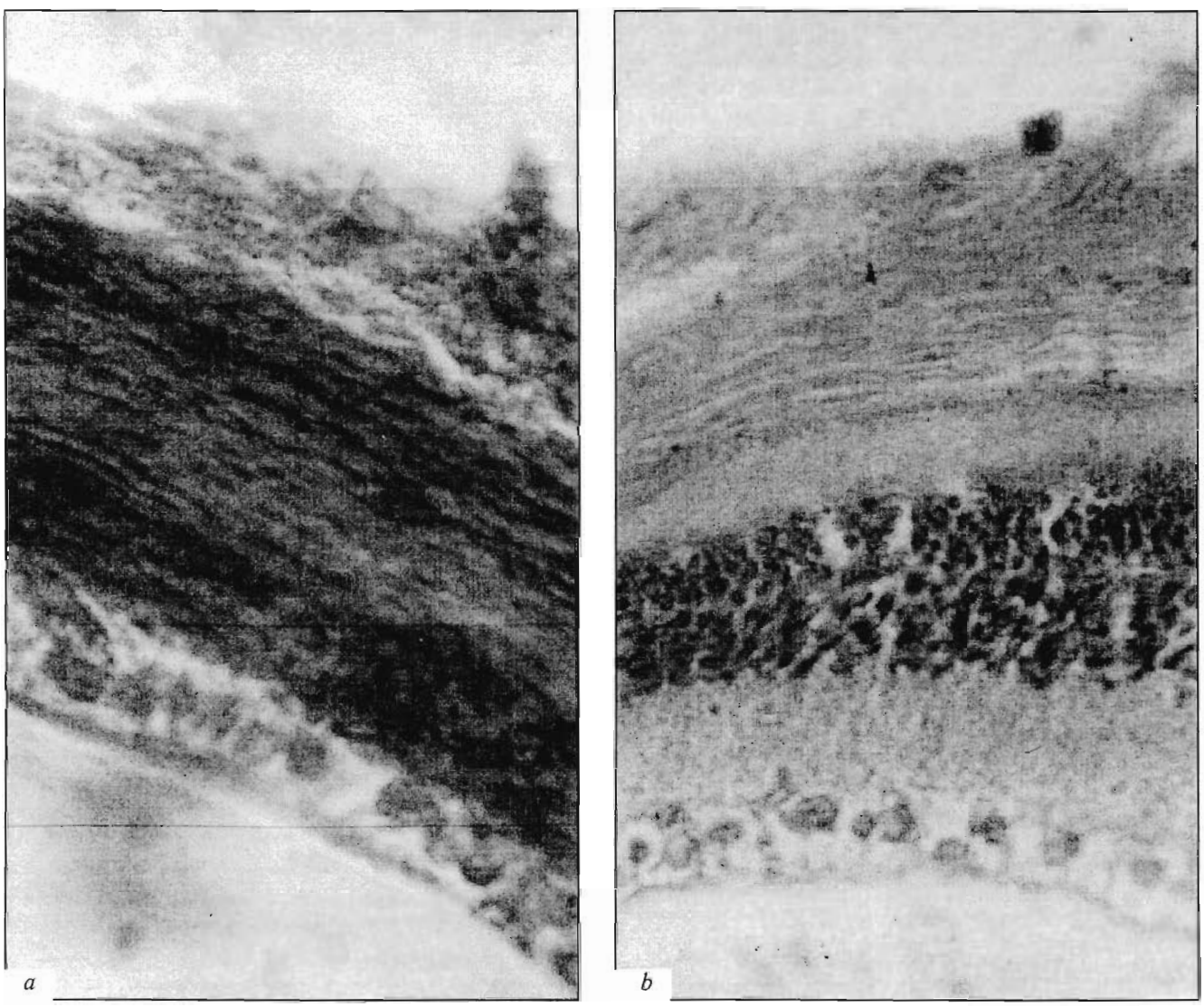

Fig. 1. Morphology of the retina in control (*a*) and epithalon-treated (*b*) Campbell rats on day 41 of life. Hematoxylin and eosin staining, ×700.

TABLE 1. Bioelectrical Activity (Summary Amplitude of ERG Waves, μV) of the Retina in Campbell Rats ($M \pm m$)

Group	Day of observation					
	17	29	35	41	56	59
Control	82.8±10.5	86.7±8.8	96.6±21.3	49.2±10.6	0	0
Experiment	56.8±13.2*	68.5±5.2*	114.9±18.7*	86.0±7.7*	69.4±15.6	37.5±10.5

Note. *$p<0.05$ compared to the control.

The data were statistically processed using Statistica software.

RESULTS

The aim of the study was general evaluation of bioelectrical activity of the retina in Campbell rats, and therefore we did not characterize individual waves and evaluated only the mean total activity of ERG waves a, b, and c.

Total bioelectrical activity increased from day 17 to day 35 in both control and experimental rats (by 16.7 and 102.3%, respectively) (Table 1). This could be due to an increase in rodopsine production during the first month of life. In control animals bioelectrical activity of the retina started to decrease on day 38 of life, and by day 43 ERG could not be recorded. In experimental animals ERG amplitude after day 40 of life remained high; on day 41 it surpassed the control by74.8%; and started to decrease only on day 60 of postnatal ontogeny. By day 62 ERG could not be recorded. Hence, Epithalon prolonged functional activity of the retina by 43.9%.

Morphological picture of the retina at birth was similar in experimental and control animals; changes in the retinal layers and structures were first detected on day 17 of postnatal development. In controls, all layers of the retina (nuclear, photoreceptor, outer plexiform layer containing rod and cone synapses with horizontal and bipolar cells) were narrowed. Hence, the inner nuclear layer containing amacrine, bipolar, and horizontal cells came closer to the outer nuclear layer consisting of cone and rode nuclei. These processes developed gradually. By day 38, the photoreceptors layer almost disappeared in controls (was not stained with hematoxylin and eosin), which probably indicates the absence or little number of photoreceptors or their low chemical and physiological activity. On day 41, complete destruction of all retinal layers was found in control rats, while in the experimental group all retinal layers were preserved (Fig. 1). In the experimental group disorders in the structure of the photoreceptor layer were observed starting from day 58 of postnatal ontogeny. The photoreceptor layer was still present in later terms, but it was gradually replaced with connective tissue, and by day 72 the retina was completely destroyed. Histological examination of the retina from control and experimental rats showed that Epithalon prolonged the period of retinal intactness by 75.6%.

Hence, in rats with hereditary pigmentary retinal degeneration Epithalon by 74.8% increased ERG amplitude on day 41 of life, prolonged the duration of functional activity of the retina by 43.9%, and prolonged the duration of morphological intactness of the retina by 75.6% in comparison with the control. These data, no doubt, attest to high efficiency of Epithalon in the treatment of some forms of retinal degeneration.

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